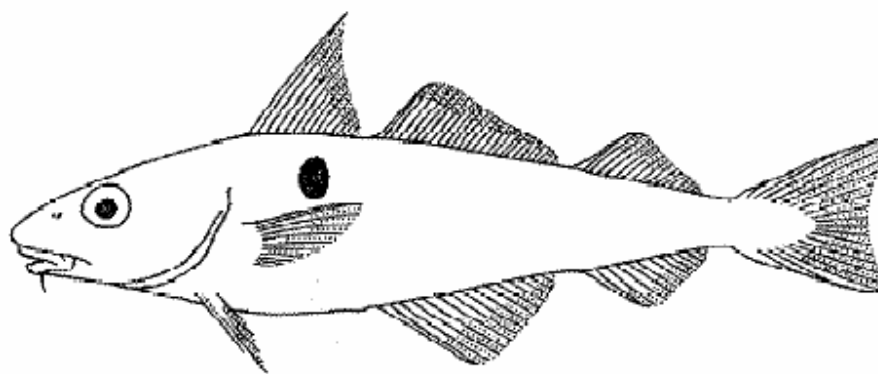


## Manual to determine gonadal maturity of North Sea haddock (*Melanogrammus aeglefinus* L)



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J. Dalskov, I. Wilhelms, A. Sell

**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
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Merete Fonn	Norway
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Rajlie Sjöberg	Sweden
Lisbet Solbakken	Norway
Yves Verin	France
Francesca Vitale	Sweden
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:

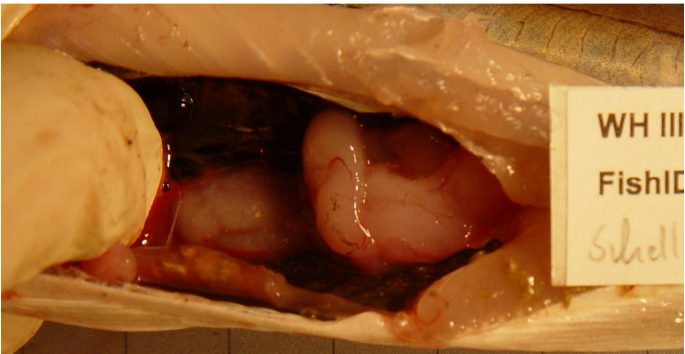
Ingo Wilhelms	Germany
Anne Sell	Germany

The editors:

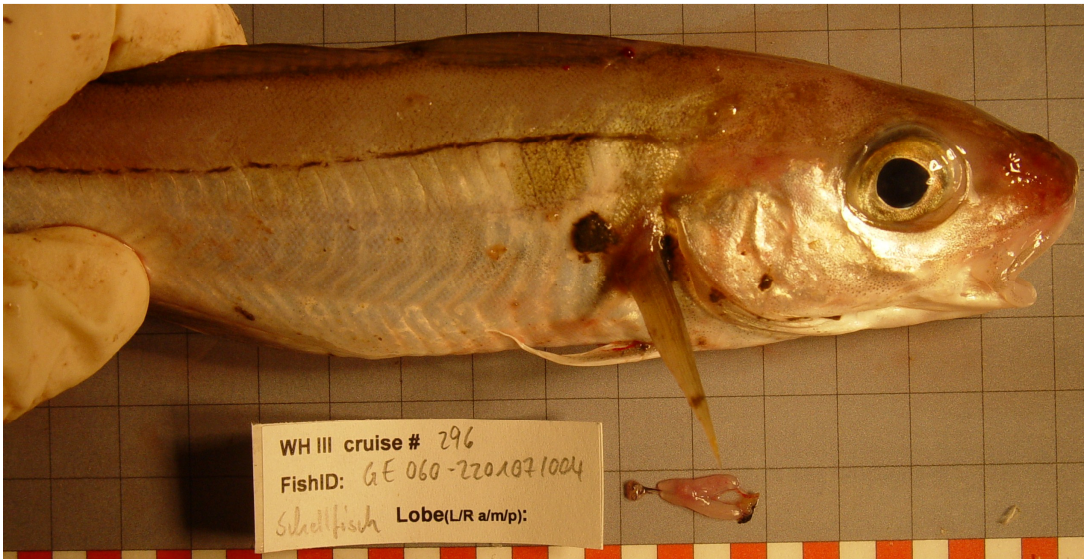
Rikke H. Bucholtz	Denmark
Gavin Power	Ireland
Jonna Tomkiewicz	Denmark
Jørgen Dalskov	Denmark



I. Juvenile/immature (early)



**Stage I (early):**  
Ovaries small paired organs; translucent but may have reddish colouration; lobes of ovaries slim.



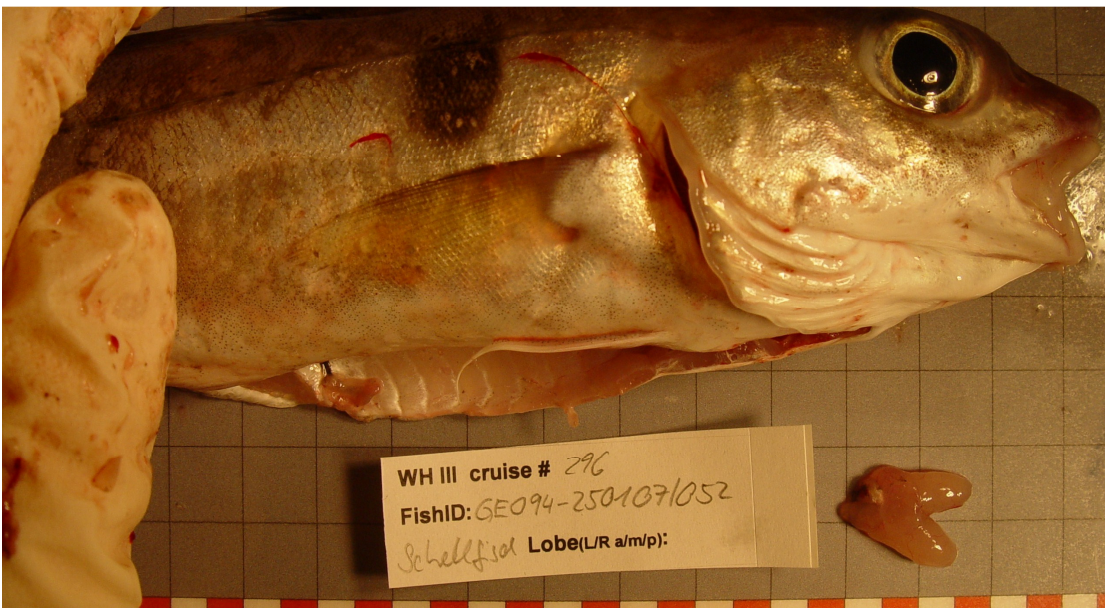
Specimen data		
L <sub>T</sub> : 21 cm	M <sub>G</sub> : 0.2 g	M: January 2007
M <sub>B</sub> : 87 g	GSI: 0.3	ID: 070718/4



II. Maturing (early)



**Stage II (early):**  
Circular aveoli; gonads swollen in appearance; translucent orange colouration; uneven surface.



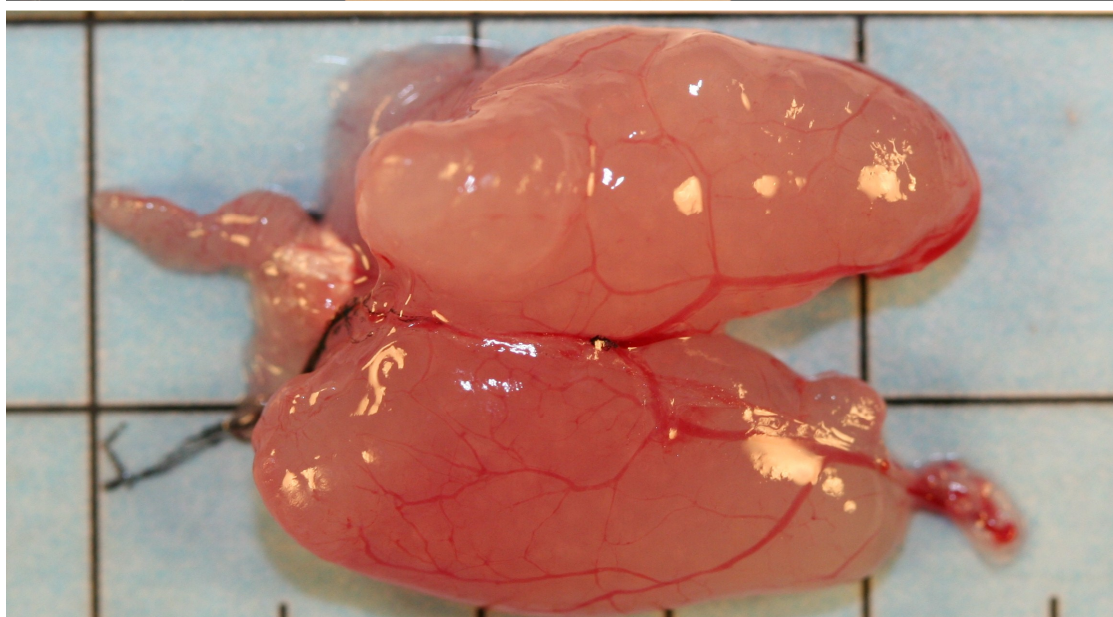
<i>Specimen data</i>		
L <sub>T</sub> : 24 cm	M <sub>G</sub> : 0.5 g	M: January 2007
M <sub>B</sub> : 132 g	GSI: 0.4	ID: 070718/2



### II. Maturing (early)



Stage II (early):  
(continued)



II

#### Specimen data

L<sub>T</sub>: 26 cm  
M<sub>B</sub>: 176 g

M<sub>G</sub>: 0.9 g  
GSI: 0.6

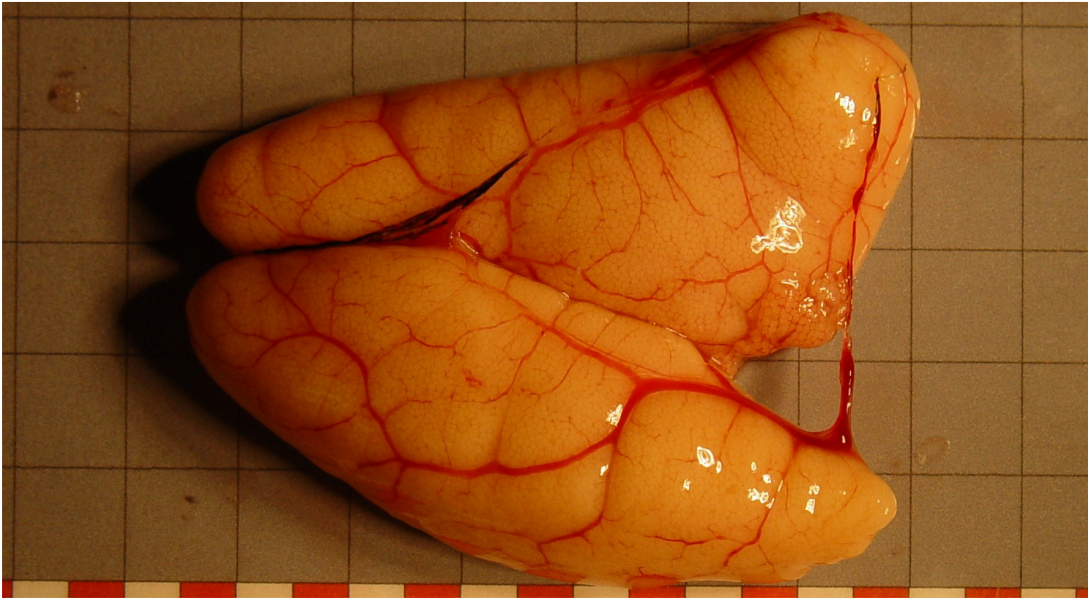
M: February 2007  
ID: 070718/247



II. Maturing (late)



**Stage II (late):**  
Gonad size much larger and predominantly or completely opaque in appearance, orange/pink colouration due to yolk accumulation; high degree of vascularisation; developing oocytes visible to the eye; no hyaline or hydrated oocytes visible.



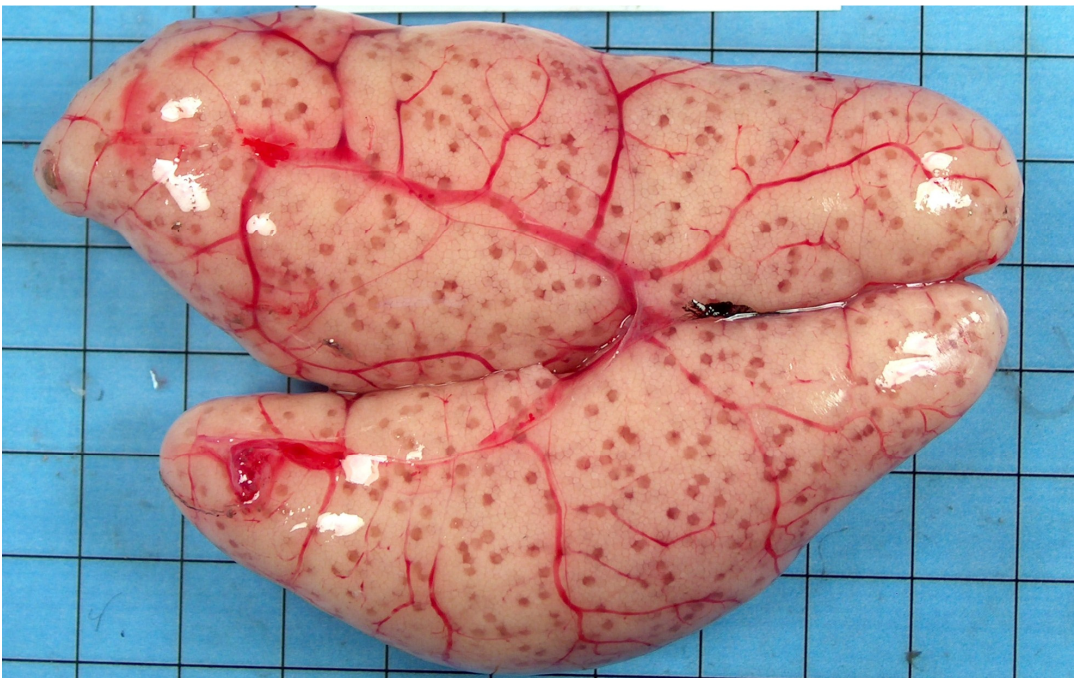
<b>Specimen data</b>		
L <sub>T</sub> : 32 cm	M <sub>G</sub> : 27.7 g	M: January 2007
M <sub>B</sub> : 328 g	GSI: 10.5	ID: 070718/8



III. Spawning (initiation)



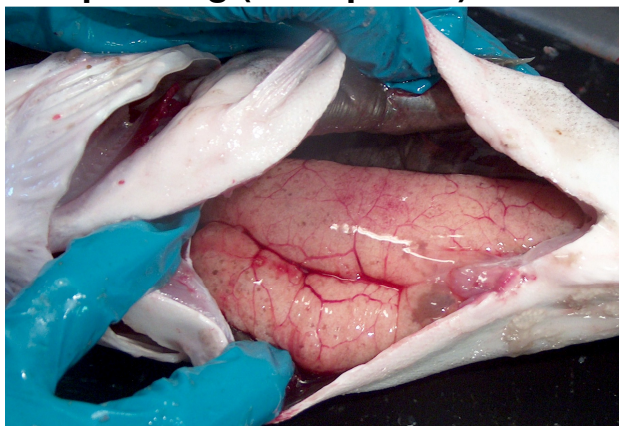
**Stage III (initiation):**  
Ovaries full with distinct yolk accumulation; orange colouration; blood vessels prominent; few to many hyaline oocytes visible; developing oocytes visible to the unaided eye.



<i>Specimen data</i>		
L <sub>T</sub> : 35 cm	M <sub>G</sub> : 62.9 g	M: February 2007
M <sub>B</sub> : 440 g	GSI: 18.1	ID: 070718/212

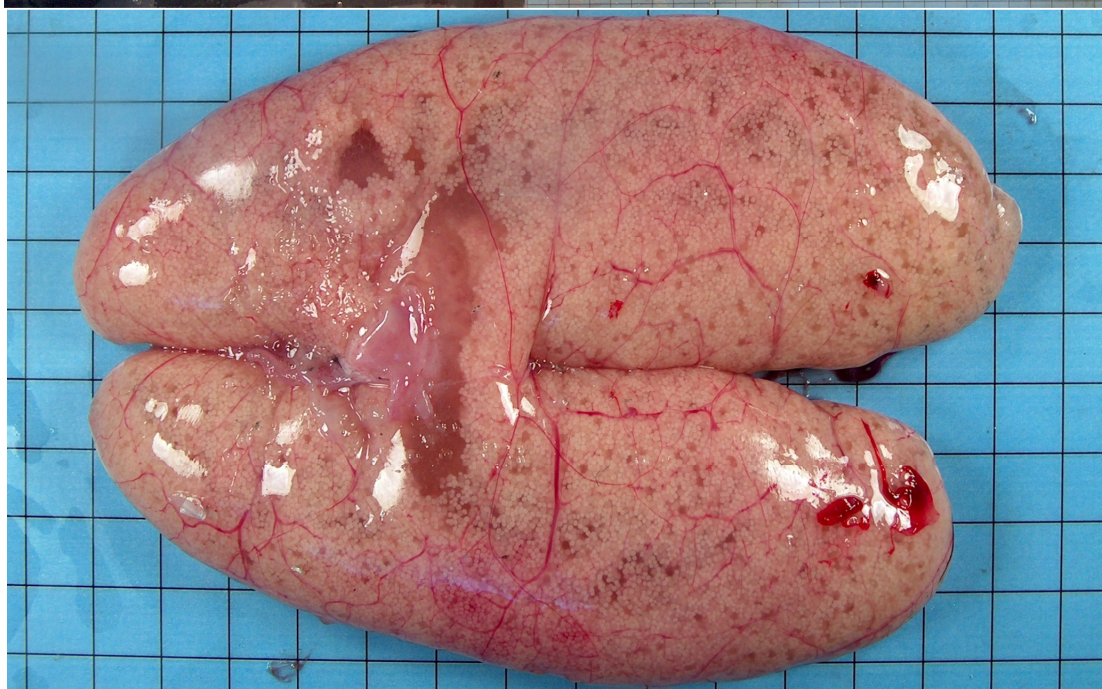
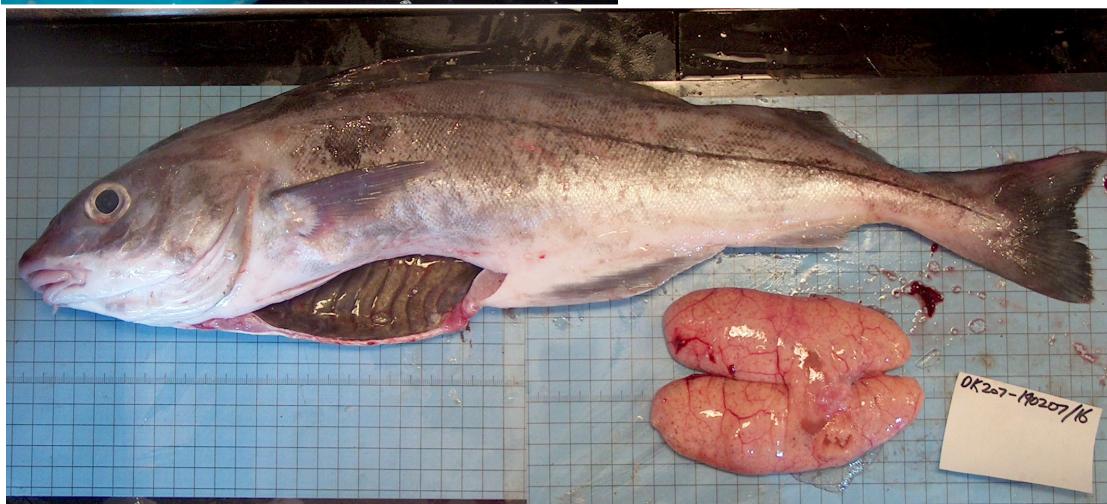


#### III. Spawning (main period)



##### Stage III (main period):

Many hydrated oocytes visible and will run from urogenital pore under moderate pressure; tissue may appear more bloodshot in appearance or display a dark pink to red colouration.



##### Specimen data

L<sub>T</sub>: 50 cm

M<sub>B</sub>: 1362 g

M<sub>G</sub>: 149.7 g

GSI: 13.4

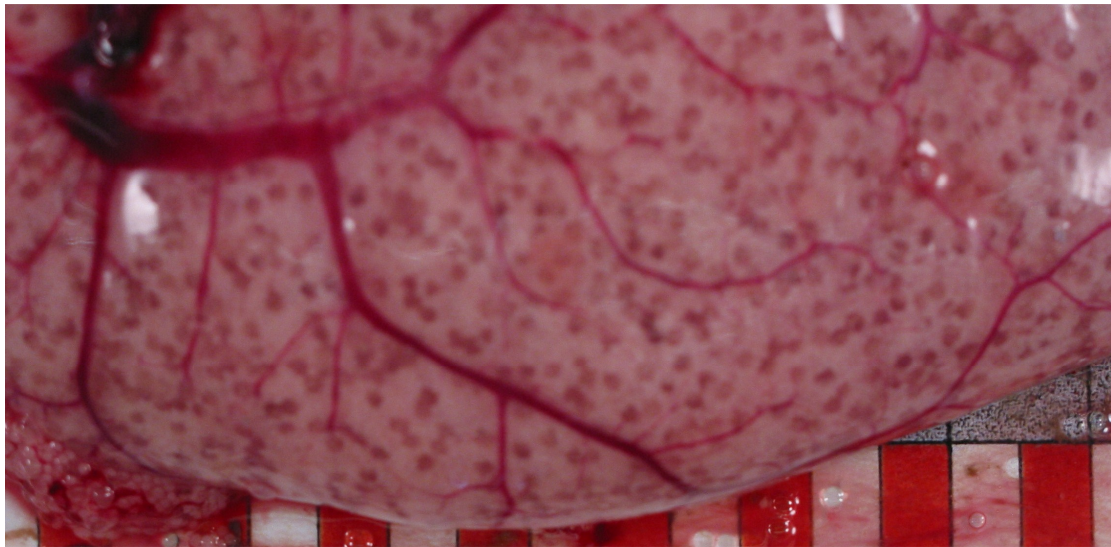
M: February 2007

ID: 070718/249



III. Spawning (main period)

Stage III (main period):  
(continued)



*Specimen data*

L<sub>T</sub>: 41 cm  
M<sub>B</sub>: 765 g

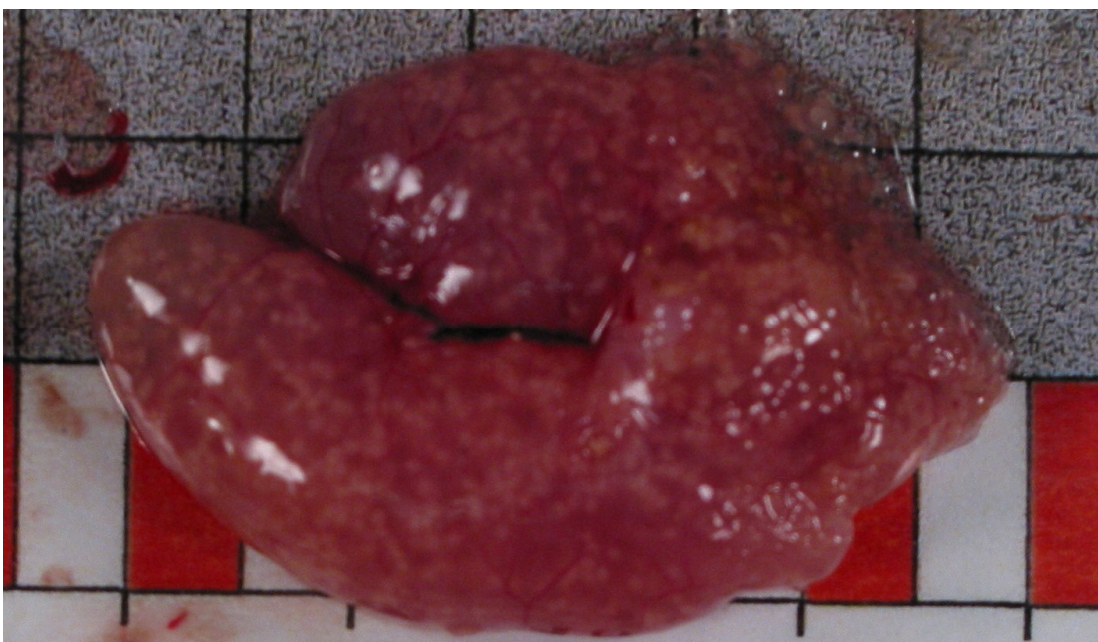
M<sub>G</sub>: 143 g  
GSI: 25.5

M: March 2007  
ID: 070718/12

### III. Spawning (cessation)

#### Stage III (cessation):

Ovaries much reduced in size but hydrated or hyaline oocytes remain visible; tissue has developed a deep red or bloodshot appearance; few opaque developing oocytes remain; high degree of vascularisation.



#### Specimen data

L<sub>T</sub>: 29 cm

M<sub>B</sub>: 204 g

M<sub>G</sub>: 5 g

GSI: 2.8

M: March 2007

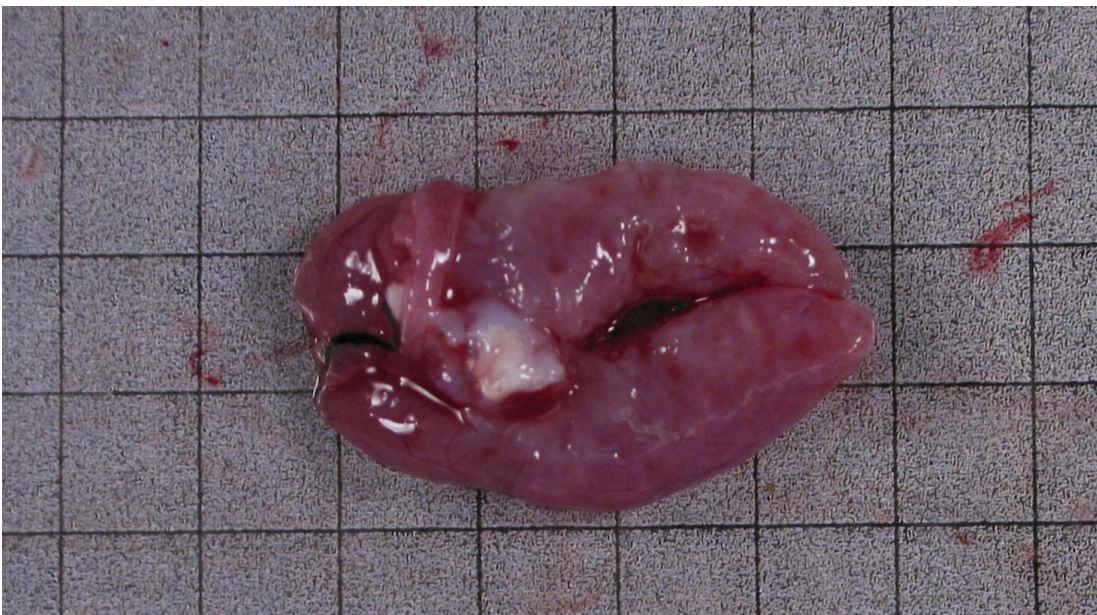
ID: 070718/11



### IV. Spent

#### Stage IV:

Tissue vastly reduced in size and appear shrunken; no oocytes visible to the eye but atretic residual oocytes may remain in some cases; deep red or purple colouration; some remaining blood vessels visible.



#### Specimen data

L<sub>T</sub>: 37 cm

M<sub>B</sub>: 522 g

M<sub>G</sub>: 16 g

GSI: 3.5

M: March 2007

ID: 070718/9

#### **V. Skip of spawning (November-February?)**

##### **Stage V:**

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



**V**

### V. Resting (March-October?)

#### Stage V:

No suitable example of a stage V specimen was available from the samples.  
(stage II) is easier seen if cut open.

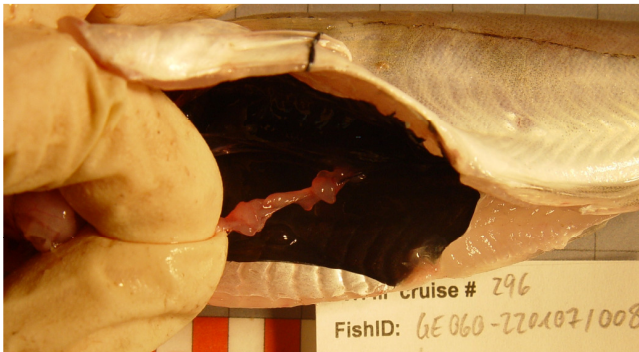
V

### **VI. Abnormal**

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

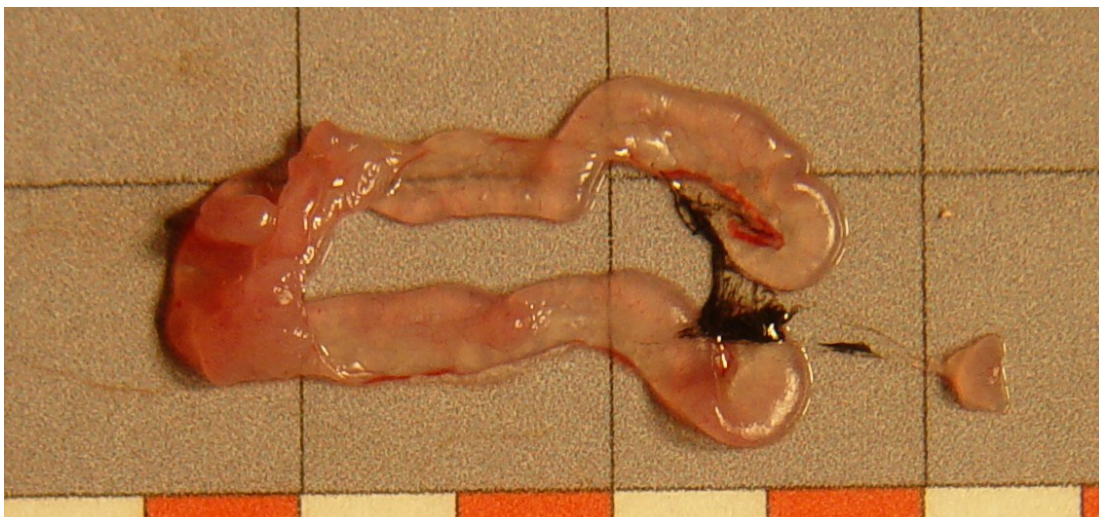


### I. Juvenile/immature (preparation)



#### Stage I (preparation):

Testes appear as paired translucent ribbons or strings; vascularisation is limited but tissue may have a reddish appearance; no frill development is visible.



#### Specimen data

L<sub>T</sub>: 18 cm

M<sub>B</sub>: 50 g

M<sub>G</sub>: 0.1 g

GSI: ~ 0

M: January 2007

ID: 070718/163

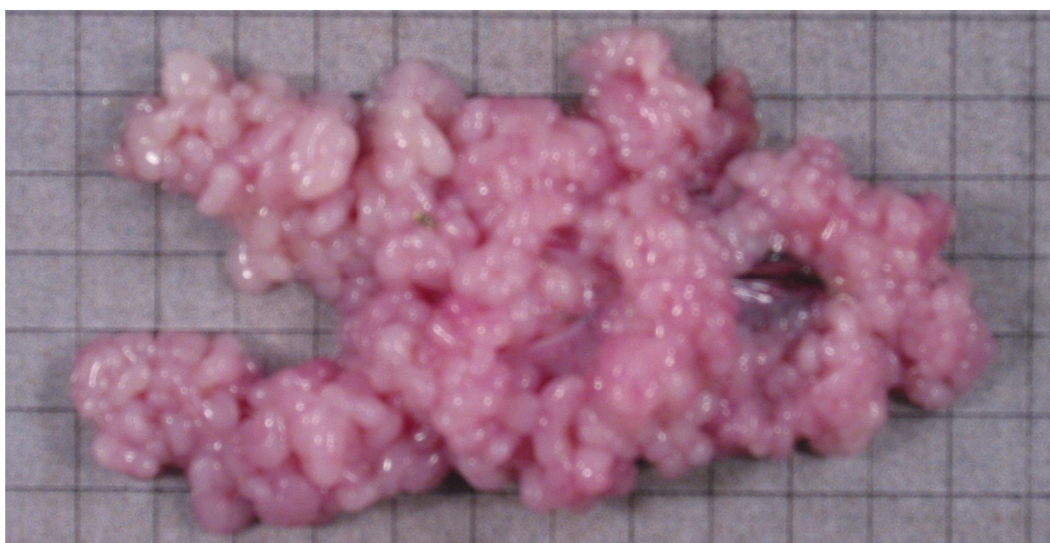


### II. Maturing (late)



#### Stage II (late):

Testes now filling and greatly increased in size; glassy to opaque appearance with distal frill well developed and convoluted; blood vessels more developed; no spermatozoa visible at urogenital pore under moderate pressure.



#### Specimen data

L<sub>T</sub>: 44 cm  
M<sub>B</sub>: 957 g

M<sub>G</sub>: 54.1 g  
GSI: 6.9

M: February 2007  
ID: 070718/121

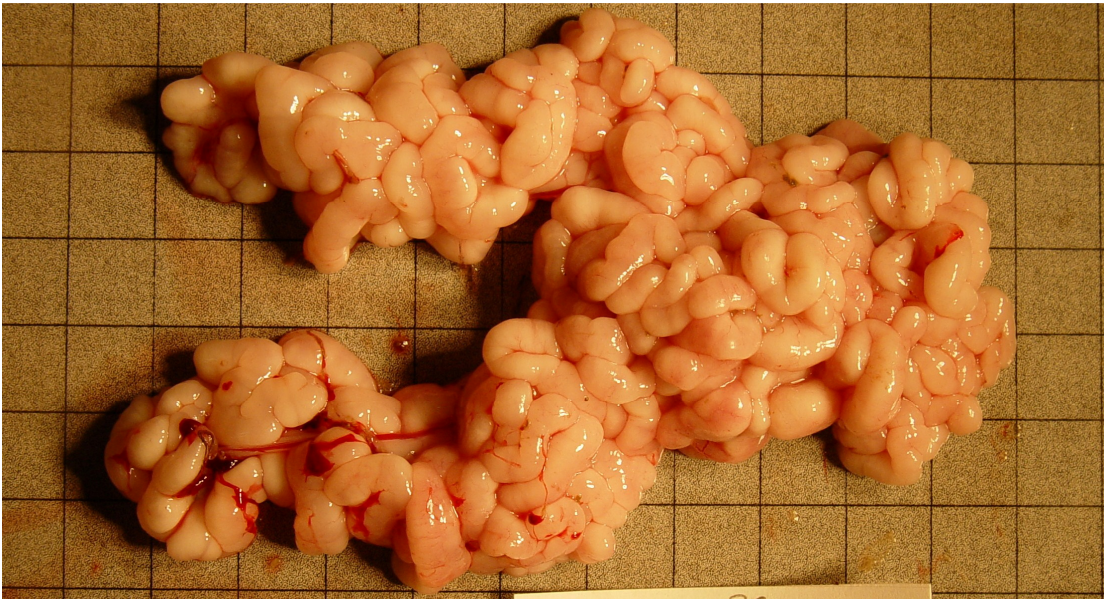
II



III. Spawning (initiation)



**Stage III (initiation):**  
Testes full and completely opaque in appearance; whitish; distal frill appears highly and tightly convoluted with many blood vessels visible; unable to identify spermatozoa in the sperm duct on picture, due to convolution of the frill, but spermatozoa visible in proximal tissue via cross section; not yet running.



Specimen data

L<sub>T</sub>: 50 cm

M<sub>B</sub>: 1292 g

M<sub>G</sub>: 40.5 g

GSI: 3.7

M: February 2007

ID: 070718/160

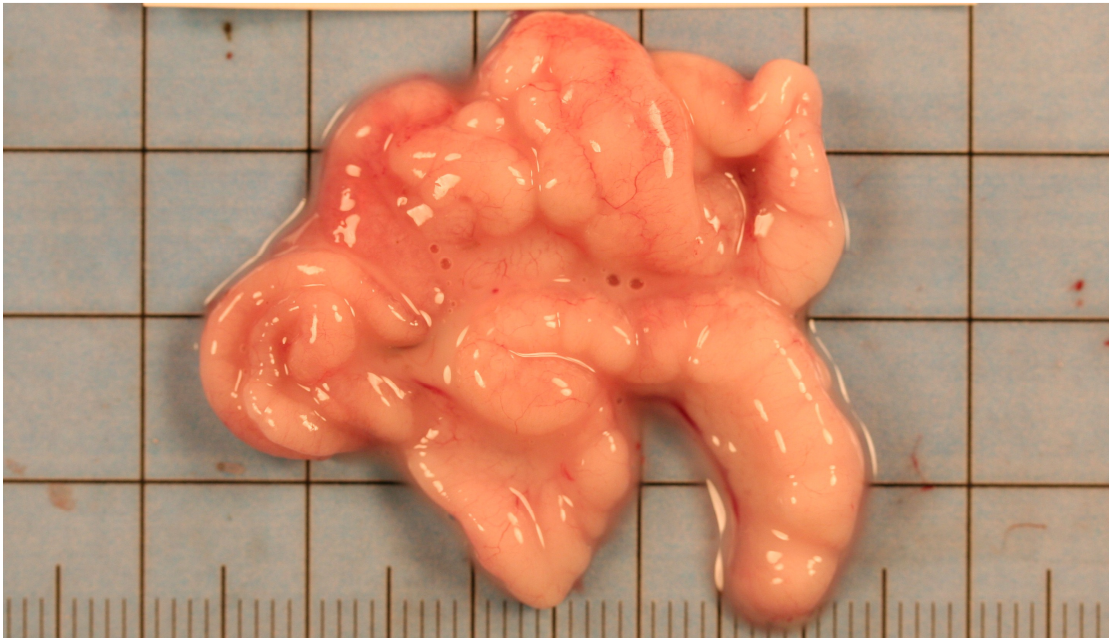




III. Spawning (main period)



**Stage III (main period):**  
Testes has a more fluid like appearance due to advanced production of spermatozoa; frills may appear less convoluted but quite creamy white and opaque; blood vessels appearance may be more intense; sperm visible at urogenital pore and running.



<i>Specimen data</i>		
L <sub>T</sub> : 27 cm	M <sub>G</sub> : 4.2 g	M: February 2007
M <sub>B</sub> : 182 g	GSI: 2.8	ID: 070718/250

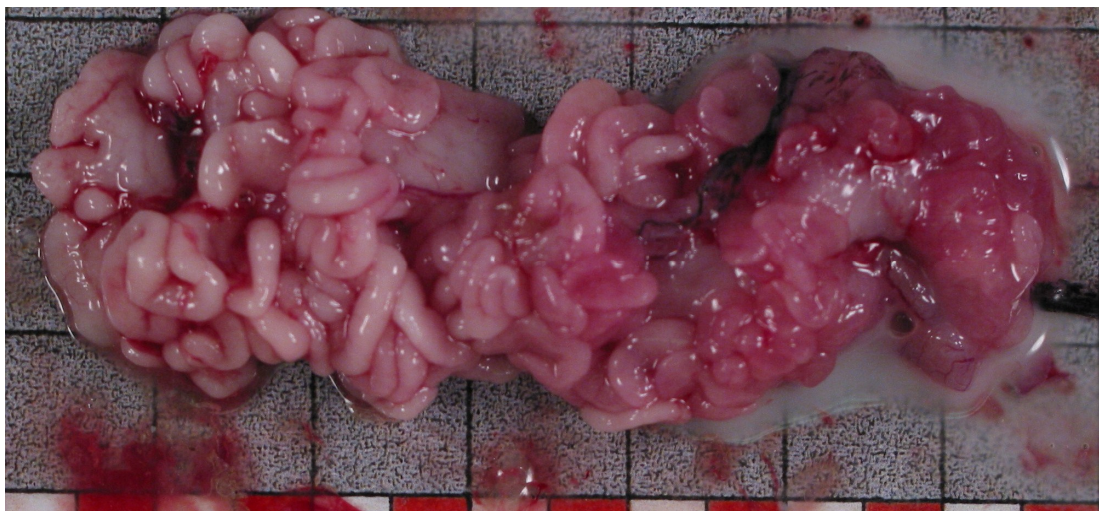




### III. Spawning (cessation)

#### Stage III (cessation):

Testes appear much reduced in size; opaque pink to dark red in appearance; distal frill is less convoluted and reduced in size (empty frills); tissue is highly vascularised and bloodshot; latter stages of spermatozoa production with sperm visible at urogenital pore under moderate pressure.



#### Specimen data

L<sub>T</sub>: 42 cm

M<sub>B</sub>: 687 g

M<sub>G</sub>: 9 g

GSI: 1.4

M: March 2007

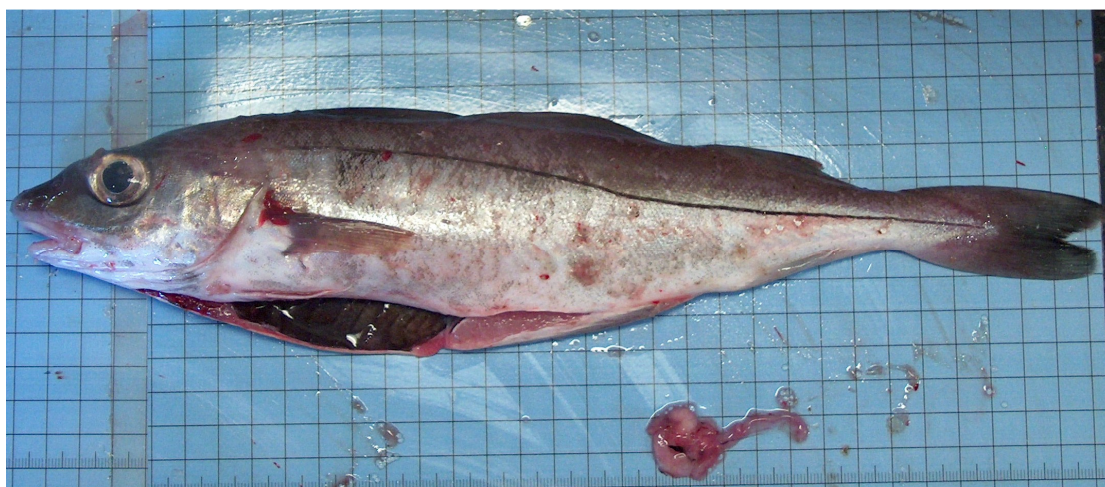
ID: 070718/123



### III. Spawning (cessation)



Stage III (cessation):  
(continued)



#### Specimen data

L<sub>T</sub>: 31 cm

M<sub>B</sub>: 290 g

M<sub>G</sub>: 1 g

GSI: 0.4

M: February 2007

ID: 070718/248

#### **IV. Spent**

##### **Stage IV:**

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

Tissue vastly reduced in size with dark red/purple bloodshot and sometimes translucent appearance.

### V. Skip of spawning (November-February?)

#### **Stage V:**

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

**V**

#### **V. Resting (March-October?)**

##### **Stage V:**

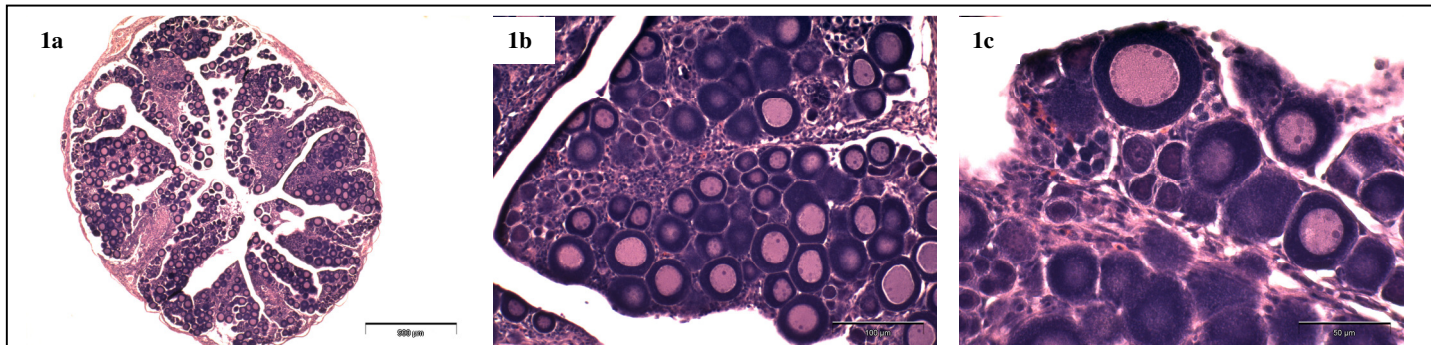
No suitable example of a stage V specimen was available from the samples.

**V**

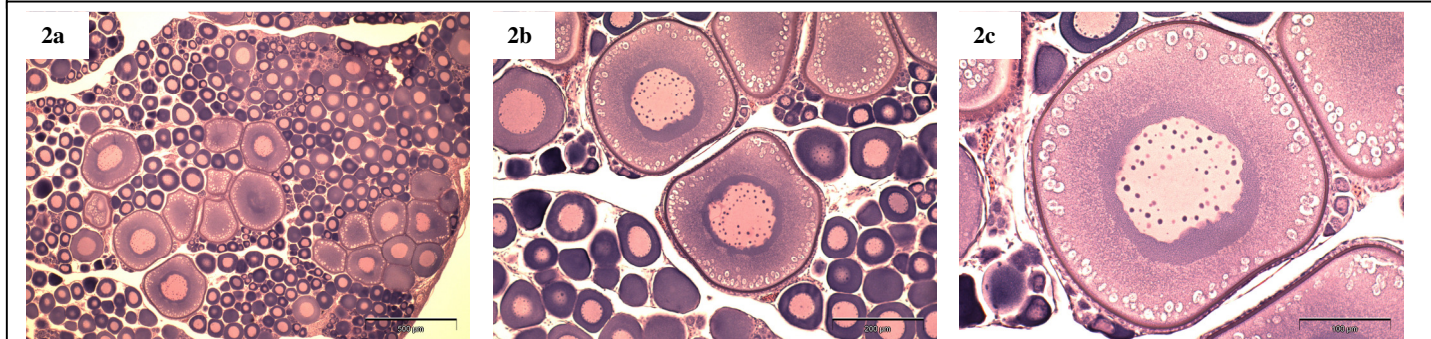
### **VI. Abnormal**

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

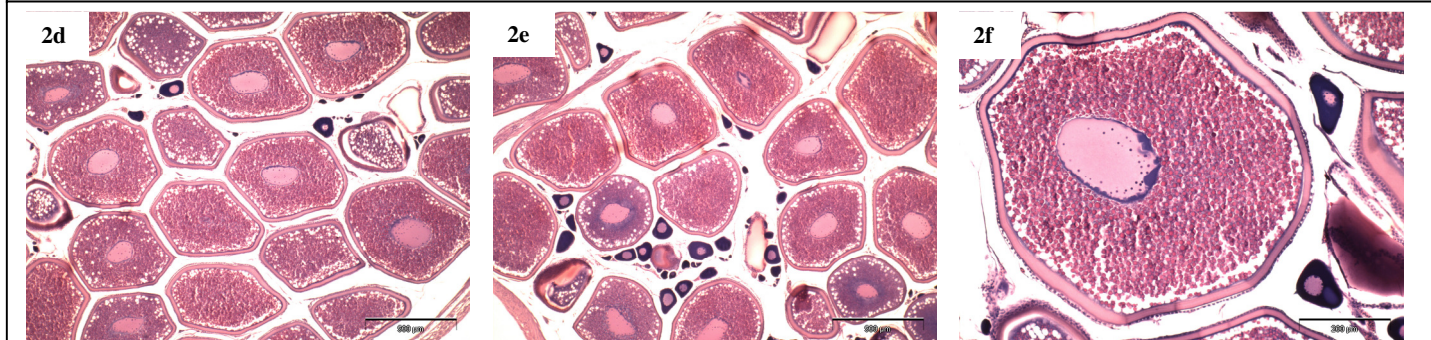




**I. Juvenile/immature (early).** Oocytes are small with dense stained cytoplasm and a central nucleus with few, large peripheral nucleoli around its edge, oogonia are always present at this premature stage although sometimes not visible. None of the oocytes have started the primary growth characterized by the circumnuclear ring (scale bars **1a.** 500 µm **1b.** 100 µm **1c.** 50 µm; specimen 070718/4)



**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 appears more lightly 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 070718/247)

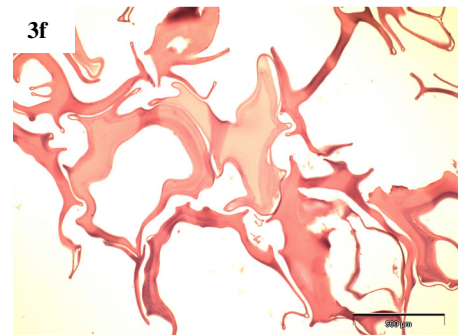
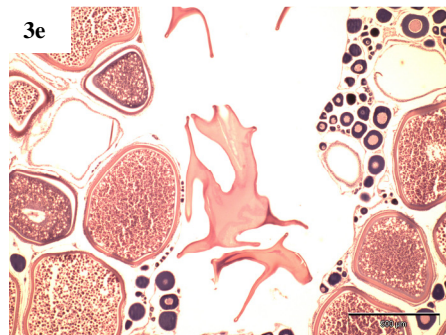
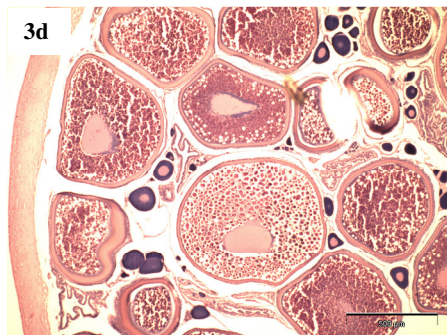


**II. Maturing (late).** Granules of yolk intensely stained, initially appear peripherally, but as they increase in number and size, they tend to spread out together with the cortical alveoli, throughout the cytoplasm. This process is termed vitellogenesis. The shape of the nucleus becomes irregular, but it is still centrally located (scale bars **2d.** 500 µm **2e.** 500 µm **2f.** 200 µm; specimen 070718/8)

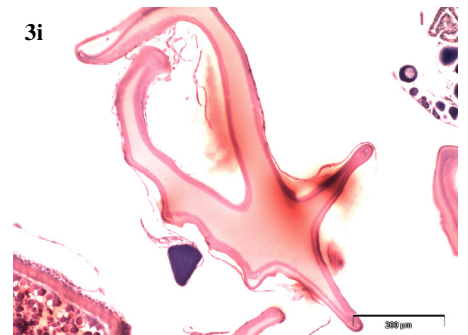
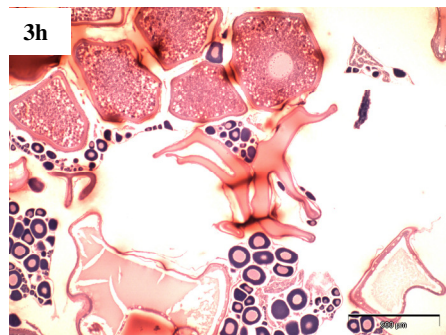
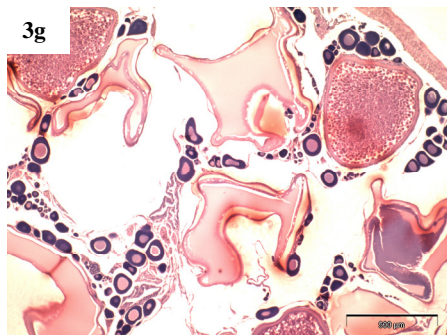


**III. Spawning (initiation).** The nucleus migrates to the periphery and breaks down, yolk granules coalesce forming large irregular spheres (**3a, 3b**), a water influx occurs and hydrated eggs (**3c**) with a completely homogeneous content are formed. At the ovulation, oocytes are released into the lumen, while the post-ovulatory follicles (**3d**) remain in the ovary (scale bars **3a.** 500 µm **3b.** 500 µm **3c.** 200 µm; specimen 070718/212)

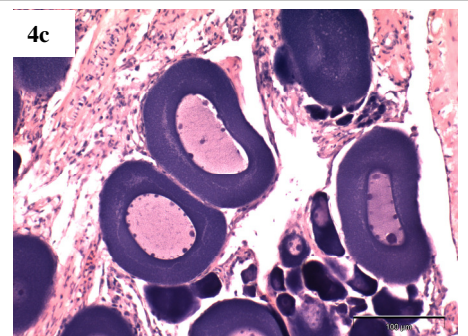
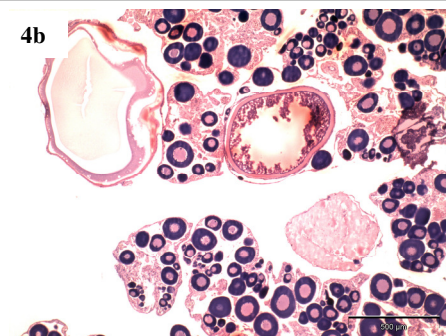
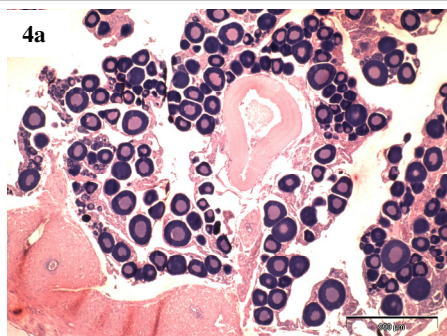




**III. Spawning (main period).** As the spawning proceeds the number of hydrated eggs and post-ovulatory follicles increases (scale bars **3d.** 500 µm **3e.** 500 µm **3f.** 500 µm; specimen 070718/249)



**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 becomes more thick and folded (scale bars **3g.** 500 µm **3h.** 500 µm **3i.** 200 µm; specimen 070718/11)

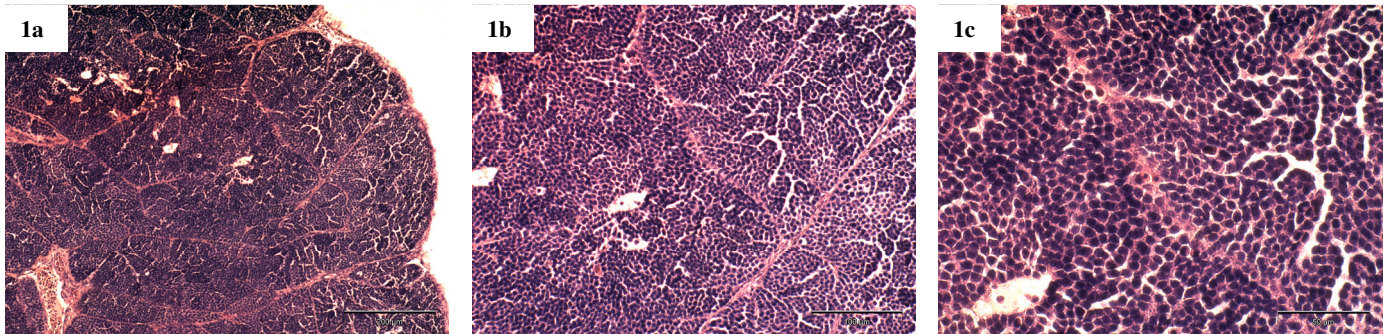


**IV. Spent.** At the end of the spawning season, the ovary enters the spent stage and post-ovulatory follicles, marking the occurred ovulation, are abundant among perinuclear or circumnuclear stage oocytes (**4c**). The development of vitellogenic oocytes sometimes fail and they undergo a process called "atresia" (**4a, 4b**) consisting in an intra-ovarian resorption (scale bars **4a.** 500 µm **4b.** 500 µm **4c.** 100 µm; specimen 070718/9)

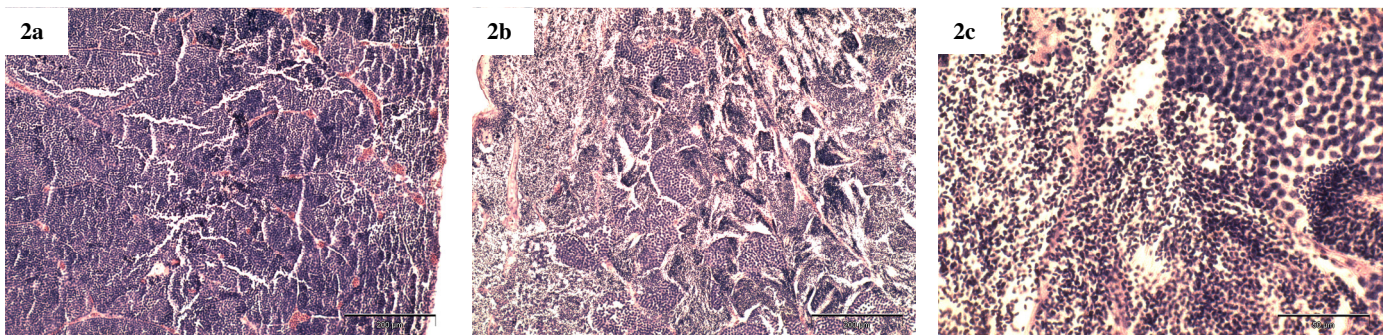




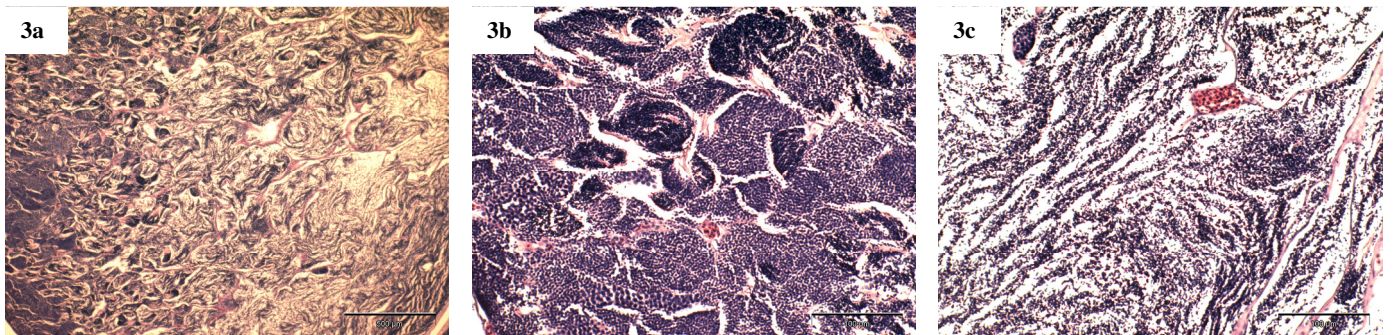




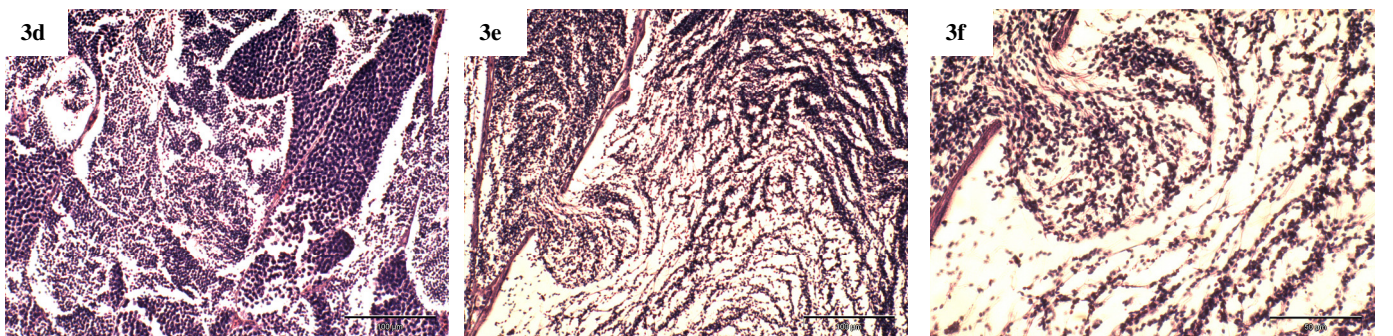
**I. Juvenile/immature (preparation).** 'Germ cells' or spermatogonia are characteristic of this stage; most are located distally. Singular germ cells lodge within a cyst and divide mitotically into groups of germ cells, which again divide into primary spermatocytes once the immature tissue is in preparation (scale bars **1a.** 200  $\mu$ m **1b.** 100  $\mu$ m **1c.** 50  $\mu$ m; specimen 070718/163).



**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  $\mu$ m-distal **2b.** 200  $\mu$ m-proximal **2c.** 50  $\mu$ m-proximal; specimen 070718/121).

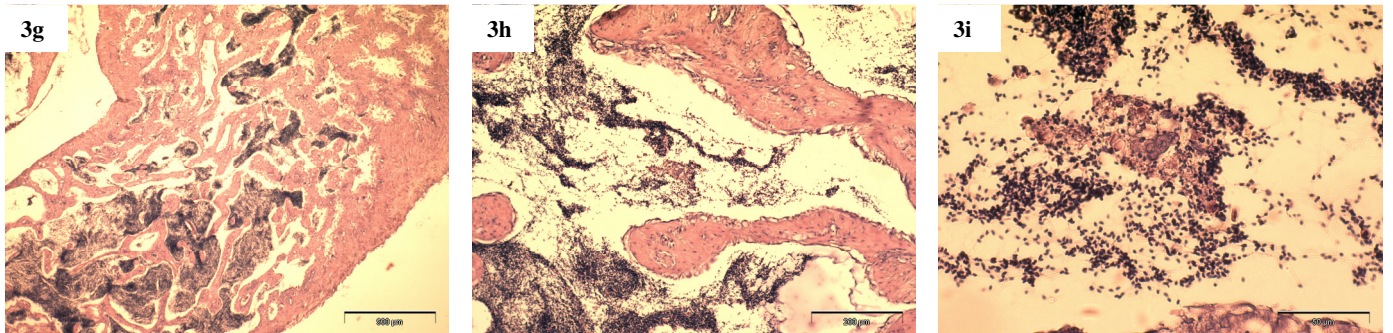


**III. Spawning (initiation).** The number of spermatozoa increases rapidly. Cyst and lobule walls disappear and long tubules are formed proximally. Mature spermatozoa become aligned. Migrating germ cells are only visible at the extreme distal edges. The sperm duct and proximal efferent duct system contains ripe spermatozoa (scale bars **3a.** 500  $\mu$ m **3b.** 100  $\mu$ m-distal **3c.** 100  $\mu$ m-proximal; specimen 070718/160)



**III. Spawning (main period).** A larger fraction of tubules now contain ripe spermatozoa (scale bars **3d.** 100  $\mu$ m-distal **3e.** 100  $\mu$ m-proximal **3f.** 50  $\mu$ m-proximal; specimen 070718/250)





**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**, 500  $\mu\text{m}$  **3h**, 200  $\mu\text{m}$  **3i**, 50  $\mu\text{m}$ ; specimen 070718/123).

