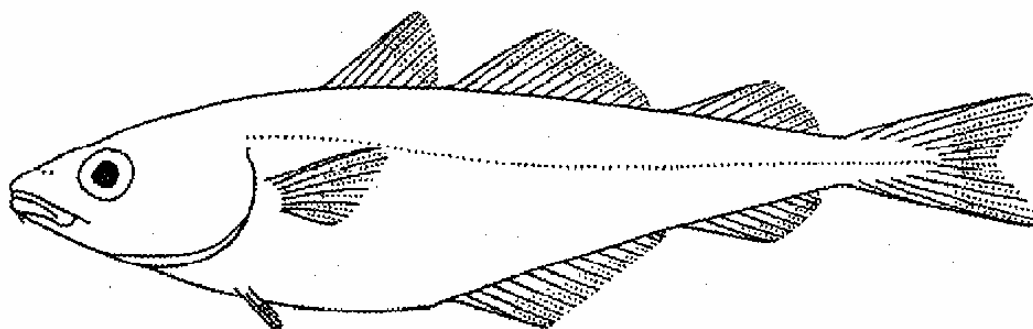


Manual to determine gonadal maturity of North Sea saithe (*Pollachius virens* L)



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J. Dalskov, I. Wilhelms, A. Sell, B. Bland, F. Vitale,
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 McCorriston	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

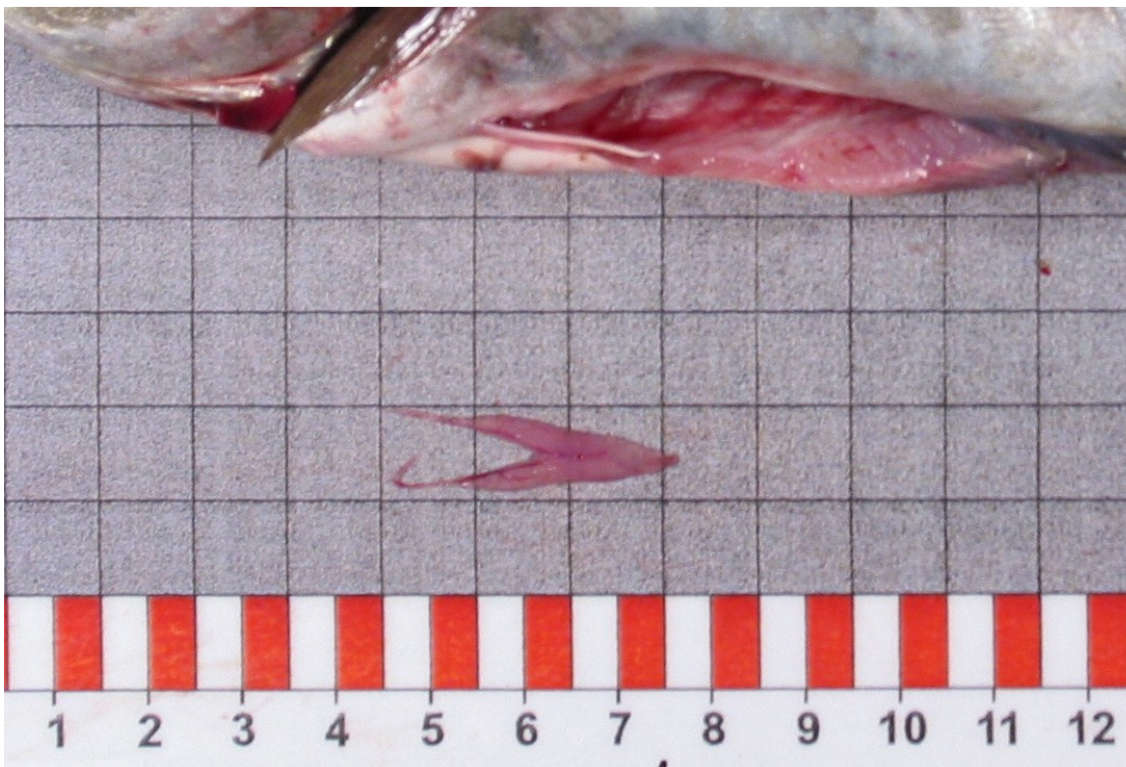
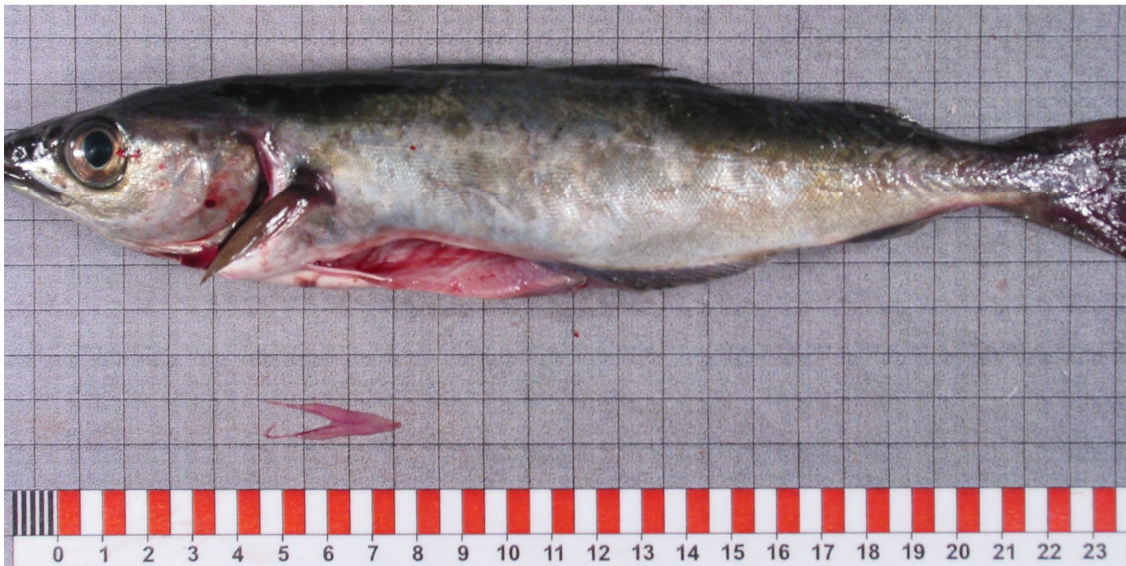
The editors:

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

I. Juvenile/immature (early)

Stage I (early):

Ovaries small paired organs posterior in body cavity close to bladder; elongated; translucent purple-reddish.



Specimen data

L_T: 27 cm

M_B: 138 g

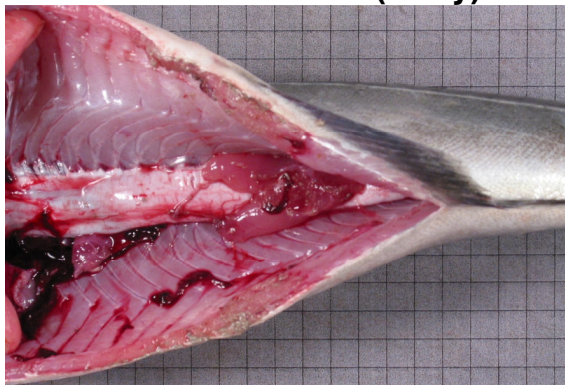
M_G: < 0.1 g

GSI: ~ 0

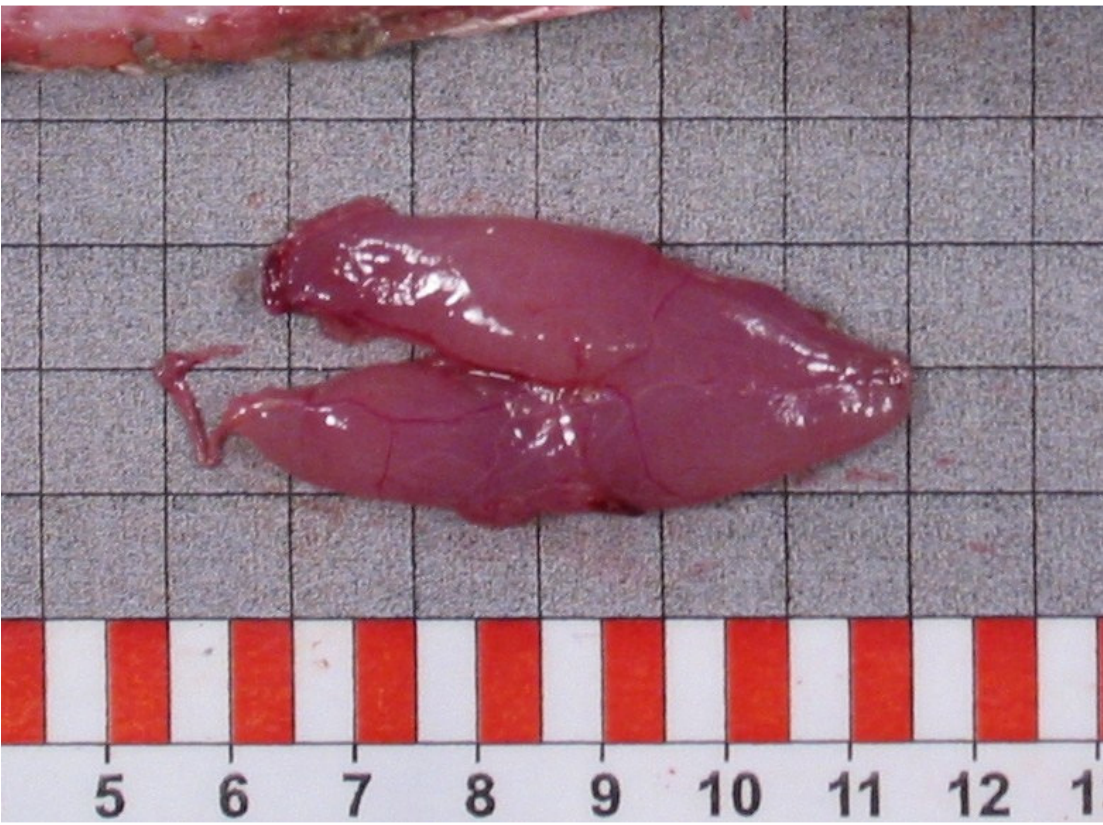
M: February 2007

ID: 070718/48

I. Juvenile/immature (early)



Stage I (early):
(continued)



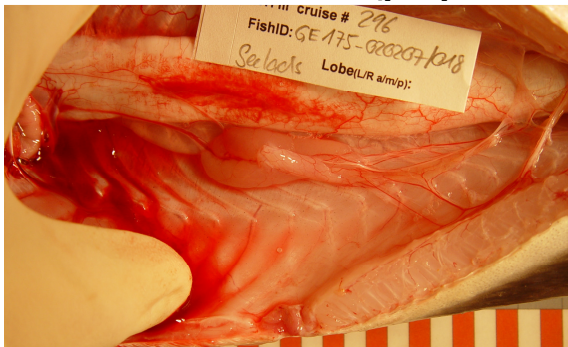
Specimen data

L_T: 50 cm
M_B: 957 g

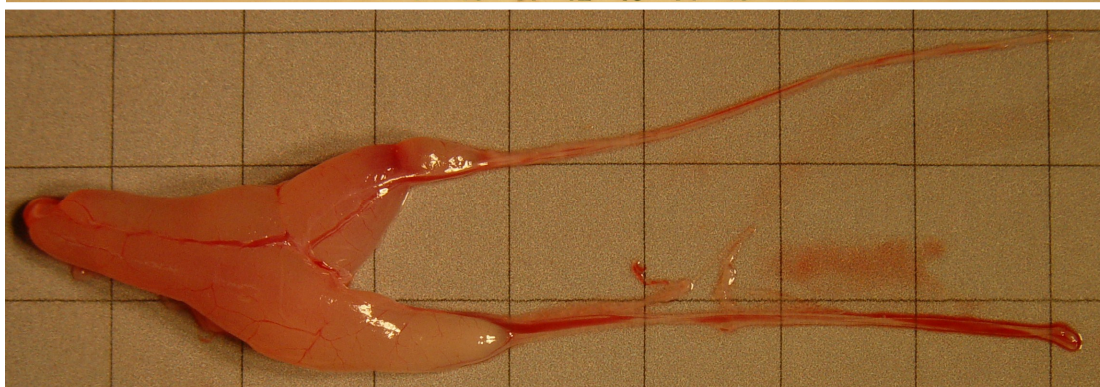
M_G: 5.7 g
GSI: 0.6

M: February 2007
ID: 070718/160

I. Juvenile/immature (preparation)



Stage I (preparation):
Ovaries small but easily distinguishable posterior in body cavity; surface is smooth; slightly translucent.



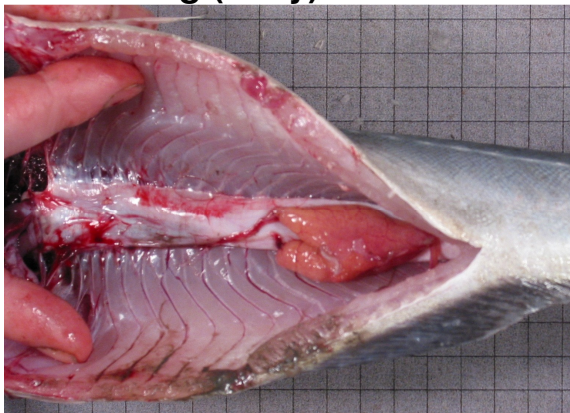
Specimen data

L_T: 44 cm
M_B: 553 g

M_G: 1.4 g
GSI: 0.3

M: February 2007
ID: 070718/185

II. Maturing (early)



Stage II (early):

Ovaries still small and restricted to posterior body cavity; firmer than in stage I, surface is slightly uneven; opaque orange.



Specimen data

L_T: 40 cm
M_B: 517 g

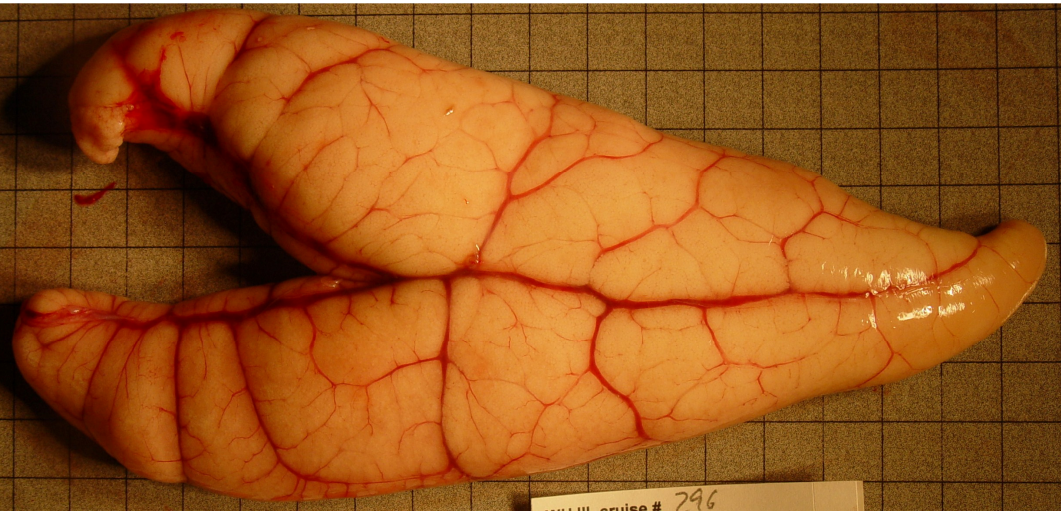
M_G: 5.4 g
GSI: 1

M: January 2007
ID: 070718/46

II. Maturing (late)



Stage II (late):
Ovaries have reached maximum size; firm with prominent blood vessels; opaque, orange to light pink; oocytes clearly visible and densely packed.

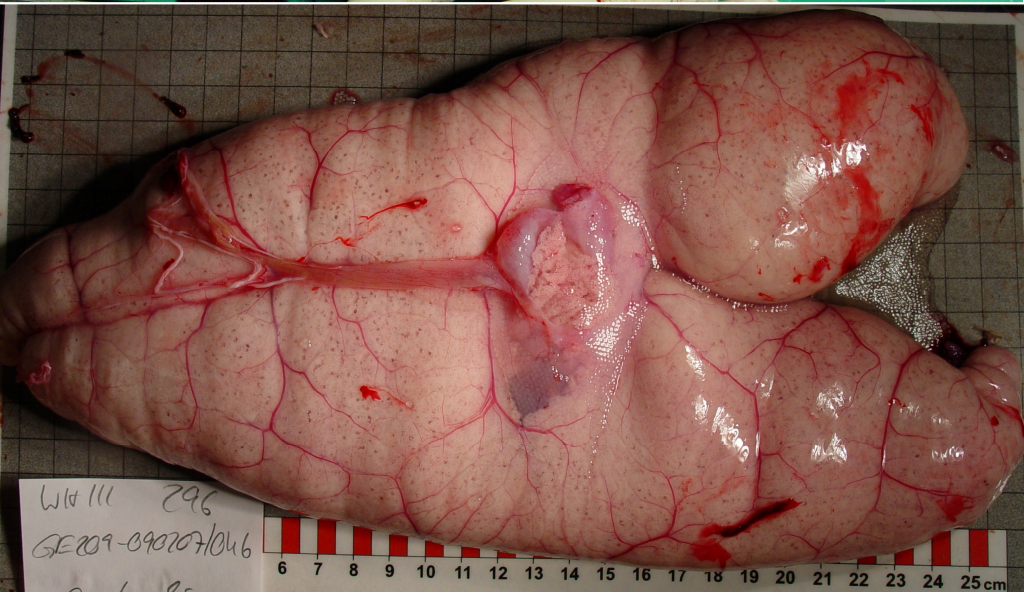


<i>Specimen data</i>		
L _T : 61 cm	M _G : 169.3 g	M: February 2007
M _B : 1482 g	GSI: 11.4	ID: 070718/187

III. Spawning (initiation)



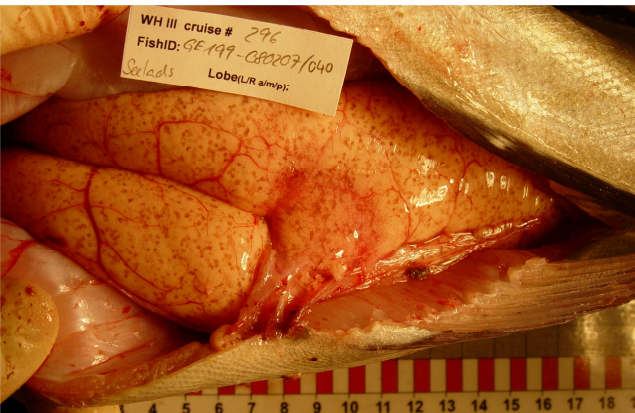
Stage III (initiation):
Ovaries extending length of body cavity; distended and soft; opaque, orange to light pink. Single glassy, hydrating oocytes among abundant opaque, vitellogenic oocytes (round and larger). Viscous fluid or hydrated eggs in lumen may occur.



Specimen data		
L _T : 82 cm	M _G : 1018 g	M: February 2007
M _B : 4062 g	GSI: 25.1	ID: 070718/188

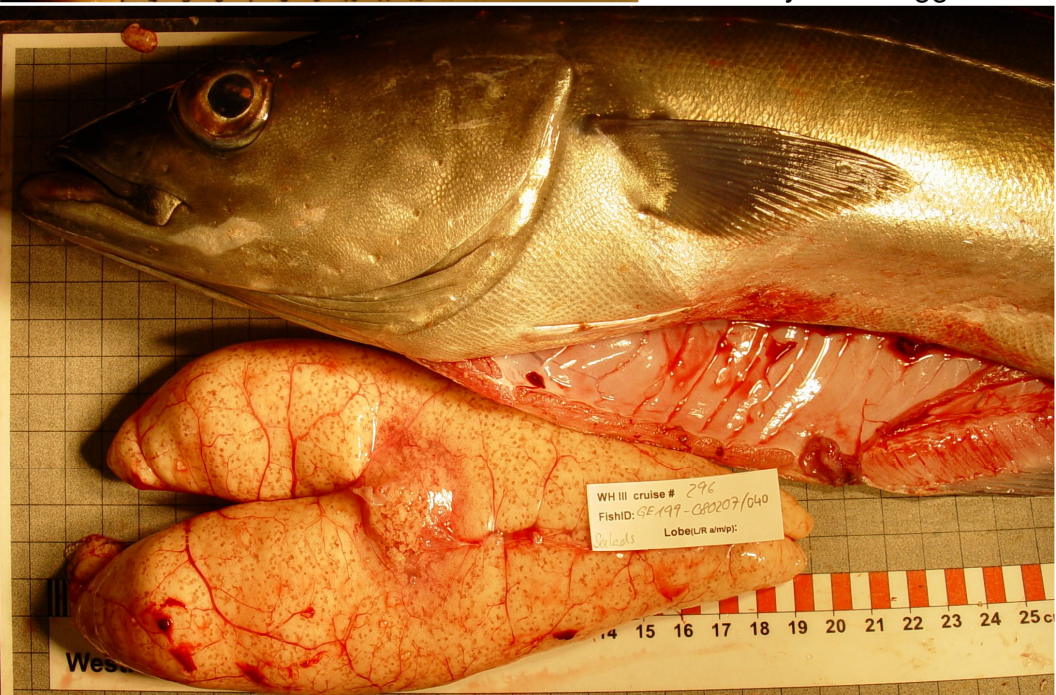


III. Spawning (main period)



Stage III (main period):
Ovaries fill most of the body cavity; very distended and soft; appear granulated orange to light pink from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs.

Stage III (cessation – no picture):
Ovaries shrunk, flabby; hydrated oocytes present, opaque oocytes few or absent. Lumen with excess fluid and hydrated eggs abundant.



Specimen data		
L _T : 59 cm	M _G : 299 g	M: February 2007
M _B : 1486 g	GSI: 20.1	ID: 070718/184

IV. Spent

Stage IV:

Ovaries contracted; slack with greyish cast; dim translucent dark red-purple. Vitellogenic oocytes absent, but single hydrated eggs or atretic oocytes (opaque, irregular granules) may occur.



Specimen data

L_T: 60 cm

M_B: 1616 g

M_G: 12 g

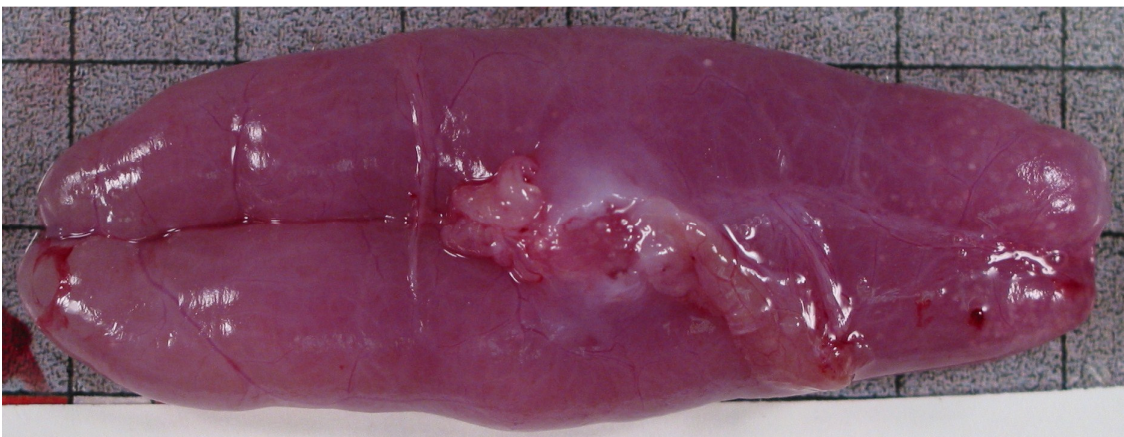
GSI: 0.7

M: March 2007

ID: 070718/50

IV. Spent

Stage IV:
(continued)



Specimen data

L_T: 52 cm
M_B: 1188 g

M_G: 18 g
GSI: 1.5

M: March 2007
ID: 070718/47

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-like colouration, indicating yolk

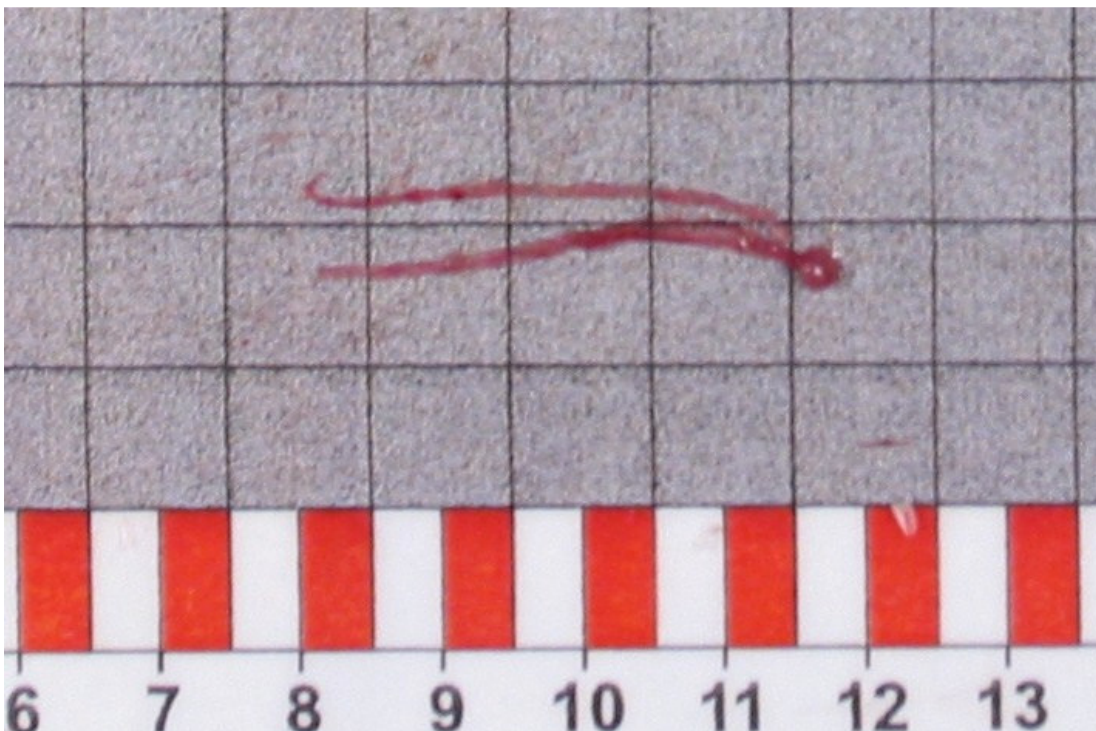
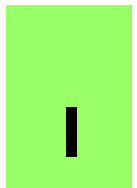
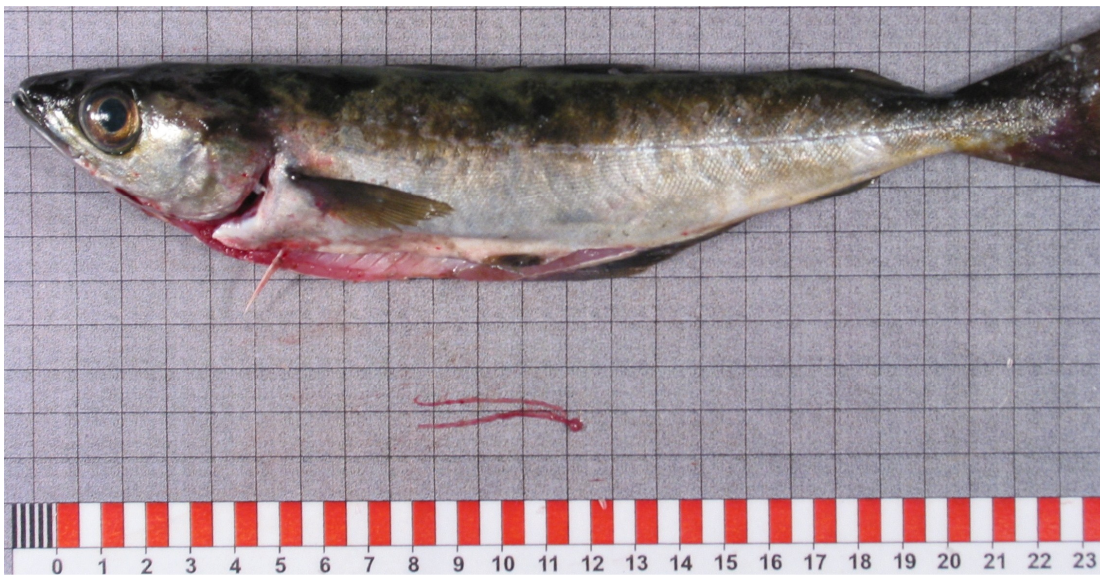
VI. Abnormal

No suitable example of a stage VI specimen was available from the samples.

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.



Specimen data

L_T: 25 cm

M_B: 133 g

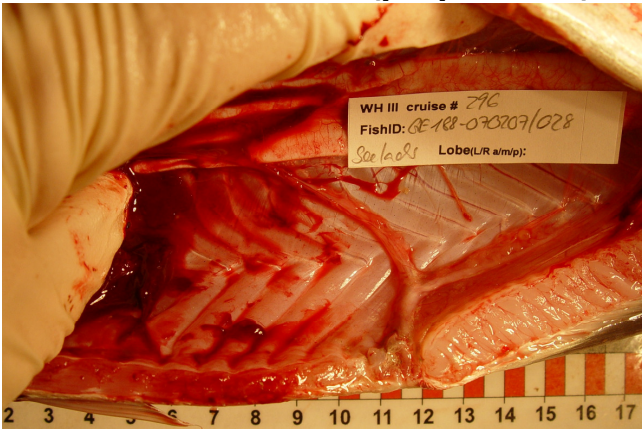
M_G: < 0.1 g

GSI: ~ 0

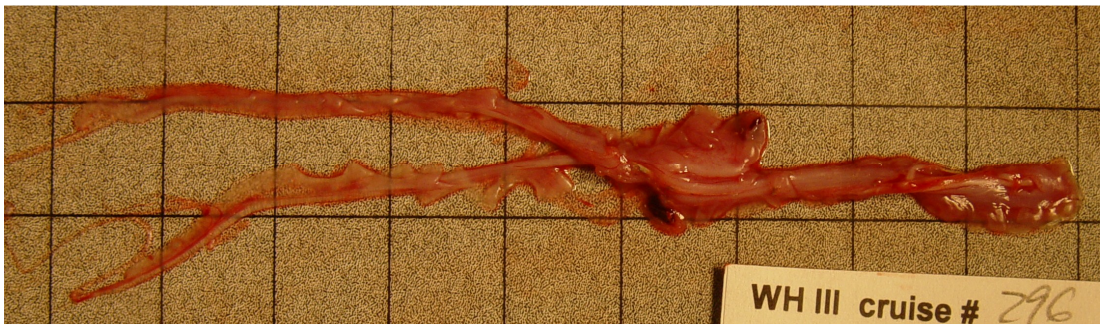
M: February 2007

ID: 070718/133

I. Juvenile/immature (preparation)



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



Specimen data

L_T: 50 cm
M_B: 914 g

M_G: 0.3 g
GSI: ~ 0

M: February 2007
ID: 070718/183

II. Maturing (late)

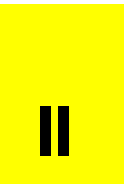


Stage II (early – **no picture**):

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

Stage II (late):

Testes enlarged and prominent dorsal in body cavity. Lobules plump and brittle; slightly translucent reddish-white. Empty transparent spermatoducts with prominent blood vessels. No sperm release.



Specimen data

L_T: 69 cm

M_B: 3134 g

M_G: 110 g

GSI: 4.1

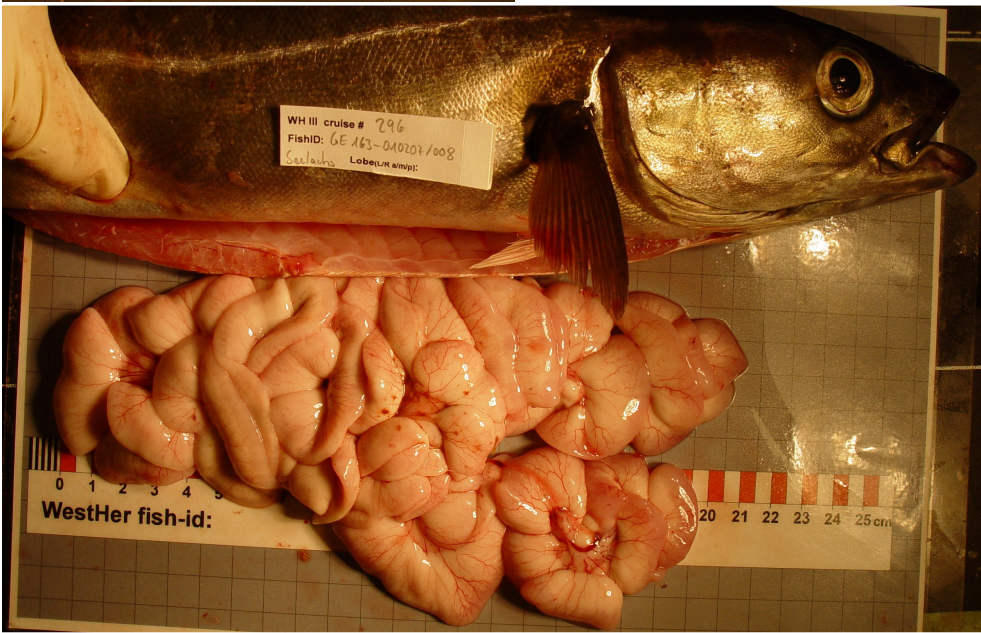
M: February 2007

ID: 070718/152

III. Spawning (initiation)



Stage III (initiation):
Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy white. Spermatoducts filled with viscous semen and a viscous droplet may be released from vent.



<i>Specimen data</i>		
L _T : 50 cm	M _G : 213.6 g	M: February 2007
M _B : 1256 g	GSI: 22.1	ID: 070718/156

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 : 55 cm	M _G : 87.1 g	M: February 2007
M _B : 1387 g	GSI: 7.2	ID: 070718/153

III. Spawning (main period)

Stage III (main period):
(continued)



Specimen data

L_T: 101 cm

M_B: 11300 g

M_G: 2430 g

GSI: 31.4

M: January 2007

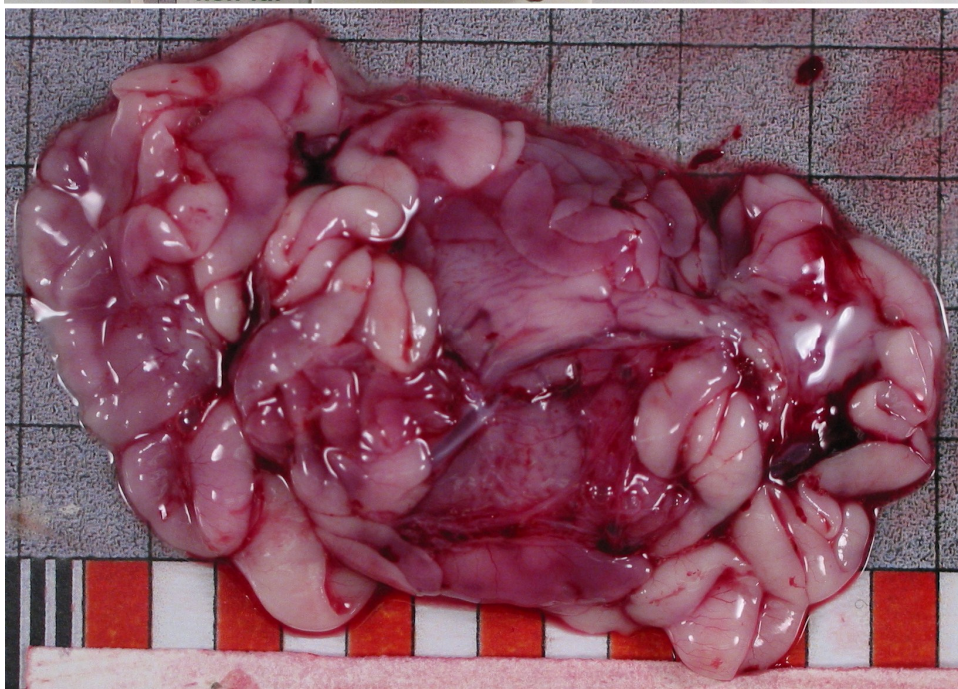
ID: 070718/132



III. Spawning (cessation)

Stage III (cessation):

Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-purple. Spermatoducts still with fluid semen, that easily flows from vent.



Specimen data

L_T: 53 cm
M_B: 1316 g

M_G: 20 g
GSI: 1.7

M: March 2007
ID: 070718/128

III. Spawning (cessation)

Stage III (cessation):
(continued)



Specimen data

L_T: 104 cm
M_B: 8085 g

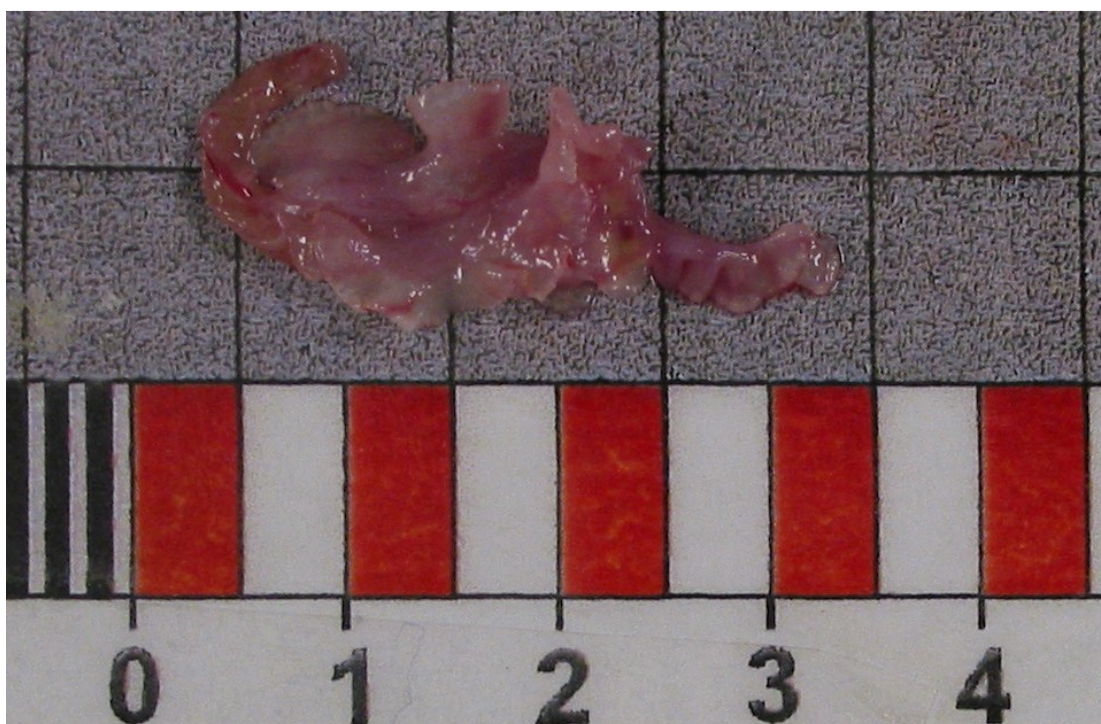
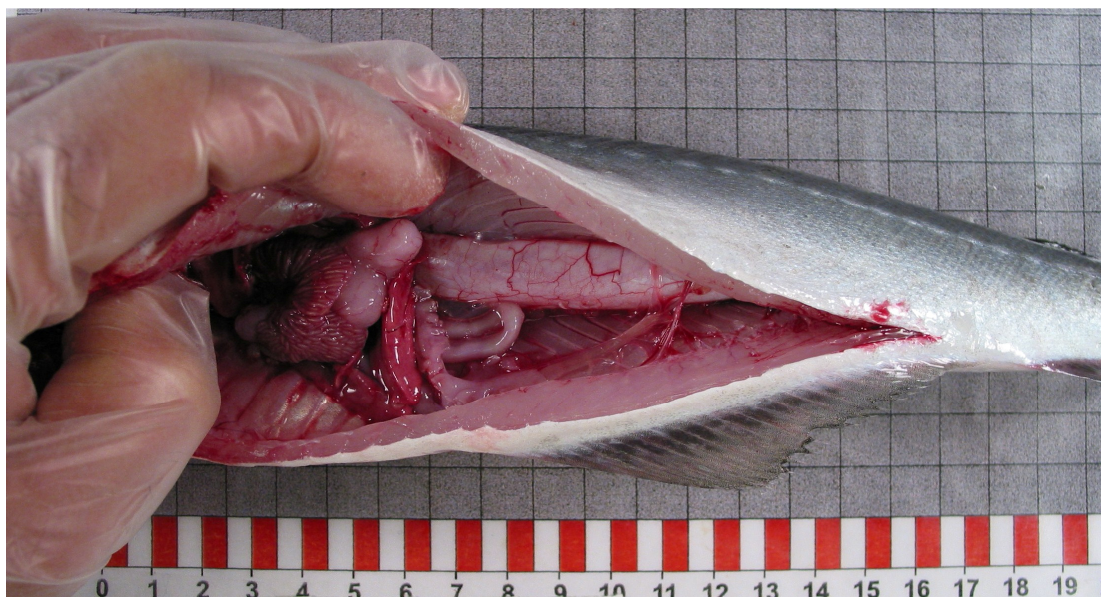
M_G: 400 g
GSI: 5.7

M: March 2007
ID: 070718/135

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 of previous distension, often with visible remains of semen.



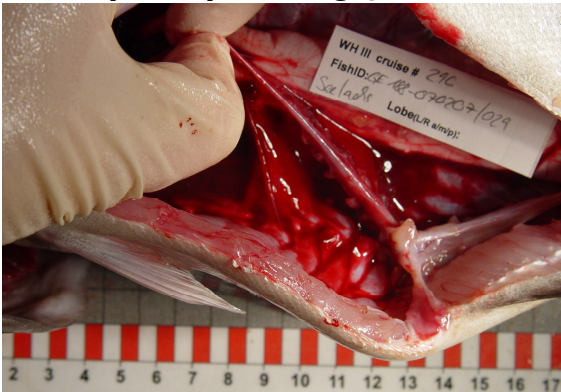
Specimen data

L_T: 52 cm
M_B: 252 g

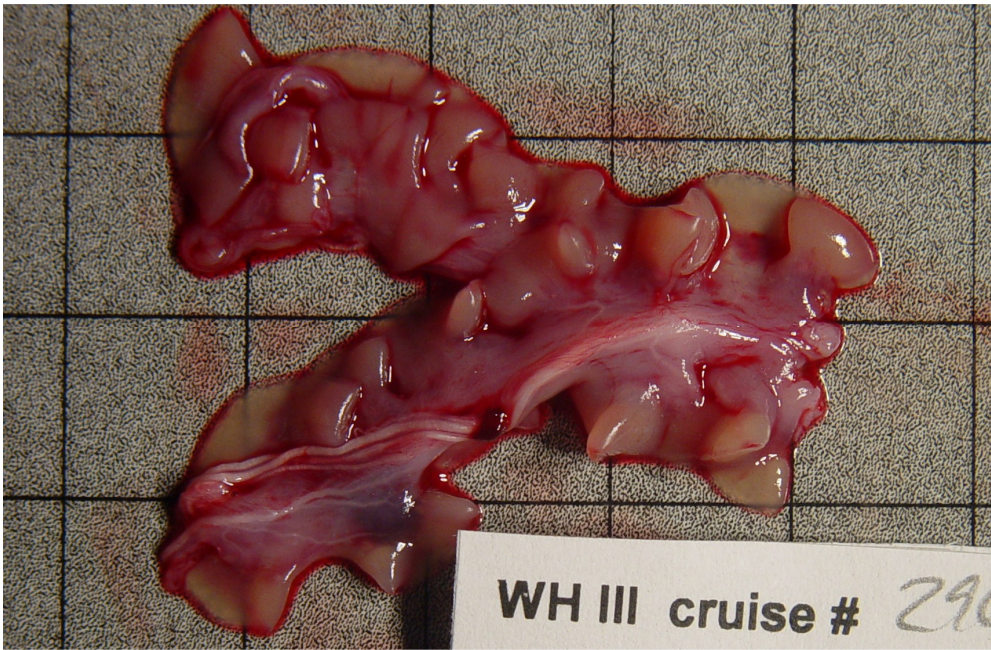
M_G: 1 g
GSI: 0.4

M: March 2007
ID: 070718/131

V. Skip of spawning (November-February)



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



Specimen data

L_T: 64 cm
M_B: 1771 g

M_G: 1.3 g
GSI: 0.1

M: February 2007
ID: 070718/155

V. Resting (March-October?)

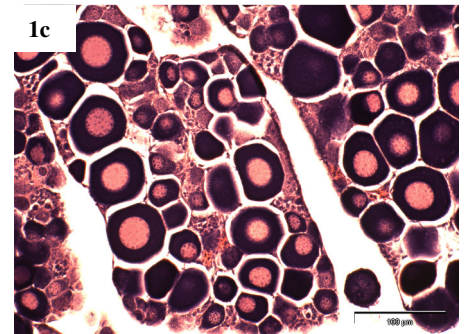
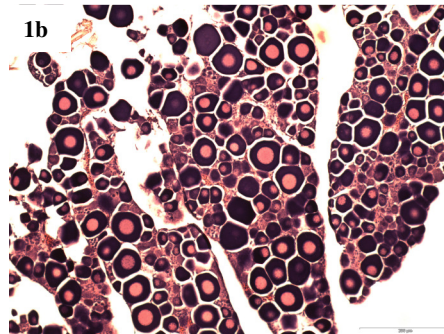
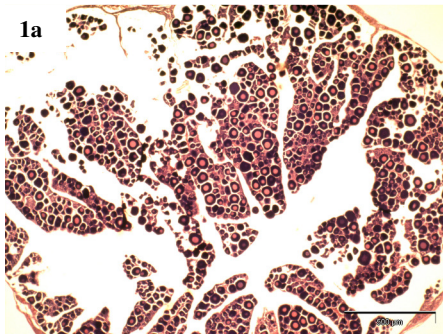
Stage V:

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

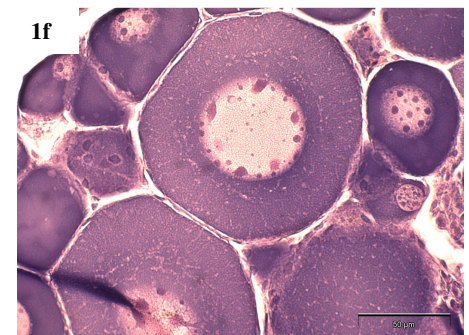
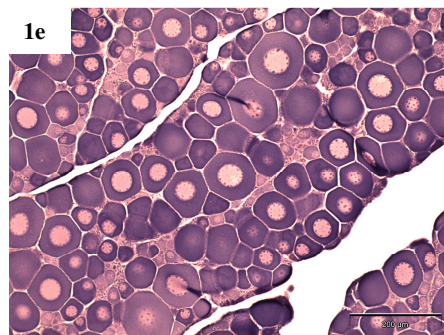
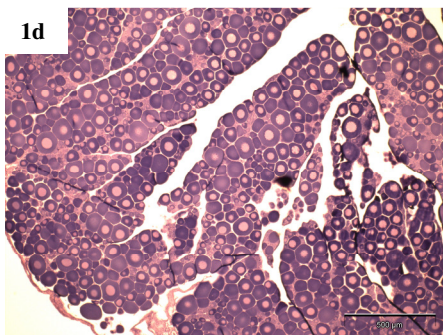
Testes small (as in stage I preparation) but with signs of previous spawning; e.g. lobules slightly larger lobules than in stage I (preparation); spermatoducts often with a greyish cast.

VI. Abnormal

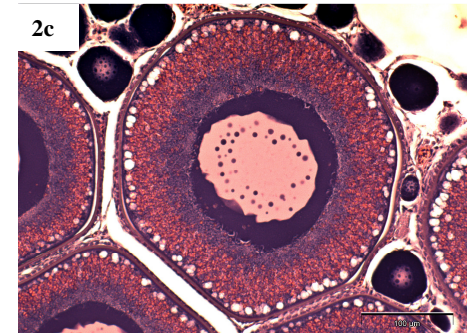
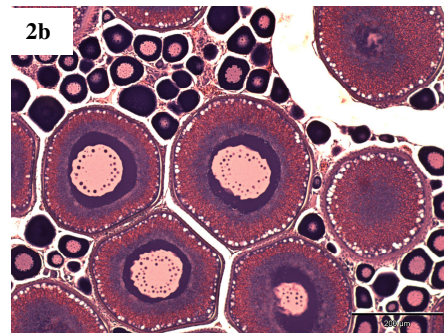
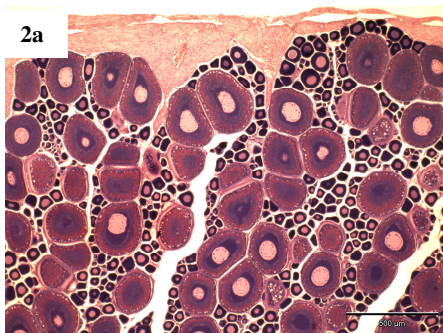
No suitable example of a stage VI specimen was available from the samples.



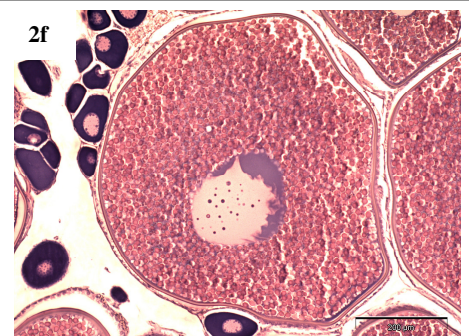
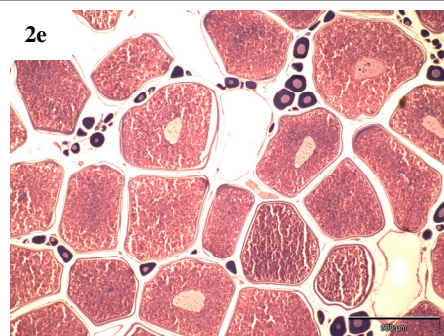
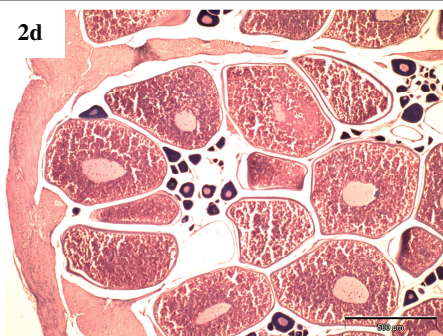
I. Juvenile/immature (early). Oocytes are small with dense stained cytoplasm and a central nucleus with few, large peripheral nucleoli around its edge, oogonia are always present at this premature stage although sometimes not visible (scale bars **1a.** 500 µm **1b.** 200 µm **1c.** 100 µm; specimen 070718/48).



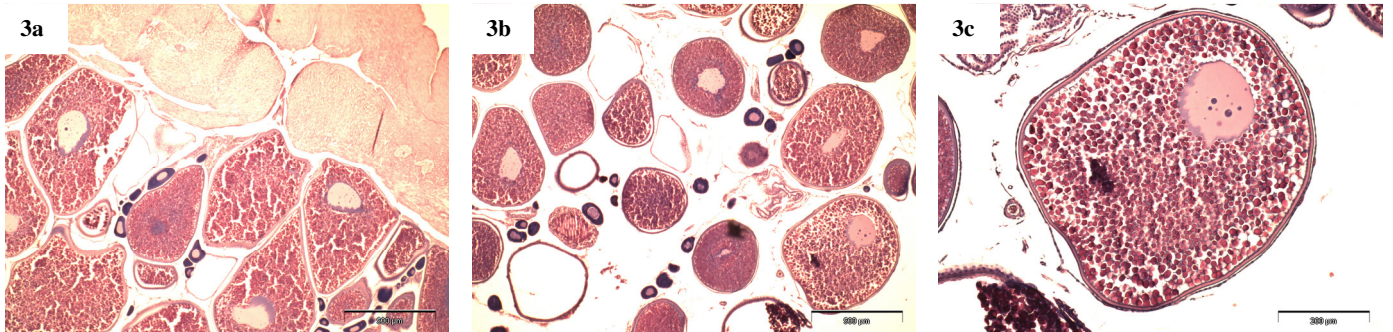
I. Juvenile/immature (preparation). A portion of the oocytes have started the primary growth (**1f**), characterized by a slight increase in size and by the presence of a light stained area around the nucleus, the so called circumnuclear ring. This ring shows that cytoplasmic changes are occurring (scale bars **1d.** 500 µm **1e.** 200 µm **1f.** 50 µm; specimen 070718/185).



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 070718/46).



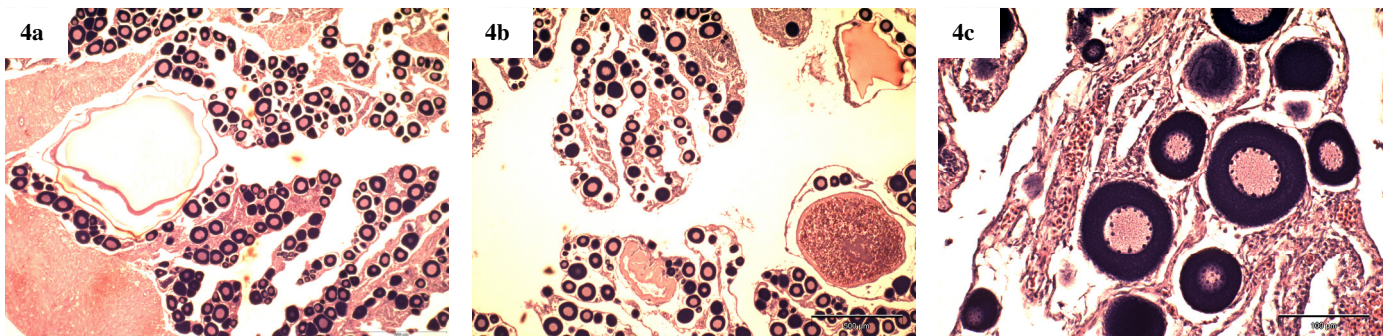
II. Maturing (late). Granules of yolk intensely stained, initially appear peripherally (**2c**) but as they increase in number and size, they tend to spread out together with the cortical alveoli, throughout the cytoplasm. This process is termed vitellogenesis. The shape of the nucleus becomes irregular, but it is still centrally located (scale bars **2d.** 500 µm **2e.** 500 µm **2f.** 200 µm; specimen 070718/187)



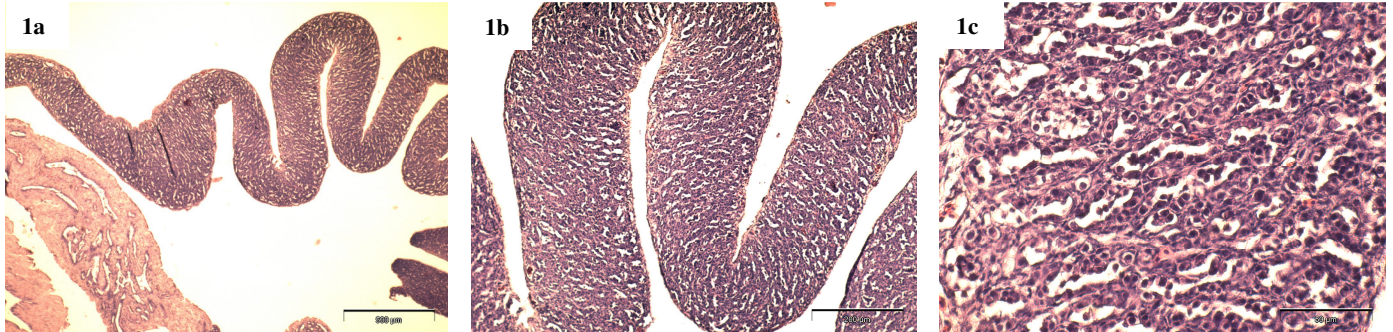
III. Spawning (initiation). The nucleus migrates to the periphery and breaks down, yolk granules coalesce forming large irregular spheres (3c), a water influx occurs and hydrated eggs (3f) with a completely homogeneous content are formed. At the ovulation, oocytes are released into the lumen, while the post-ovulatory follicles (3b, 3c) remain in the ovary (scale bars 3a. 500 µm 3b. 500 µm 3c. 200 µm; specimen 070718/188).



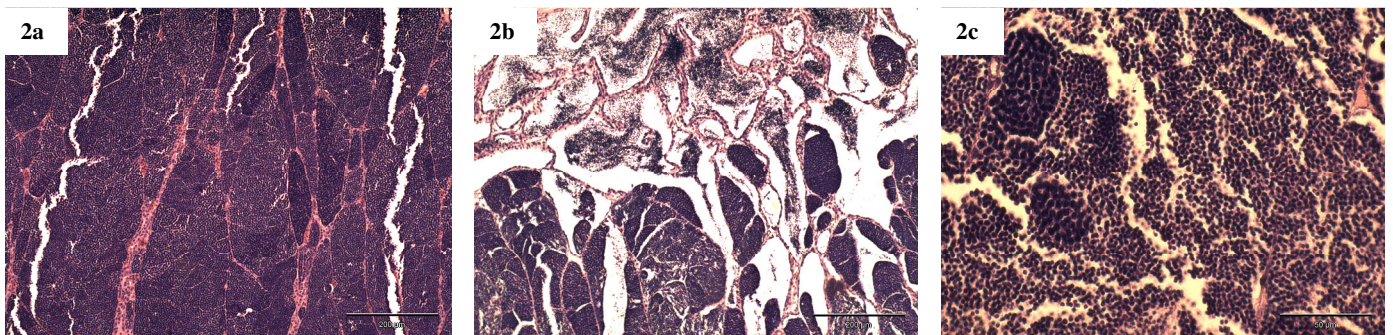
III. Spawning (main period). As the spawning proceeds the number of hydrated eggs and post-ovulatory follicles increase and at the late stage of spawning the hydrated eggs, post-ovulatory follicles and resting oocytes in the perinuclear and circumnuclear stages are dominant and the ovary wall (3g) becomes more thick and folded (scale bars 3d. 500 µm 3e. 500 µm 3f. 200 µm; specimen 070718/184)



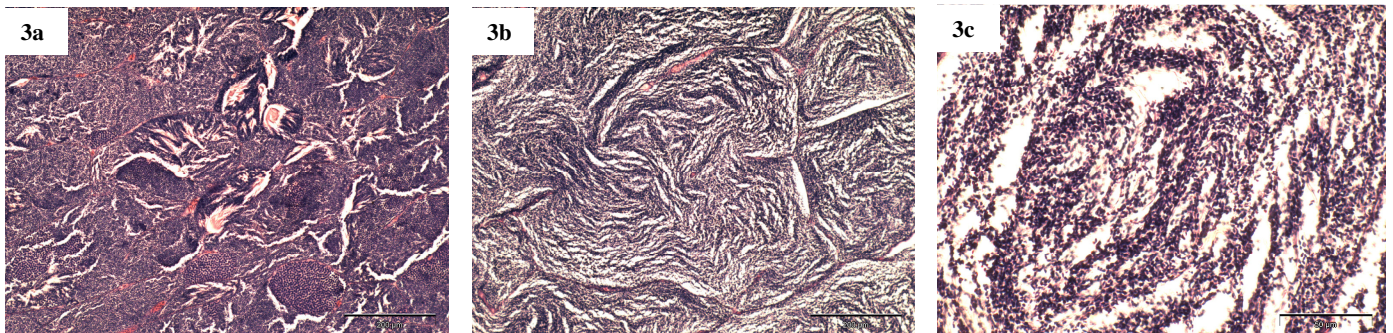
IV. Spent. At the end of the spawning season, the ovary enters the spent stage and post-ovulatory follicles, marking the occurred ovulation, are abundant among perinuclear or circumnuclear stage oocytes. The development of vitellogenic oocytes sometimes fail and they undergo a process called "atresia" (4b) consisting in an intra-ovarian resorption (scale bars 4a. 500 µm 4b. 500 µm 4c. 100 µm; specimen 070718/50)



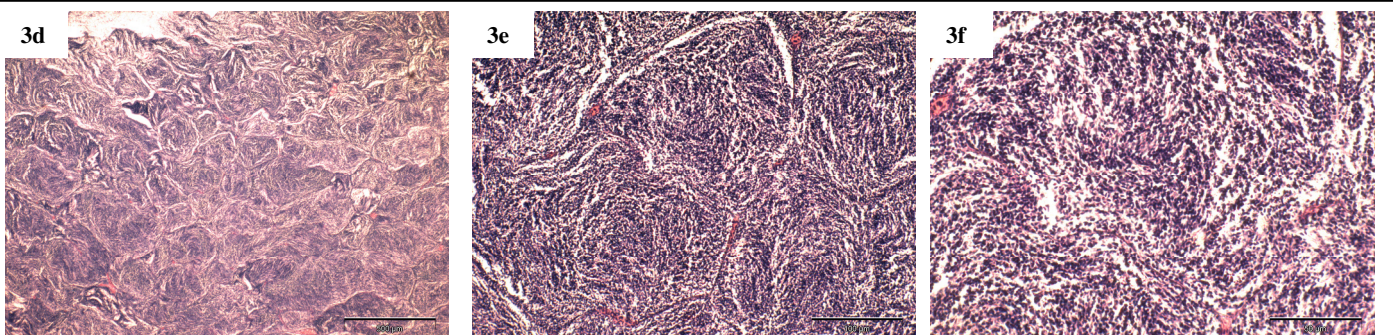
I. Juvenile/immature (preparation). ‘Germ cells’ or spermatogonia are characteristic of this stage; most are located distally. Singular germ cells lodge within a cyst and divide mitotically into groups of germ cells, which again divide into primary spermatocytes once the immature tissue is in preparation (scale bars **1a**. 500 μ m **1b**. 200 μ m **1c**. 50 μ m; specimen 070718/183).



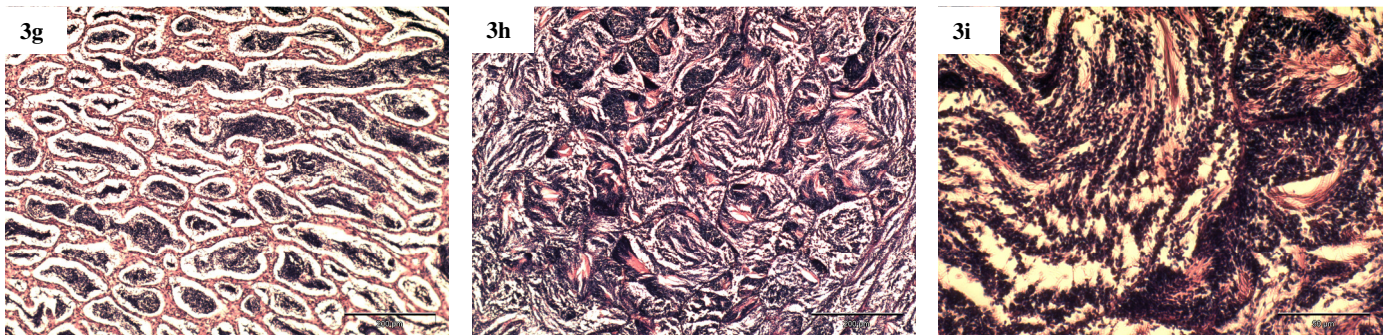
II. Maturing (late). In late maturing tissue the numbers of spermatids and flagellate spermatozoa increases rapidly, but no motile sperm are present in the sperm duct. Flagellate spermatozoa are present in higher numbers in the more rapidly developing proximal tissue (scale bars **2a**. 200 μ m-distal **2b**. 200 μ m-proximal **2c**. 50 μ m-proximal; specimen 070718/152).



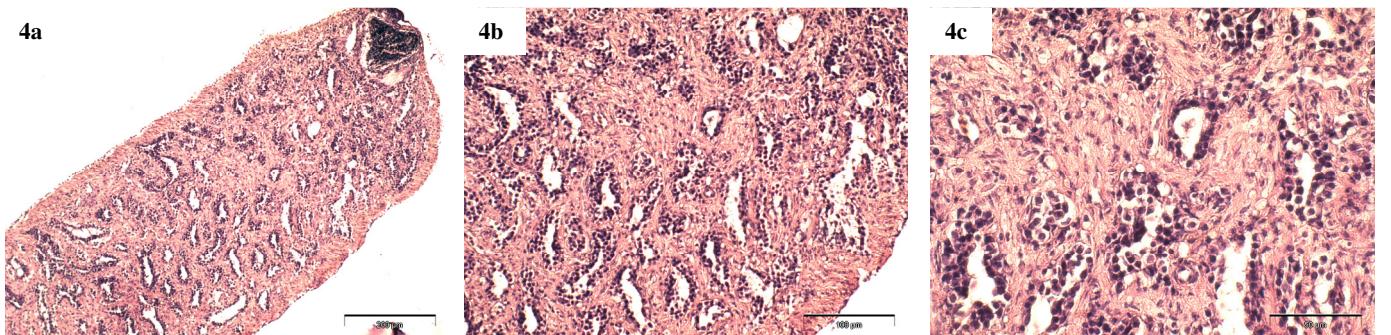
III. Spawning (initiation). The number of spermatozoa increases rapidly. Cyst and lobule walls disappear and long tubules are formed proximally. Mature spermatozoa become aligned. Migrating germ cells are only visible at the extreme distal edges. The sperm duct and proximal efferent duct system contains ripe spermatozoa (scale bars **3a**. 200 μ m-distal **3b**. 200 μ m-proximal **3c**. 50 μ m-proximal; specimen 070718/156).



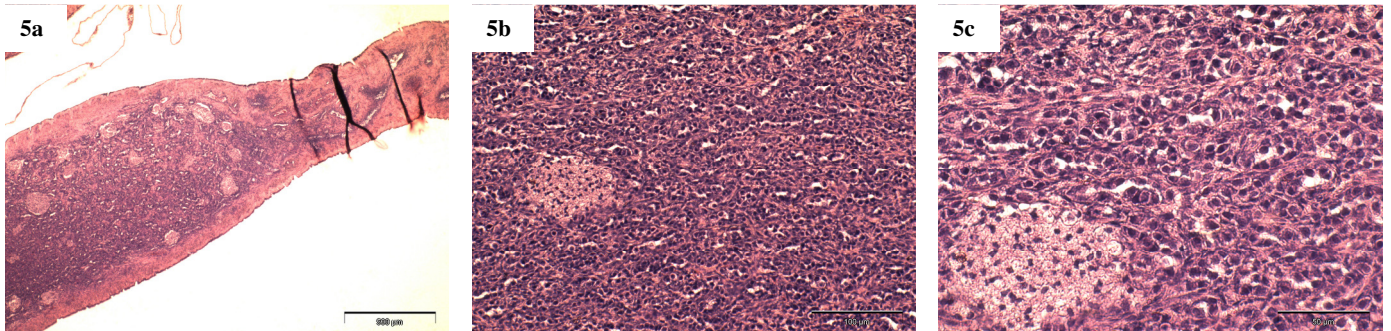
III. Spawning (main period). A larger fraction of tubules now contain ripe spermatozoa (scale bars **3d**. 500 μ m-distal **3e**. 100 μ m-proximal **3f**. 50 μ m-proximal; specimen 070718/132).



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 **3g**, 200 µm-distal **3h**, 200 µm-proximal **3i**, 50 µm-proximal; specimen 070718/128)



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**, 200 µm **4b**, 100 µm **4c**, 50 µm; specimen 070718/131).



V. Resting. Tissue appears quite dense and a reorganization of the tissue is in progress. New cysts are being formed and lobule walls contain many migrating germ cells or spermatogonia. Resting cysts of spermatogonia or primary spermatocytes may also be visible. Phagocytes reabsorb relict atretic spermatozoa and stain a lighter colour (scale bars **5a**, 500 µm **5b**, 100 µm **5c**, 50 µm; specimen 070718/155).

