

Characterisation and histopathology grading system of gill lesions using flatfish and Mediterranean fish species as models.

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¿Why are we doing this?

- Improve **quality, accuracy and intercalibration potential** of the **histopathological studies and diagnostics** in gill health assessment on four of the most important fish species reared in the Mediterranean and South-European Atlantic areas

Following previous background

- **Human histopathology:** many scoring systems well developed and implemented for many diseases (mainly neoplasia)
- **Veterinary Histopathology:** some approaches and systems
 - Klopffleisch, R (2013): Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology - a systematic review. BMC Veterinary Research, 9:123.
 - Gibson-Corley et al. (2013). Principles for Valid Histopathologic Scoring in Research. Veterinary Pathology. Vol 50 (6)
- **Fish Histopathology**
 - Bequalm Liver histopathology and External Fish Disease Work programme (CEFAS)
 - Bernet et al (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis., 22: 25-34.
 - Mitchell et al. (2012) Development of a novel histopathological gill scoring protocol for assessment of gill health during a longitudinal study in marine-farmed Atlantic salmon (*Salmo salar*). Aquaculture International, 20, 813 – 825
 - Jeffrey et al. (2014) Pathology working group review of histopathologic specimens from three laboratory studies of diclofenac in trout. Aquatic Toxicology, 146, 127-136.

Yes, but....

- *A priori* main constraints:

Available work and data:

- Limited number of references (books, journals, technical papers) on gill pathology
- Few scientific references on diseases / histopathology on seabream, seabass, turbot or sole.

Rev Fish Biol Fisheries (2011) 21:423–440
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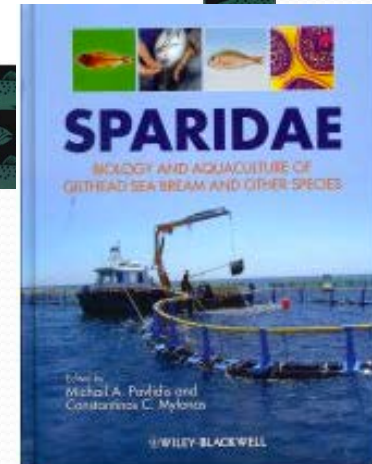
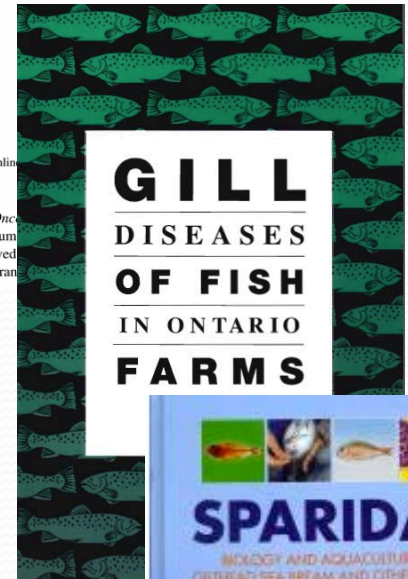
REVIEWS

Non-infectious gill disorders of marine salmonid fish

Hamish D. Rodger · Louise Henry ·
Susan O. Mitchell

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Abstract Gill disorders present a significant challenge in salmon (*Salmo salar* and *Oncorhynchus* sp.) farming regions throughout the world. This review of gill disorders and diseases of marine fish is focused on the most common and economically important disorders. The review covers the following: gill anatomy and function, gill pathology, and the role of gill disorders in the development of other diseases. The review is intended for fish farmers, veterinarians, and researchers in the field of fish pathology.





- *A priori* main constraints:

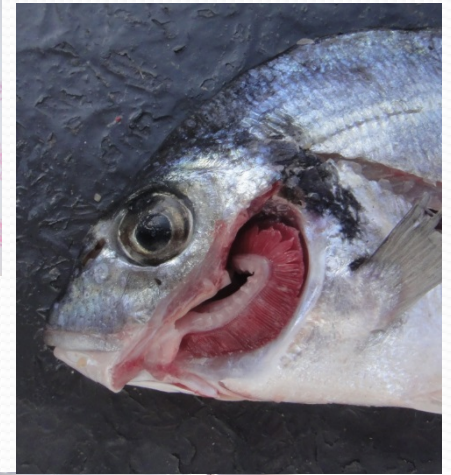
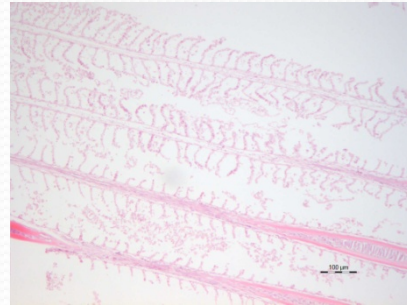
- Staff selection

- Experience in fish histopathology

- Knowledge about the specific biology and rearing process of the selected species

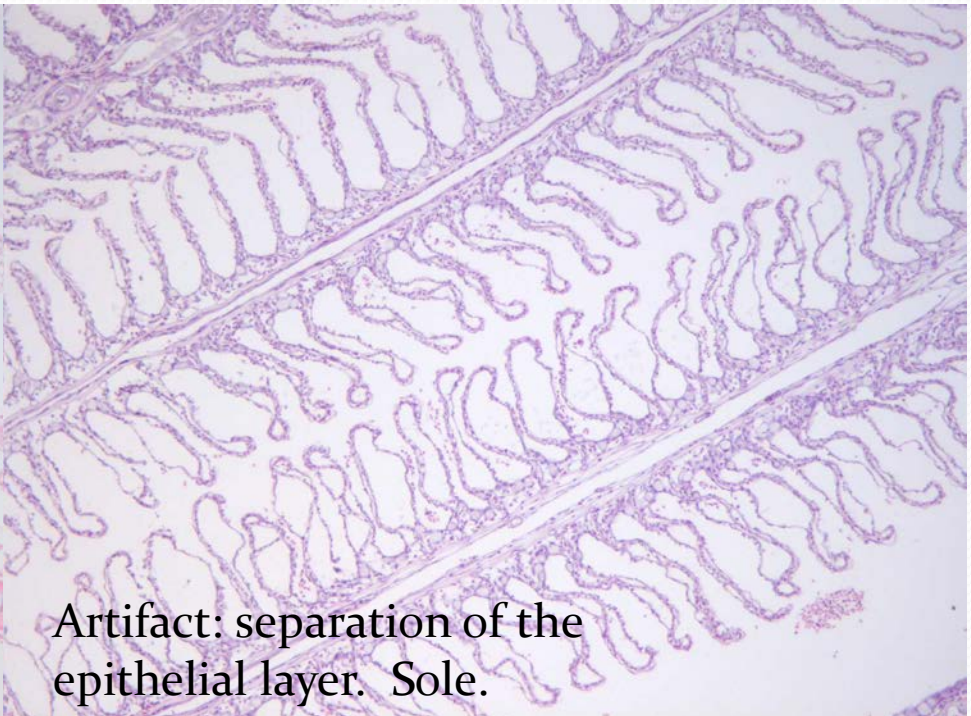
An also....

- *A posteriori* main constraints:
 - Sample quality:
 - CRITICAL!
 - Why?
 - Sampler skills : not always well trained: sample selection (sick fish, advanced lesions...) adequate fixation.
 - Land based farms vs offshore cages
 - Hatchery/ Nursery samples vs offshore cages
 - Warmer temperatures in the Mediterranean





Autolysis: seabass



Artifact: separation of the epithelial layer. Sole.

Evaluation process description

- Material: cases from SDPP & Ictiovet (2004-2014) : slides, blocks & relevant clinical data.
 - Work on database and a lot of dust!
- Selection of the most suitable cases (anonymous samples)
- Check the quality of the material (re-sectioning, new slides if necessary)



Intercalibration exercise

- Codified blind cases
- 4 observers with different levels of experience
- General instructions
- Worksheet with categories
- 10-15 minutes for each
- General discussion
- **Characterization of each case**
 - Description of main pathological categories
 - Quantification of the changes
 - Chronobiology of the lesions (if possible)
 - Main lesional characterization

**Abnormal
growth**

Inflammation

**Hemodynamic
changes**

**Degenerative
changes**

**Necrosis/
apoptosis/
anoikis**

Repair

**Agents/
Foreign
elements**



Tbr (repression) Vand. utahensis

259/13 A 521/12 A 36/14 11/13

mini RING TEST - 9/5/14

	1	2	3	4
Abnormal growth	Si requires → No opac Furby Hypoglycemia (wild) no Masses white (woc): (1) (2) (3)	Zones hyperplasia 1 (woc) (1) → 30% → des. (1) (2)	Alga autolysis x	Masses hyperplasia spots 117 (woc) on 2 good cells spot-grains idesq.
Inflammation	Algae focus also w/... Fix. woc... (1) (2)	Lesions a v. v. v. zones a flau... (woc) (1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (23) (24) (25) (26) (27) (28) (29) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74) (75) (76) (77) (78) (79) (80) (81) (82) (83) (84) (85) (86) (87) (88) (89) (90) (91) (92) (93) (94) (95) (96) (97) (98) (99) (100)	Basics and flau interpath low < 10% w/... (1) (2)	Minimal - 1 secid → Acids pgs
Hemodynamic changes	Algae some w/ flau. w/... (1) (2)	Exudates neurospines (woc) (1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (23) (24) (25) (26) (27) (28) (29) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74) (75) (76) (77) (78) (79) (80) (81) (82) (83) (84) (85) (86) (87) (88) (89) (90) (91) (92) (93) (94) (95) (96) (97) (98) (99) (100)	?	Helicospores a v. v. 2 one → infect. prec. (1) (2)
Degenerative changes	No.	Si → lesions punct. at assoc. in lesion	NO	NO
Necrosis/apoptosis/anokis	Si - zones opac flau... Desq. opac. hien	(1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (23) (24) (25) (26) (27) (28) (29) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74) (75) (76) (77) (78) (79) (80) (81) (82) (83) (84) (85) (86) (87) (88) (89) (90) (91) (92) (93) (94) (95) (96) (97) (98) (99) (100)	NO	NO.
Repair	?		NO	NO
Agents/Foreign elements	ERC + / - - Diplomon Masses po < Tauri	Spargocyst + Ternatococcus: + Bact. over 70-80%.	Cis hyper Hypermon.	(1) Formes cludant flau... w/... (1) (2)

Uthrao. > 90%
Silvan B&D <
2. Dissect. → ERCS

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unknow. edictio.
Tauri

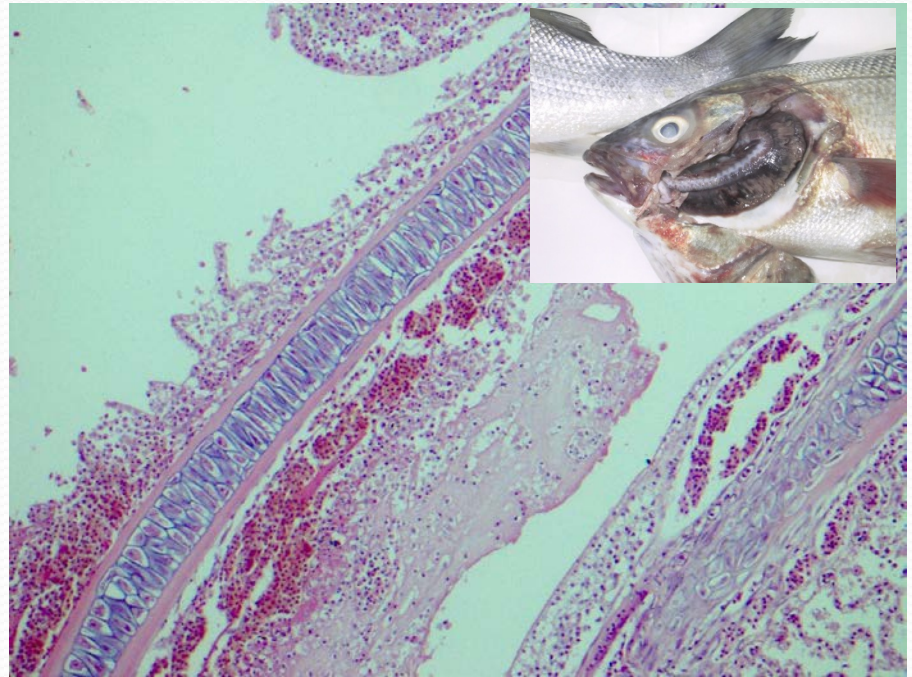
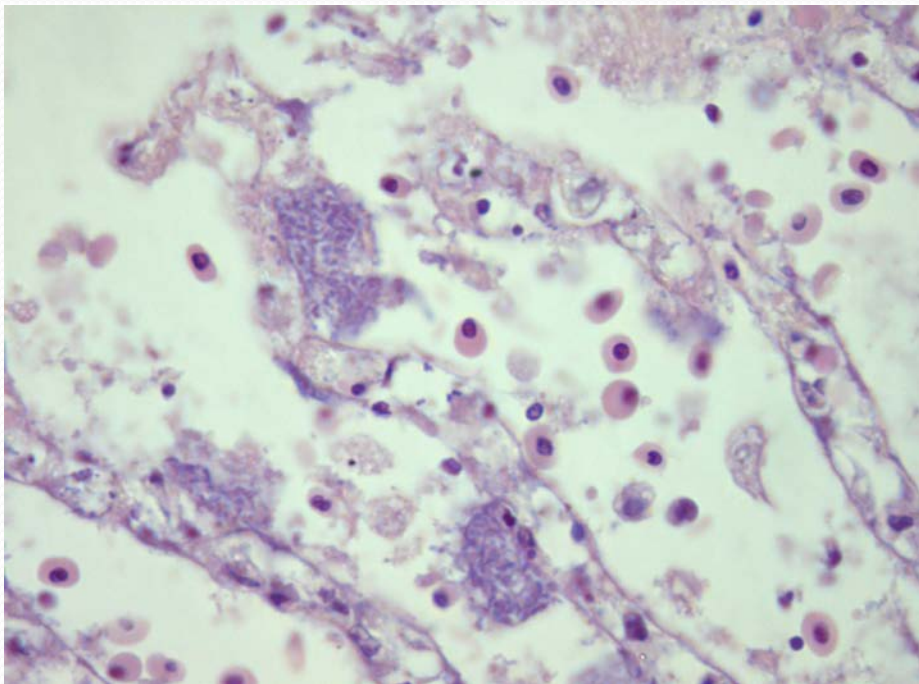
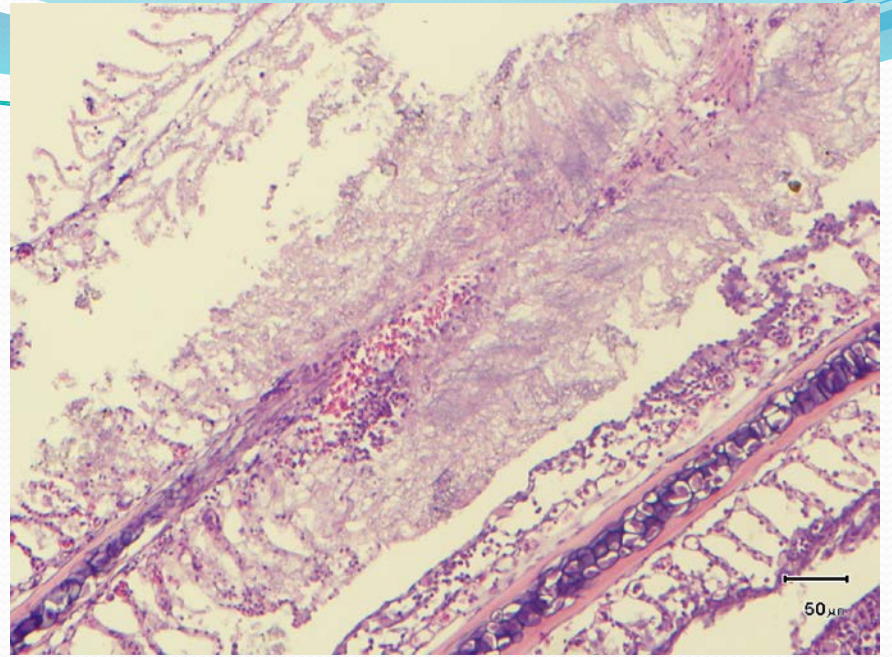
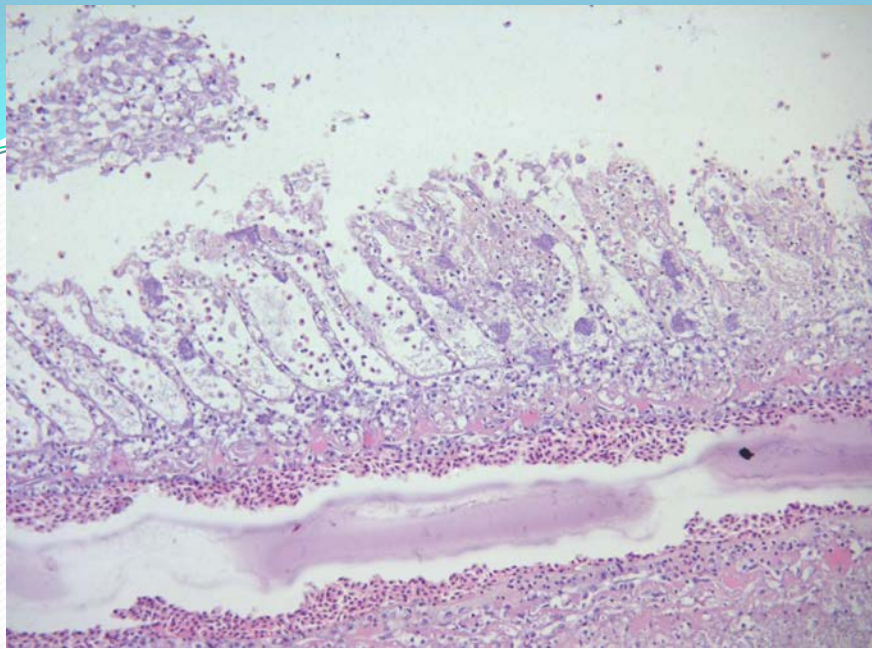
Code	Species	Main Histopath. Charact.	Main final Diagnostic	2ary lesion	3ary	qQUALITY (5*/1*)
136-1999	Turbot	Severe chronic proliferative branchyitis	AGD	Trichodina	BDG	*****
34-2013	Sea bream	Multifocal severe chronic necrotic/proliferative branchyitis	Monogenean infection (Sparicotyle)	Bacterial infection (Tenacibaculum)	–	***
262/13	Sea bass	Extensive severe subacute necrotic/haemorrhagic branchyitis	Unknown	–	–	***
14/14	Sole	Scattered mild subacute epithelial hypertrophy/mild branchyitis	Herpesvirus	–	–	*****
244/07	Sea bream	Extensive severe chronic proliferative branchyitis	Epitheliocytis	Trichodina	–	****
153/12	Sea bass	Multifocal moderate chronic proliferative branchitis	Monogenean (Diplectanum)	Copepod (Learnonthropus)	–	****
351/11	Turbot	Extensive moderate chronic proliferative branchyitis	Water quality (suspended solids)	–	–	*****

Pathological categories used in the database

- A) Necrosis / apoptosis / anoikis
- B) Degenerative changes
- C) Hemodynamic changes
- D) Inflammation
- E) Abnormal growth
- F) Repair
- G) Agents/ Foreign elements

Necrosis / Apoptosis / Anoikis

- Criteria / differential diagnostic
- Necrosis
 - Coagulative?
 - Liquefactive?
 - Caseous?



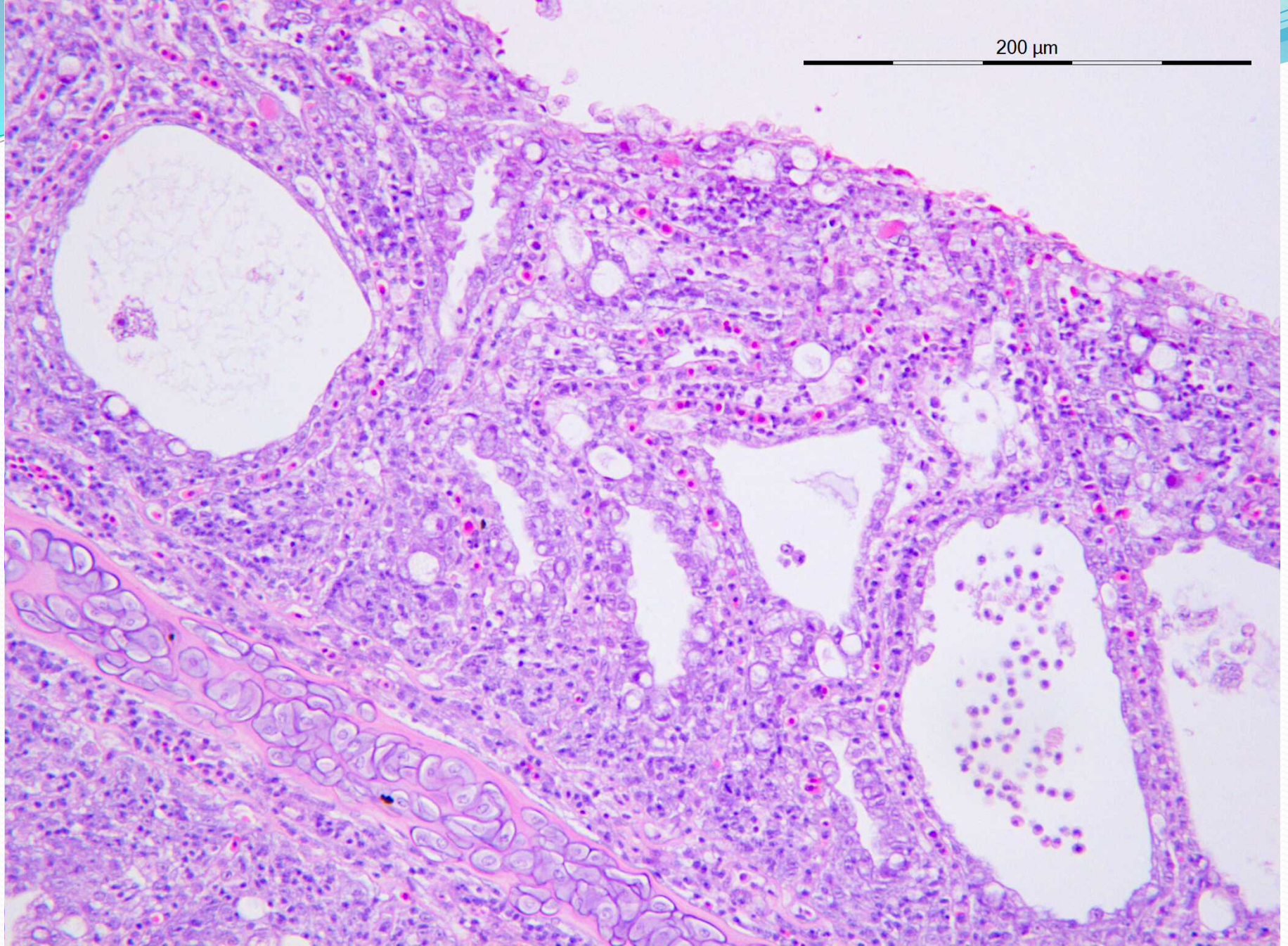
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Degenerative changes

- Epithelial spongiosis
- Epithelial desquamation
- Calcification
- Abnormal intracellular deposits
- Abnormal extracellular deposits
- Changes associated to starvation

200 μ m

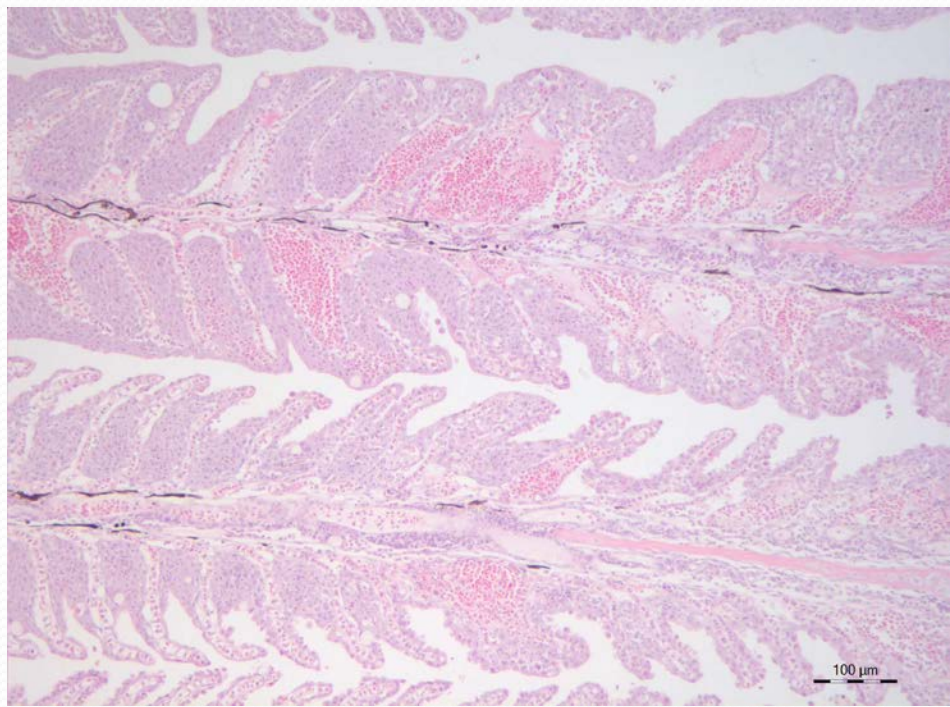
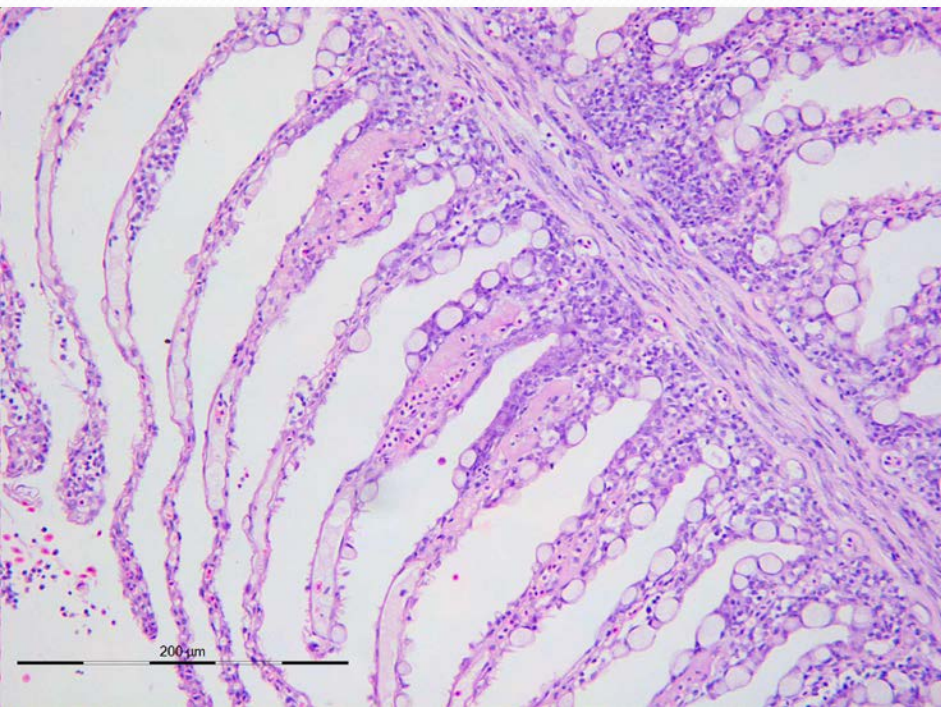
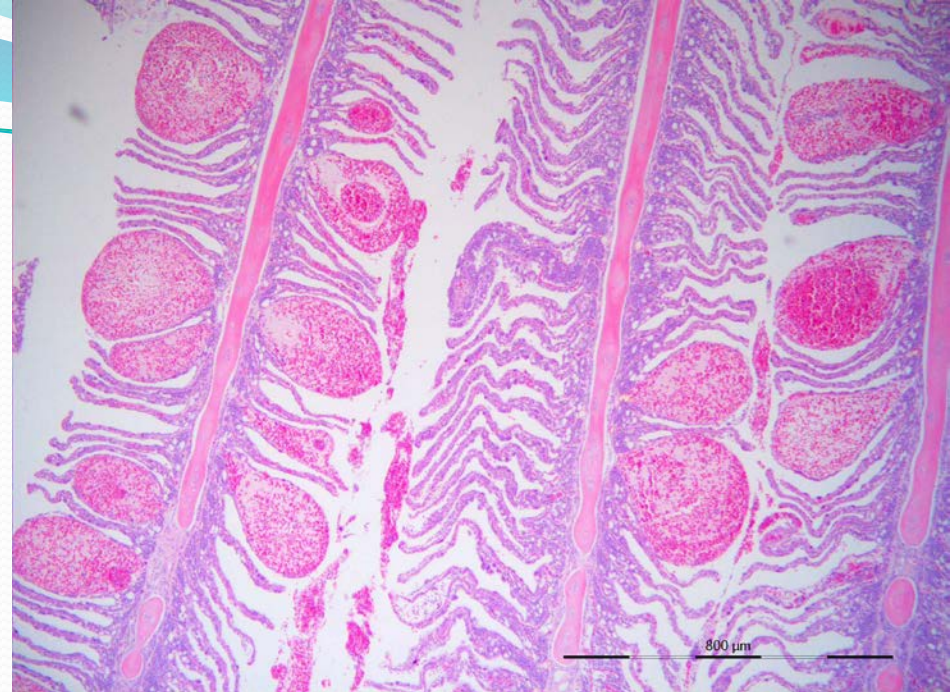
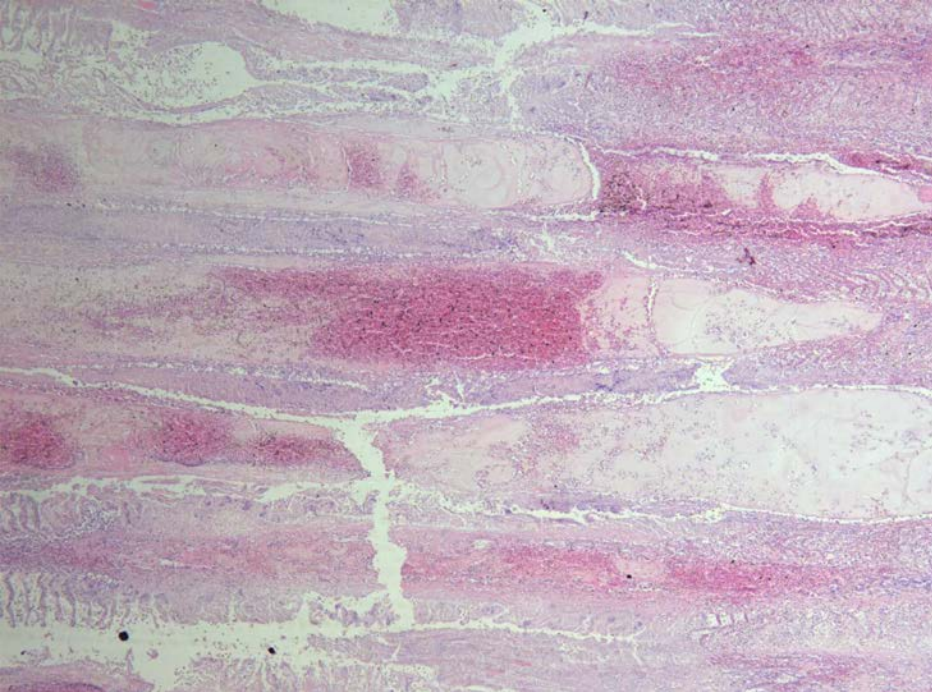


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Hemodynamic changes

- Blood congestion
- Haemorrhages
- Telangiectasis
- Thrombi
- Edema
- Exudates (fibrinous?)

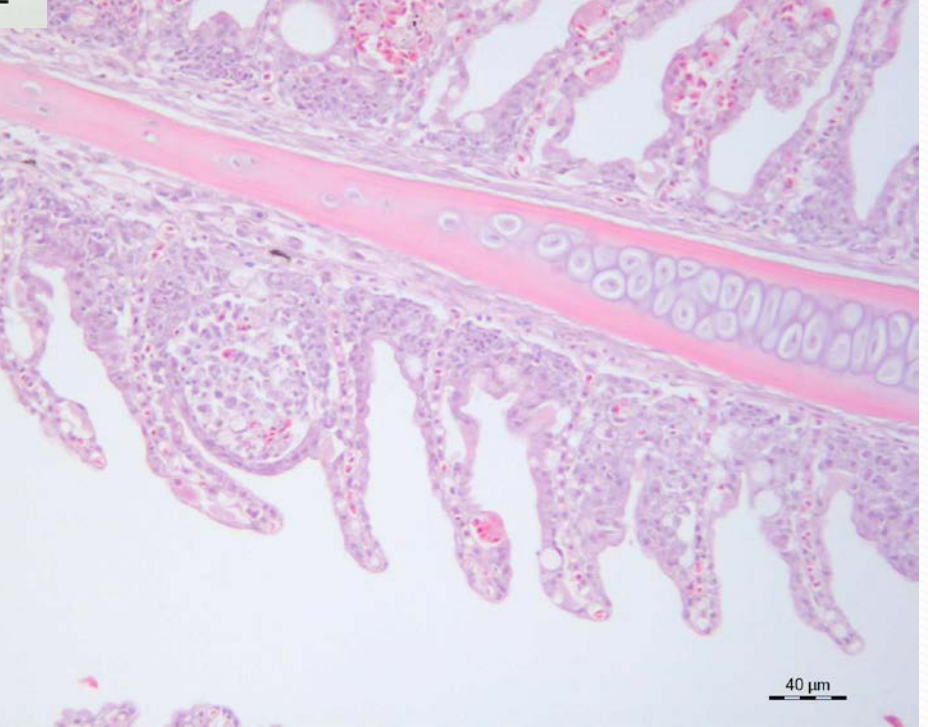
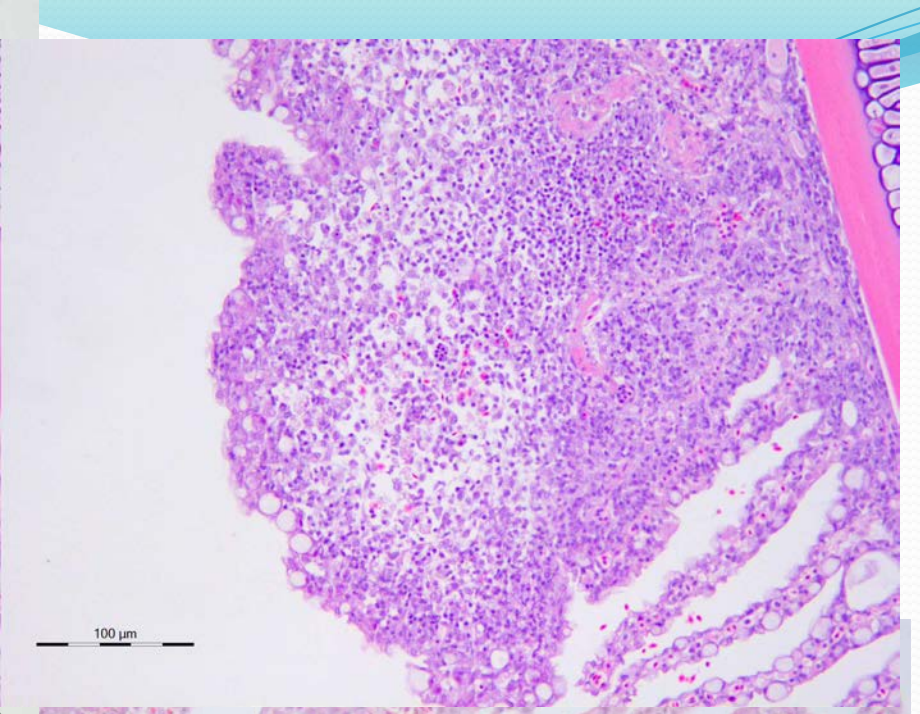
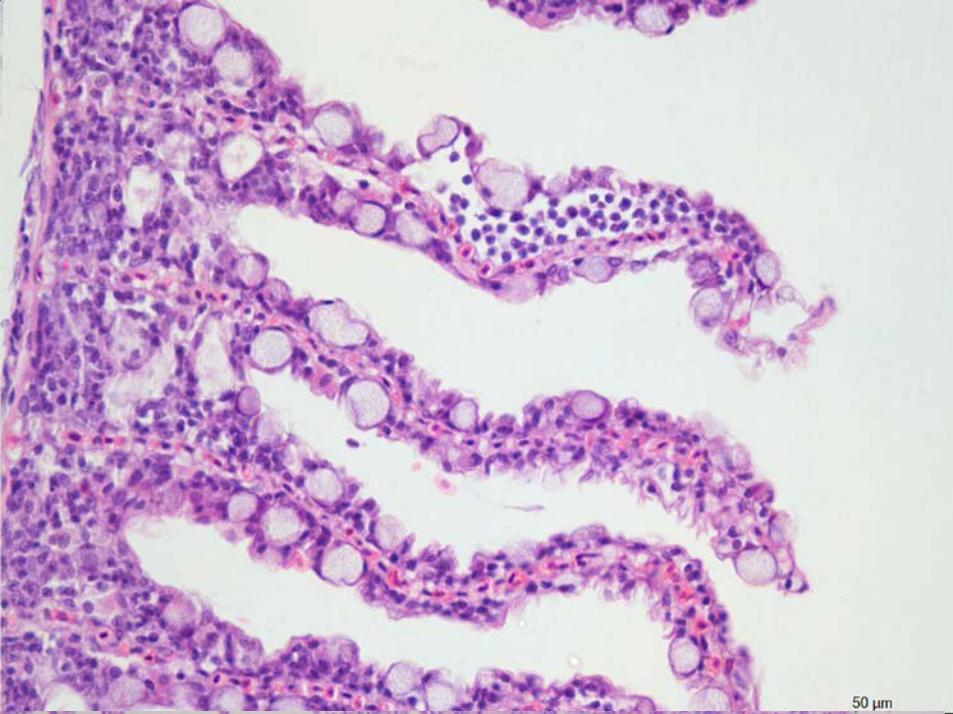


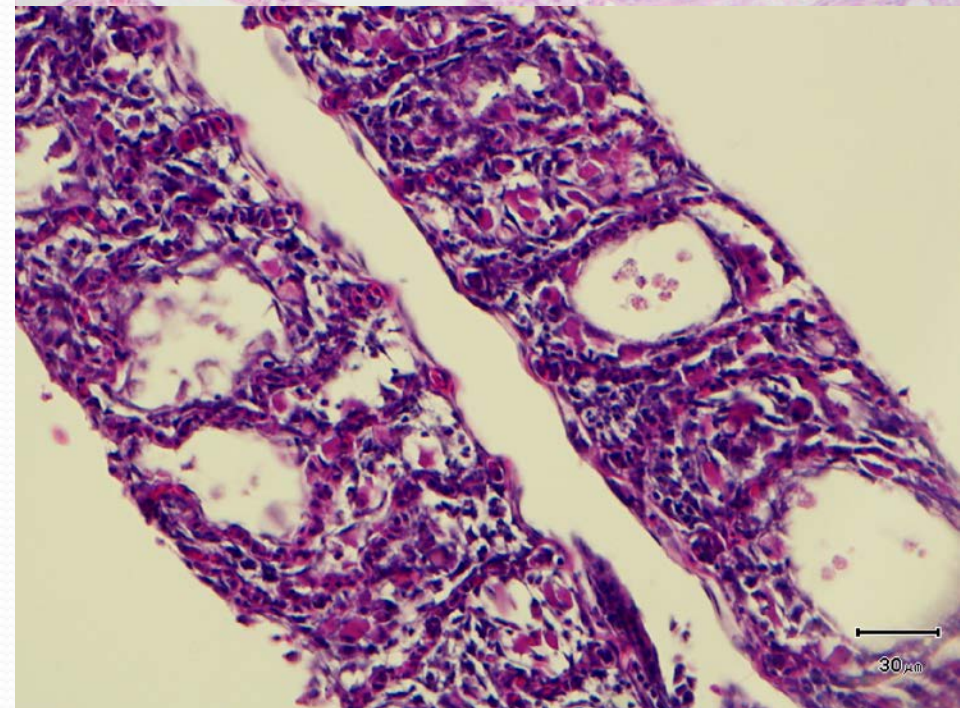
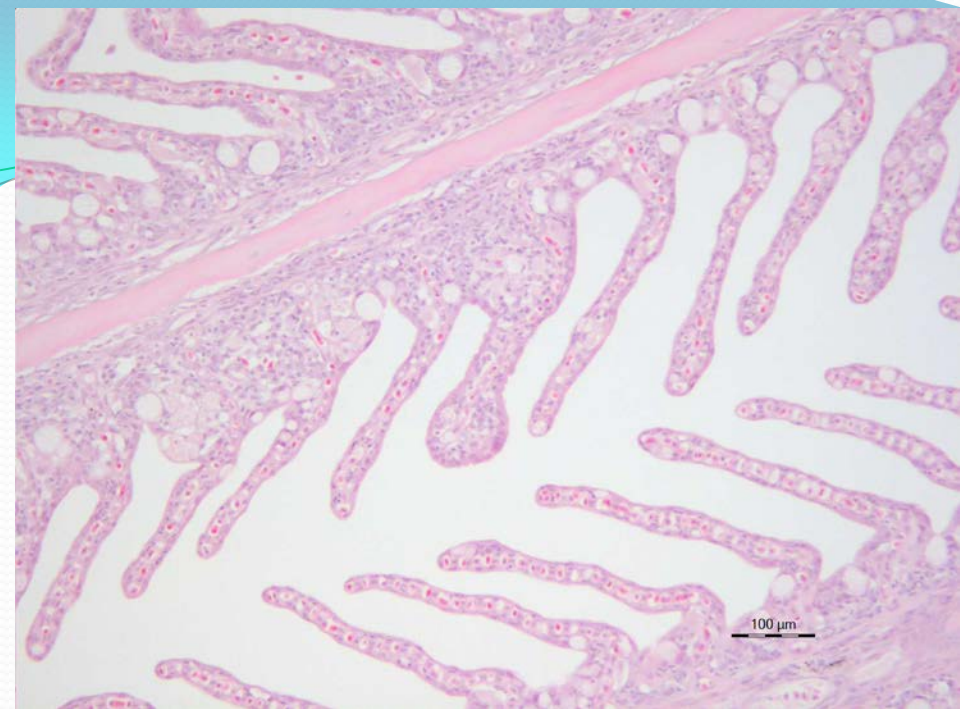
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Cellular Inflammatory changes

- Infiltration
 - Macrophages
 - Macrophage aggregates
 - Giant Cells /Syncitial cells
 - Lymphocytes
 - Granulocytes
 - Plasmacells
 - Resident Granular cells
 - Dendritic-like cells
 - Rodlets





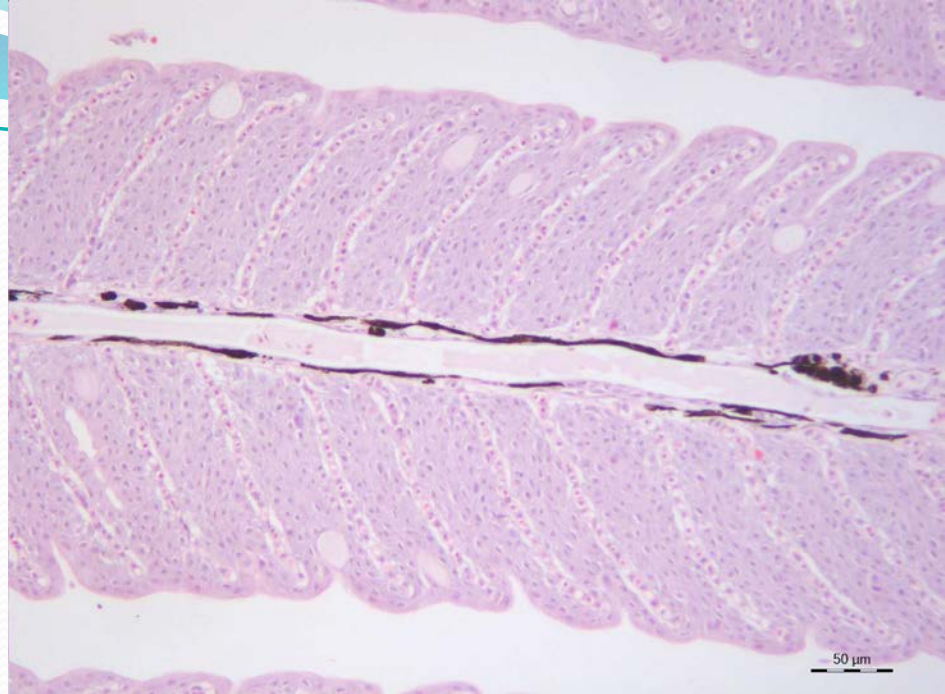
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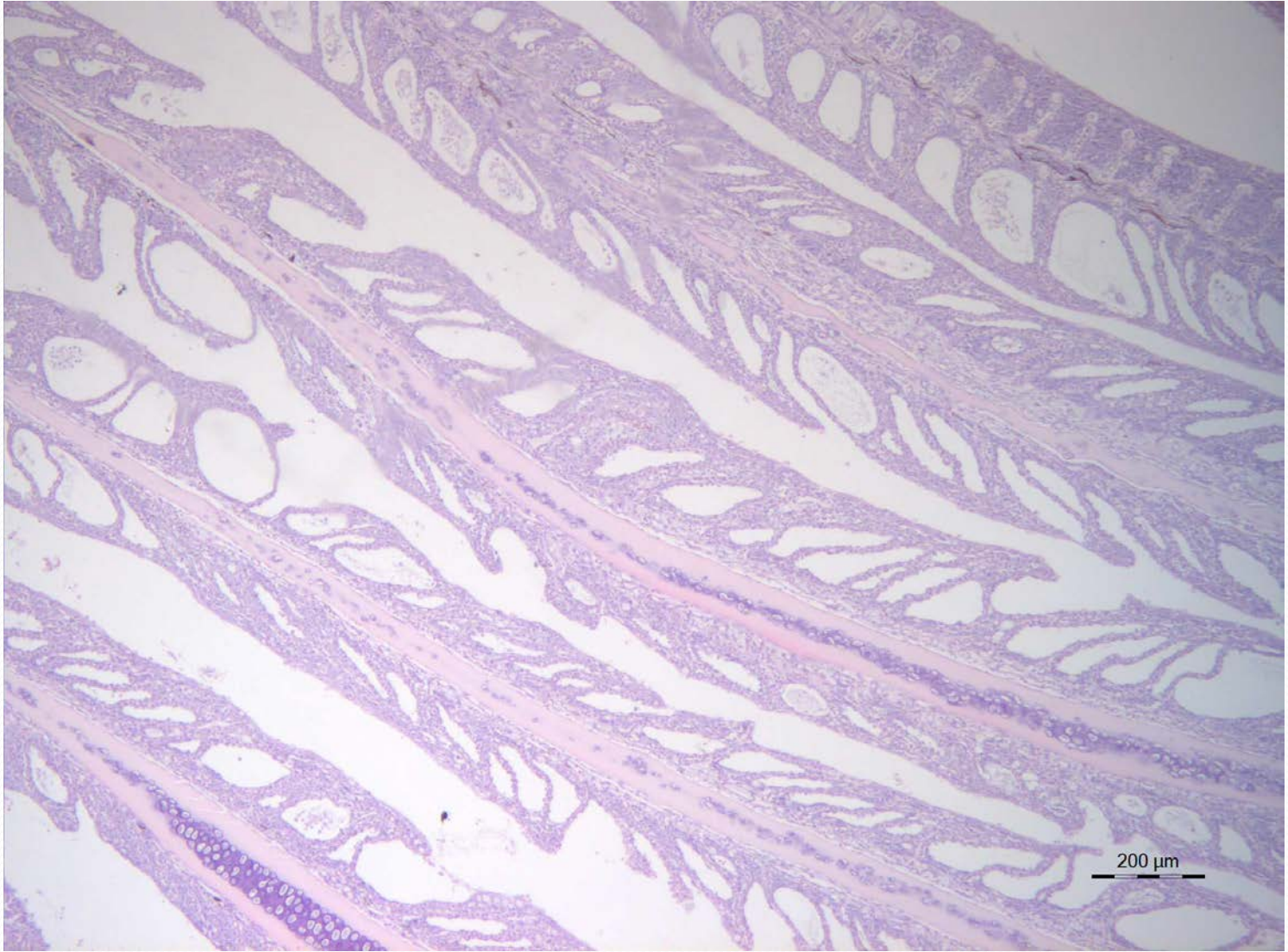
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Disturbances of growth:

Hypertrophy /hyperplasia /Abnormal growth

- Hypertrophy*
- Atrophy /lack of structures
- Hyperplasia (specific cell type)
- Synechia
- “*Metaplasia*”
 - Lymphocystis-infected fibrocytes*
 - Herpesvirus-infected epithelial cells*
- Atypical tissue/cells
 - Thyroid follicles
- FCA
- Neoplasia
- Malformation





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Regeneration / Repair

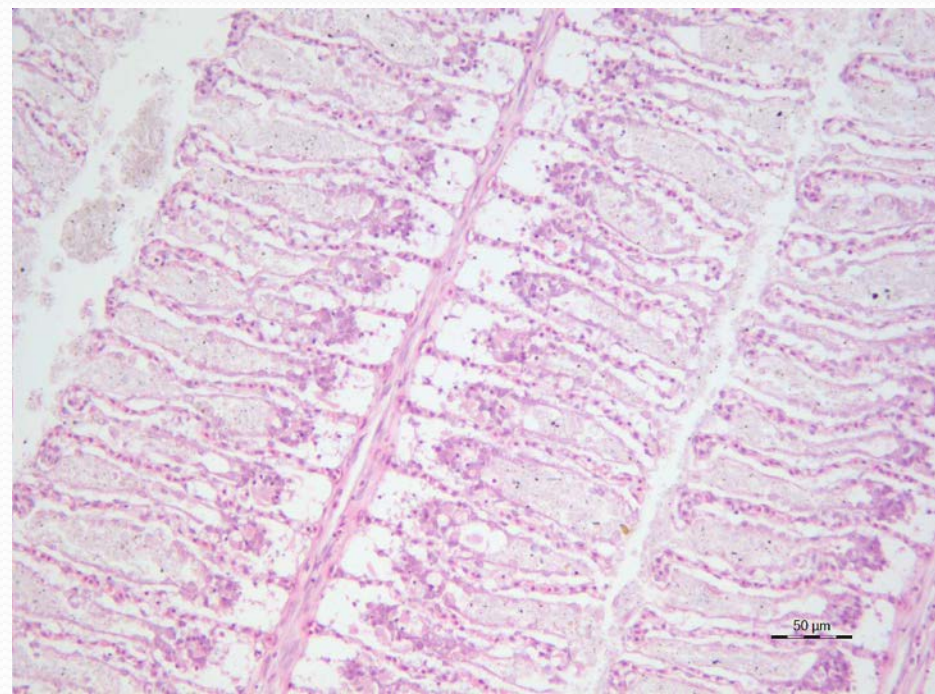
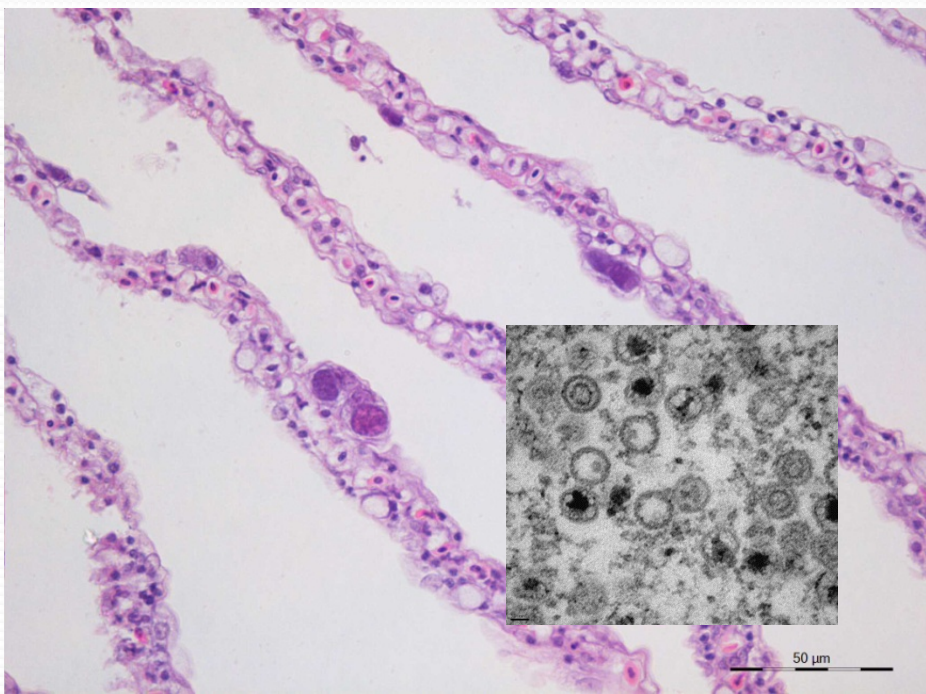
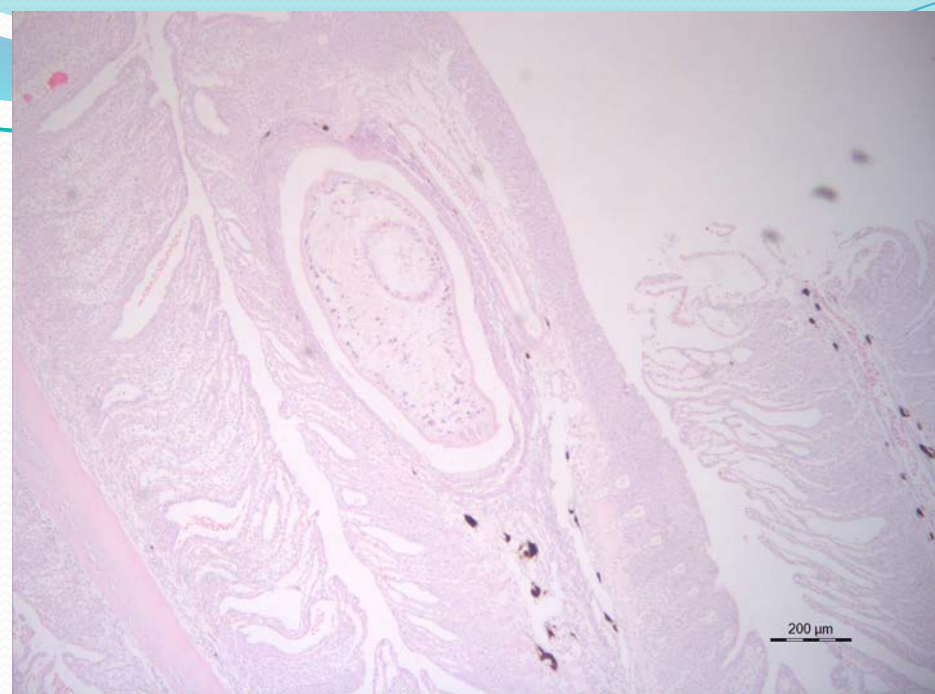
- Regeneration
 - Epithelia
 - Neovascularisation
- Repair
 - Gill plasticity
 - Fibrosis

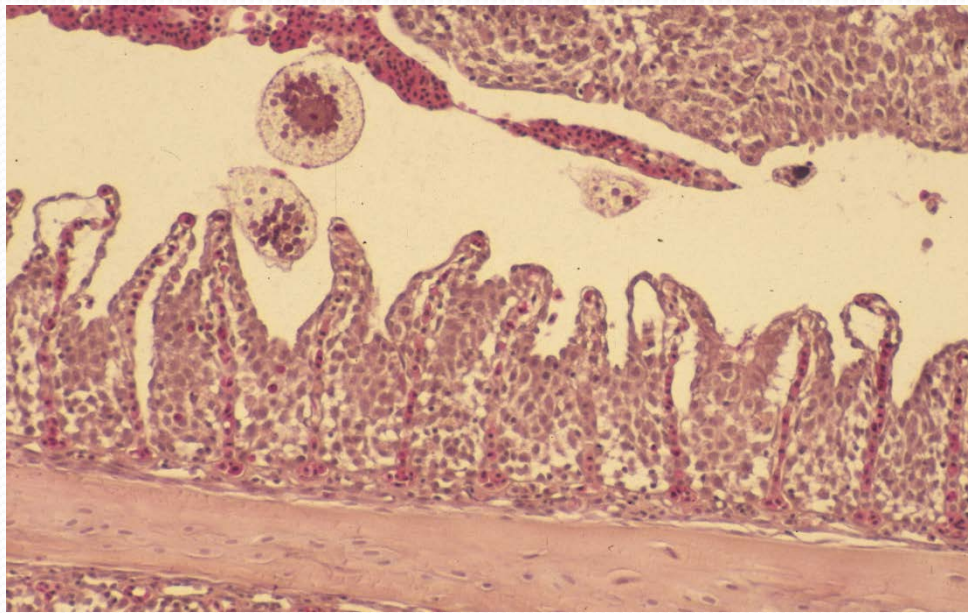
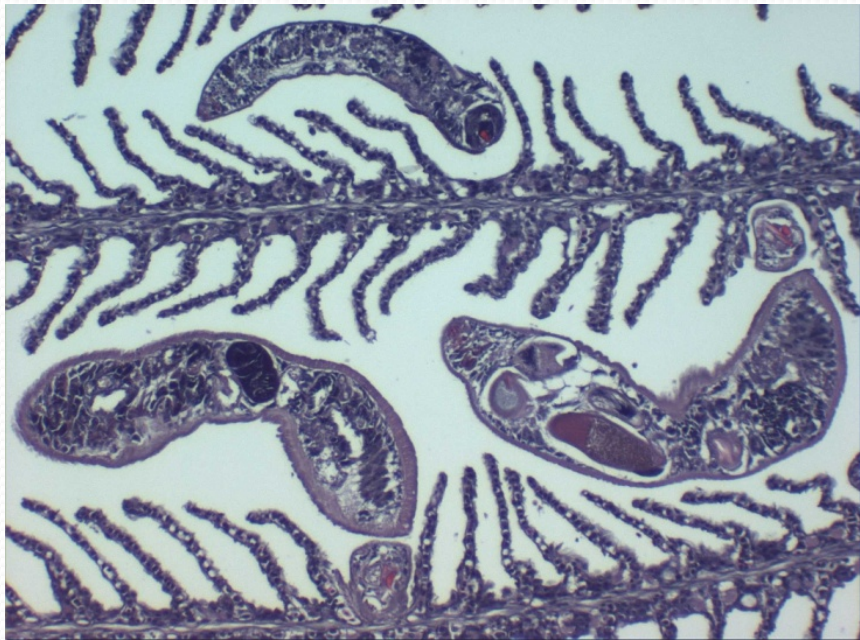
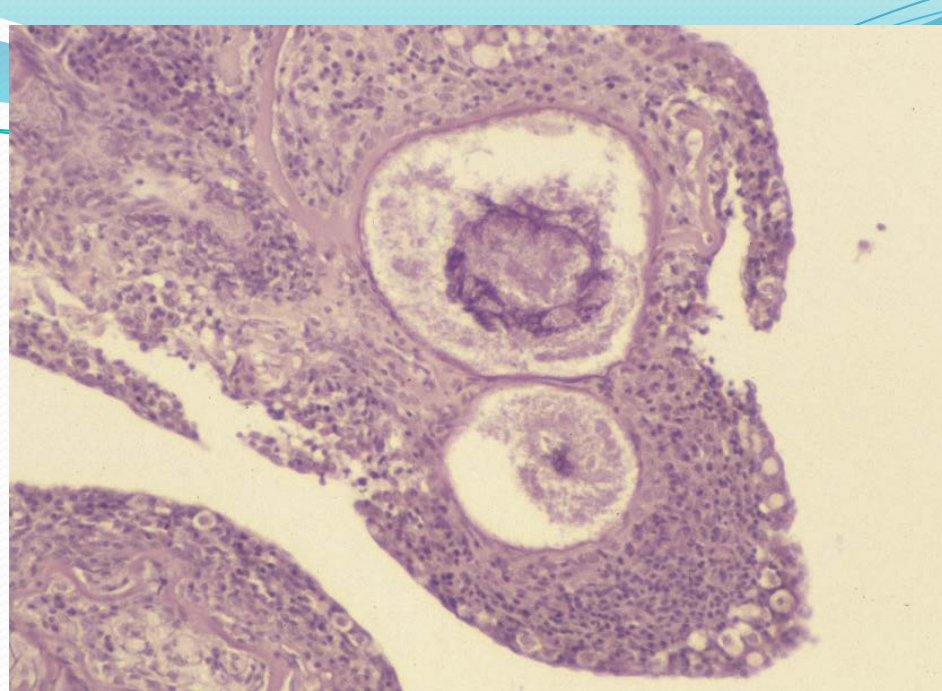
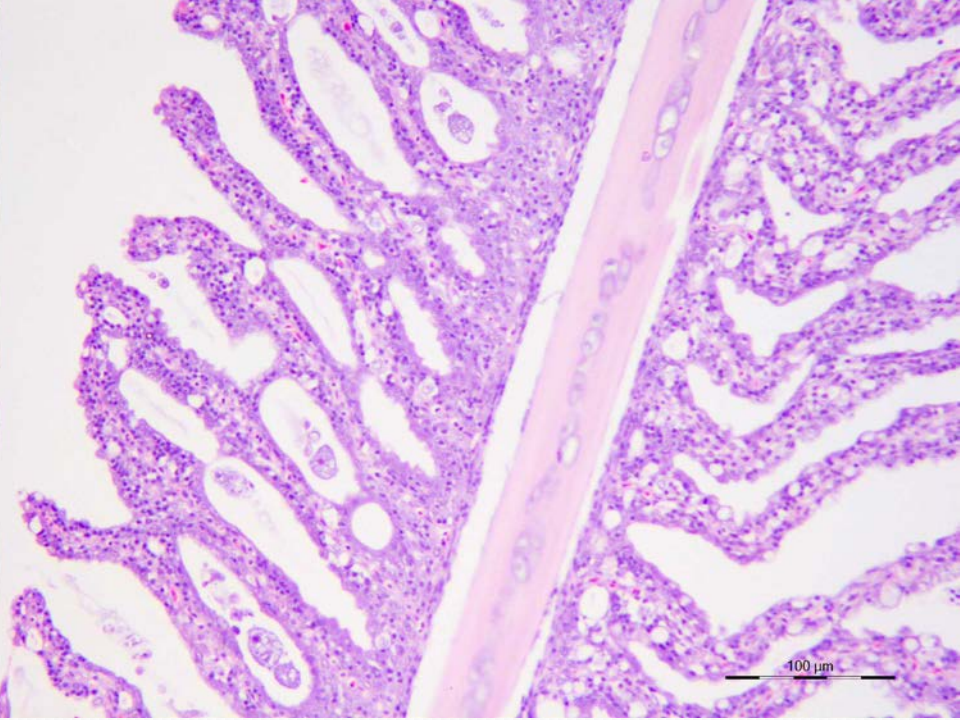
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Agents / Foreign elements

- Agents
 - Bacteria: external / internal lesions
 - External Parasites (ectoparasites)
 - Protozoan
 - Monogenean
 - Copepods
 - Parasites inside gill structures
 - Blood parasites
 - Chlamydia (EPC)
 - Viral inclusions
 - Rickettsia
- Foreign element
 - CUE's (enigmatic bodies)
 - Gas expansions
 - Interlamellar material





Quantification of the changes/lesions

- Respiratory area affected
 - Ex: % of affected lamellae per section
- Intensity of the lesions
- Any quantification should be associated to a standardized sampling methodology!
 - Gill arches? Whole gill? Orientation?....

Final histopathological characterisation

- 1) Grade: mild, moderate, severe
- 2) Acute / chronic
- 3) Main histopathological feature(s)
- 4) Topographical and quantitative evaluation
- 5) Regenerative capacity (?)
- 6) Prognostic (???)
- 7) Clinical approach (?????????)

DELIVERABLES

- Basic criteria for the normal histology of the four species and 'normal' changes associated to age or specific rearing conditions
- Fish quality evaluation: artifacts
- Characterisation of the main lesions/changes observed
- Progressive development of reference criteria for diagnostics

Future

- Increase the number of cases in the database
- Increase the number of pathologists involved in the intercalibration exercises and expand the number or the intercalibration exercises
- Use of digital scanned slides using Aperio technologies
- Application of specific histopathology techniques (IHC, ISH...) to improve evaluation and diagnostics
- Generation of reference material (website, CD's...)

FUTURE RING TESTS: BEHAVIOURAL ENRICHMENT OF THE PARTICIPANTS DURING THE TESTS

