Melanin - sequential experimental study of salmon

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The aim of this part of the project is to study the development of the pathology and the possible presence of agents, for the purpose to reveal the cause(s) of black spots

Material and sampling

0+ smolt transferred to SW in autumn 2013	1+ smolt transferred to SW in spring 2013
2013 July – FW before vacciantion	2013 Mars – FW before vaccination
2013 September – FW after vaccination	2013 May – FW after vaccination
2014 January – SW	2013 September - SW
2014 Mars - SW	2014 January - SW
2014 June – SW	2014 Mars - SW
	2014 June - SW
	2014 August – SW

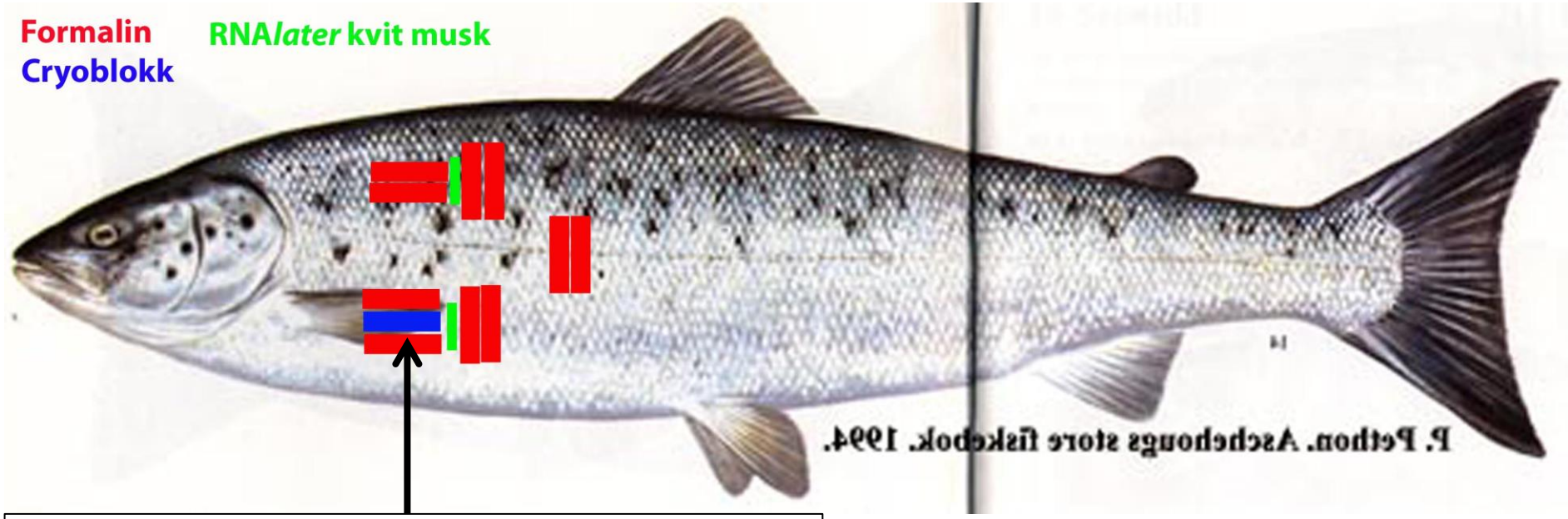
FW – Sunndalsøra, SW – Nofima / Marine Harvest Ekkilsøy/Averøy

Salmon at normal diet, vaccinated and unvaccinated

Predetermined sampling, according to protocol

Also internal organs

Additional sampling from melanin spots and other pathological changes



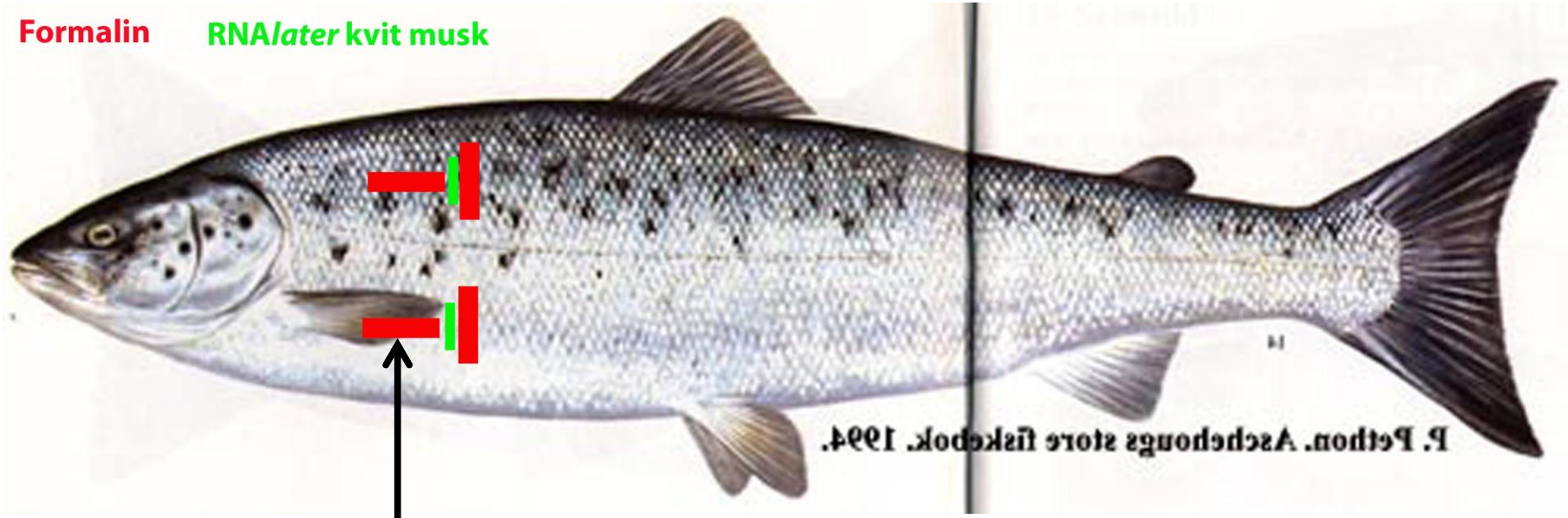
Histology
of muscle from the cranial abdominal wall

Salmon at diet supplemented with Zn, Cu or antioxidants, all vaccinated

Predetermined sampling, according to protocol

Also a few internal organs

Additional sampling from melanin spots and other pathological changes

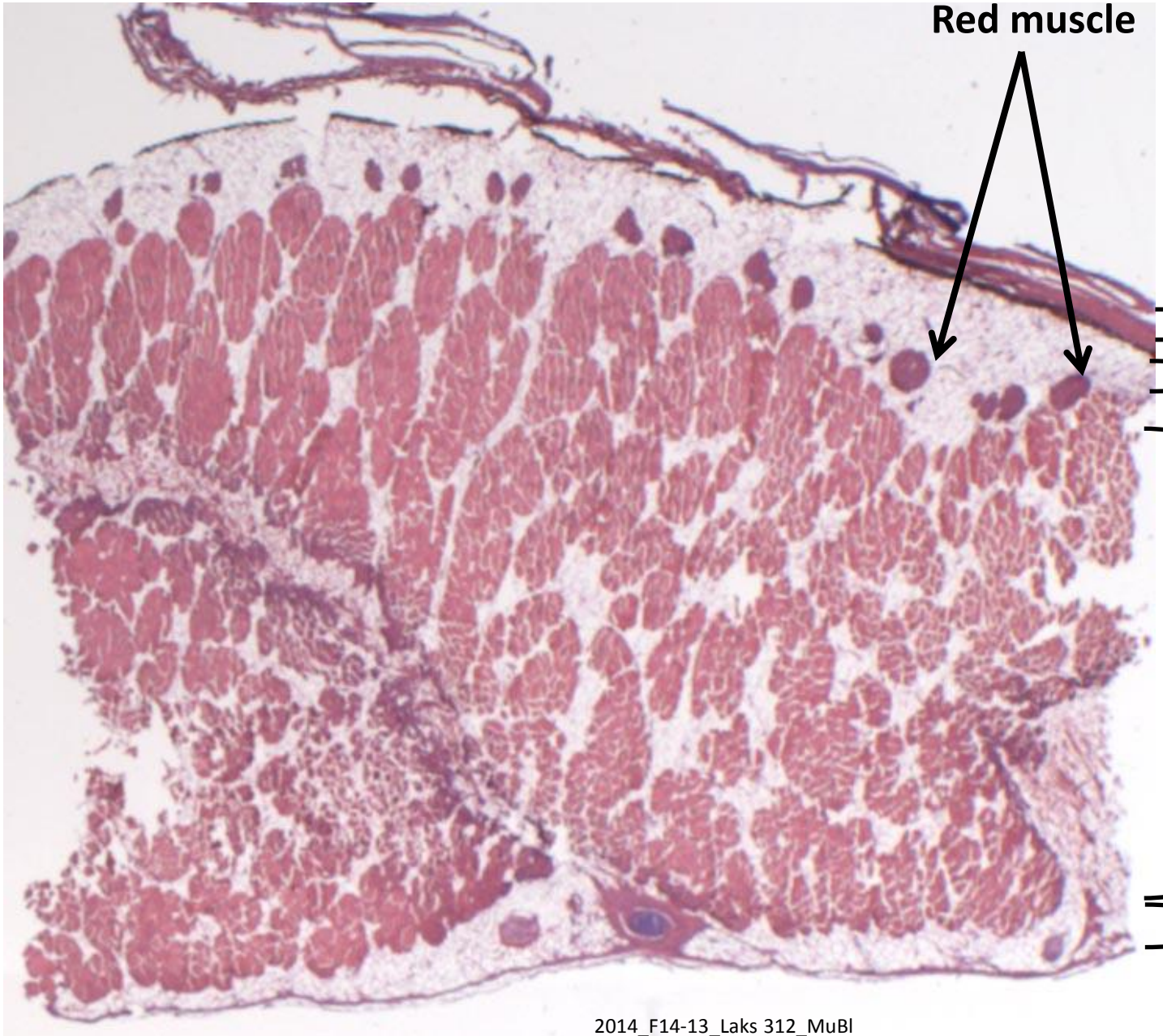


Histology
of muscle from the cranial abdominal wall

Histological examination

- **Longitudinal section which included all the layers of the cranial abdominal wall:**
  - **Skin**
  - **Subcutis (underhud, subcutaneous tissue, mainly fatty tissue)**
  - **Red muscle**
  - **White muscle**
  - **Fatty tissue**
  - **Connective tissue sheet (bindevevslag) containing the ribs**
  - **Peritoneum (bukhinne)**
- **Blood vessels, connective tissue, nerves and fatty tissue are present within all these layers, but in varying amounts**
- **The sections were stained with haemalum («haematoxylin») & eosin, and next evaluated by light microscopy**

A histological section showing the layers of the abdominal wall



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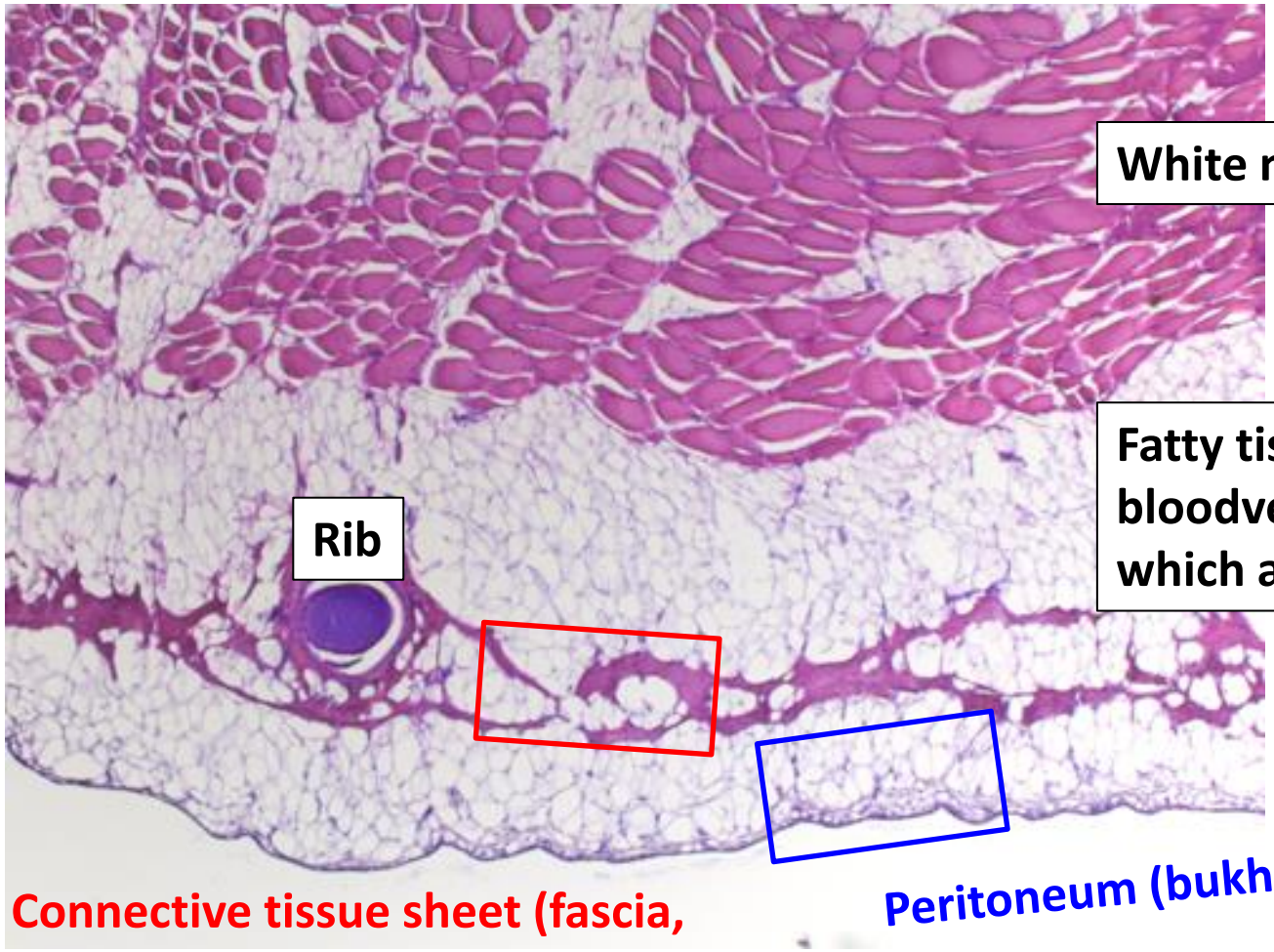
Details of the inner abdominal wall



Black spots in salmon fillet
From a farm with high prevalence and extensive changes

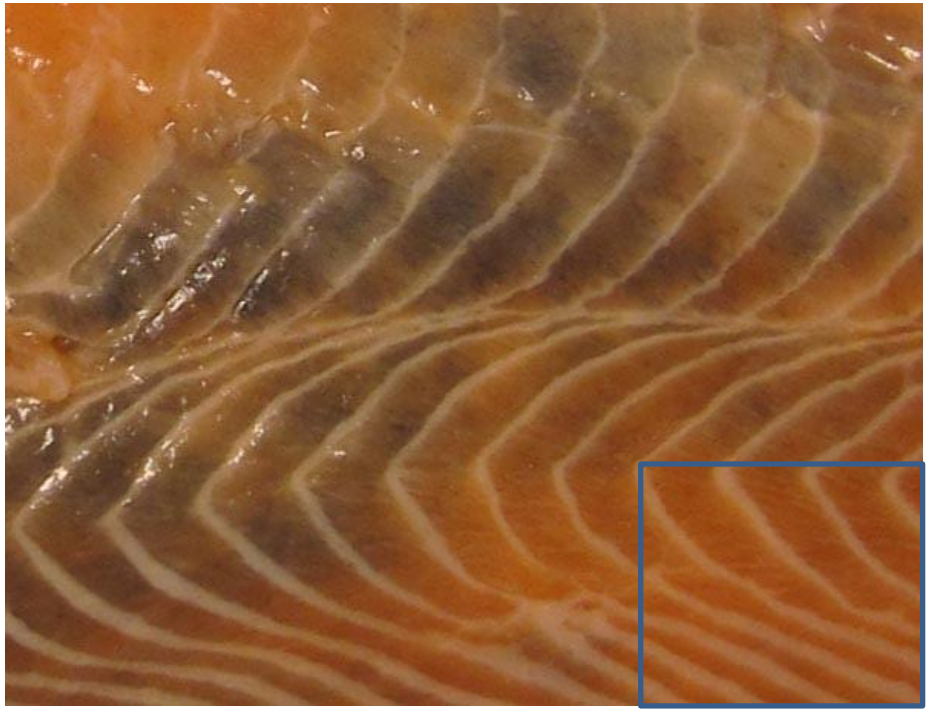


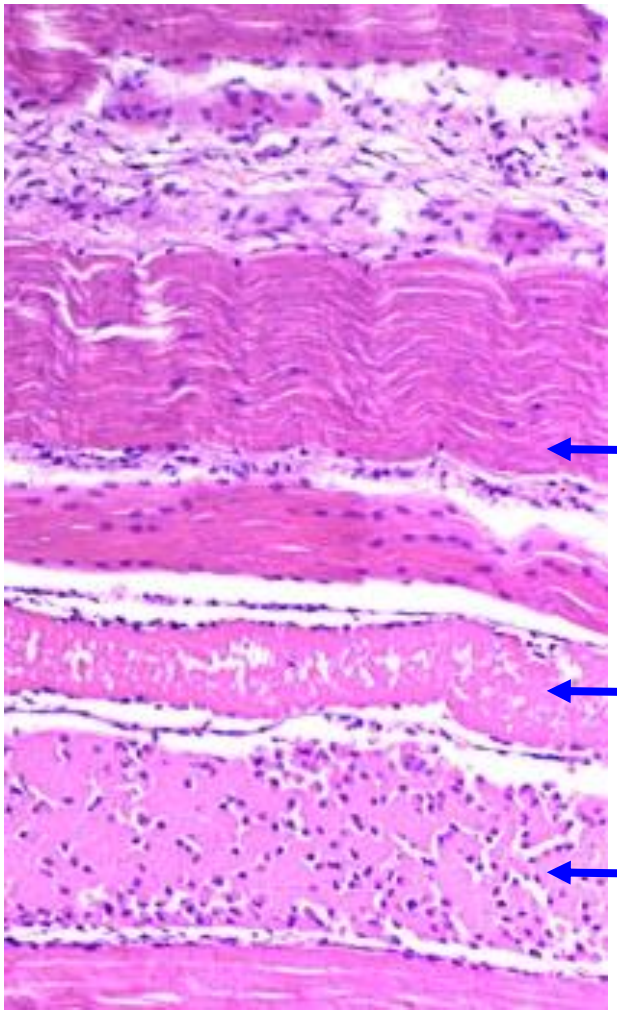
Photo: Local Fish Health Service

Detail from lower right corner showing more normal tisse, with the myosepta (arrows) and the myomeres between (double arrow in red)

Myosepta consist of connective tissue
Myomeres (myotomes) contain the muslce tissue between the myosepta

Photographs from a histological section through the black sport depicted in the previous slide

Numbers 1-3 indicate the sequence in which the different types of changes may occur



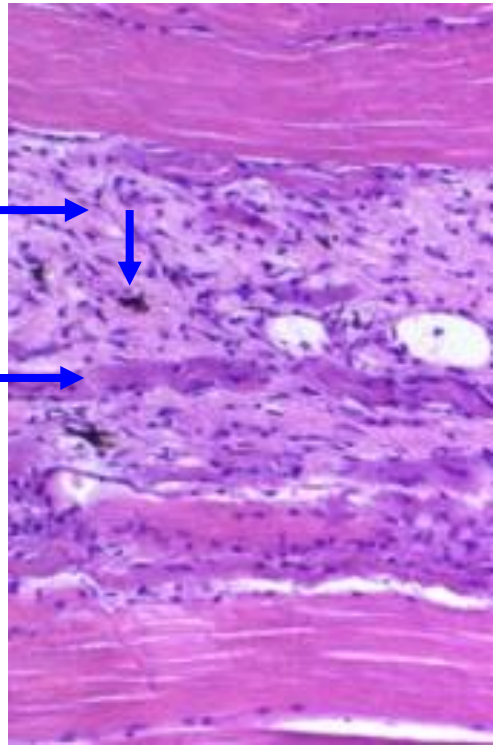
3) Inflammation of the interstitium (connective tissue between the sides of the muscle cells). Cells with melanin are present (vertical arrow)

3) Regeneration (formation of a new muscle cell)

Normal muscle cell

1) Degeneration (death of a muscle cell)

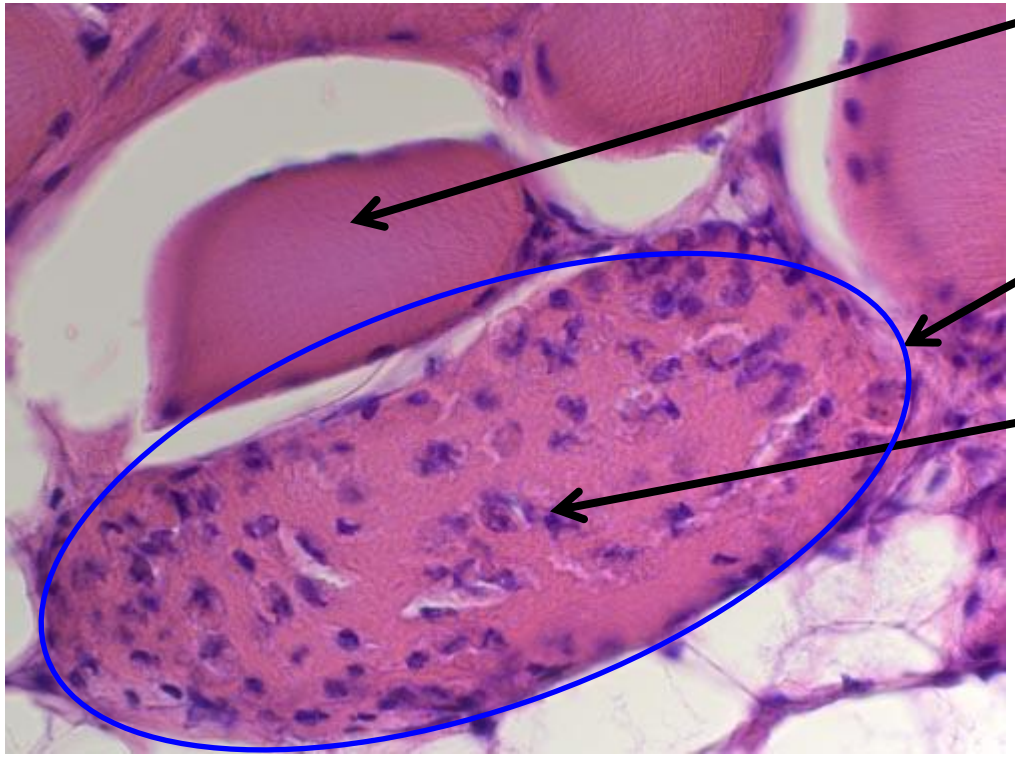
2) Inflammation: Leukocytes remove the remnants of a dead muscle cell



Degeneration & chronic inflammation with melanin-containing cells

Pathology in white muscle of the cranial belly

Among changes observed were dead muscle cells, in which leukocytes (macrophages) were present and cleaned up (i.e. inflammation)
Very few such altered muscle cells were present where this type of change was observed



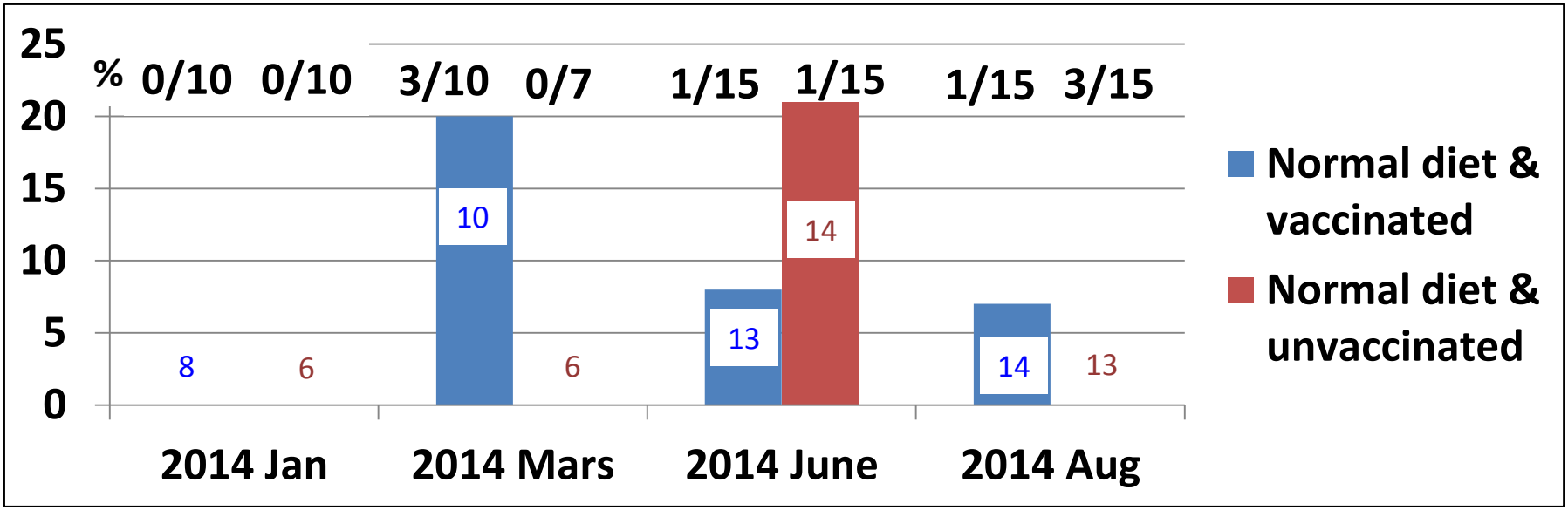
Normal muscle cell in cross section

Degenerated (dead) and inflamed (betent) muscle cell

Macrophages cleaning up (i.e. eating the remnants of the cell)

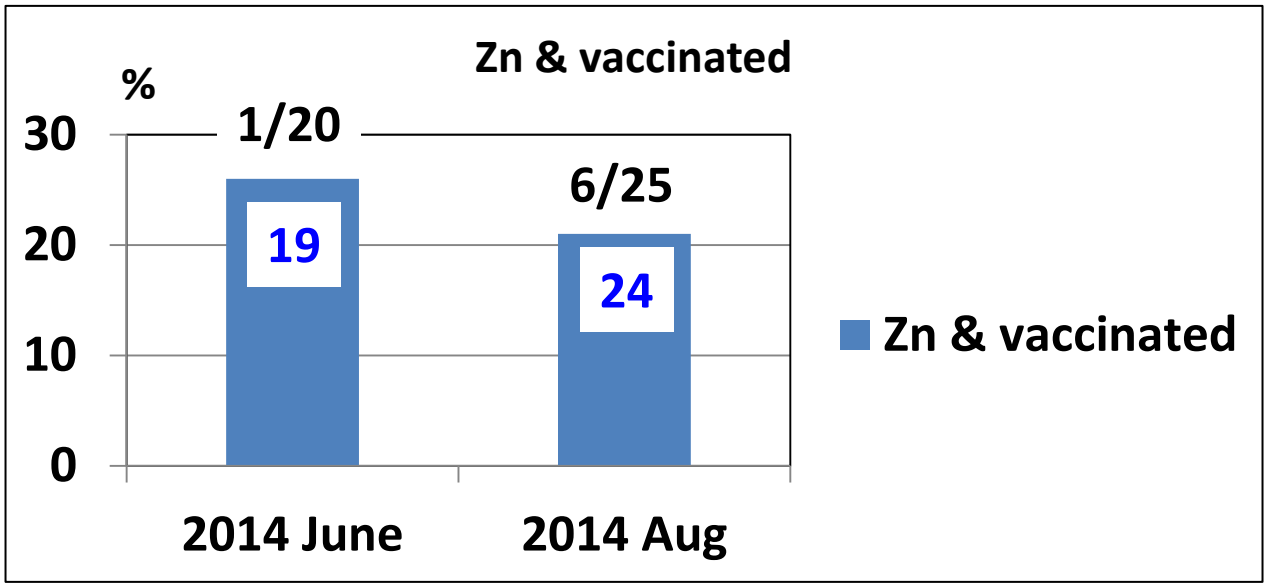
The following slides present the percentage of random sampled fish (1+ smolt in 2014) in which this type of change was observed.
This material will be used in further studies

Pathology in muscle
- salmon fed the normal (standard) diet



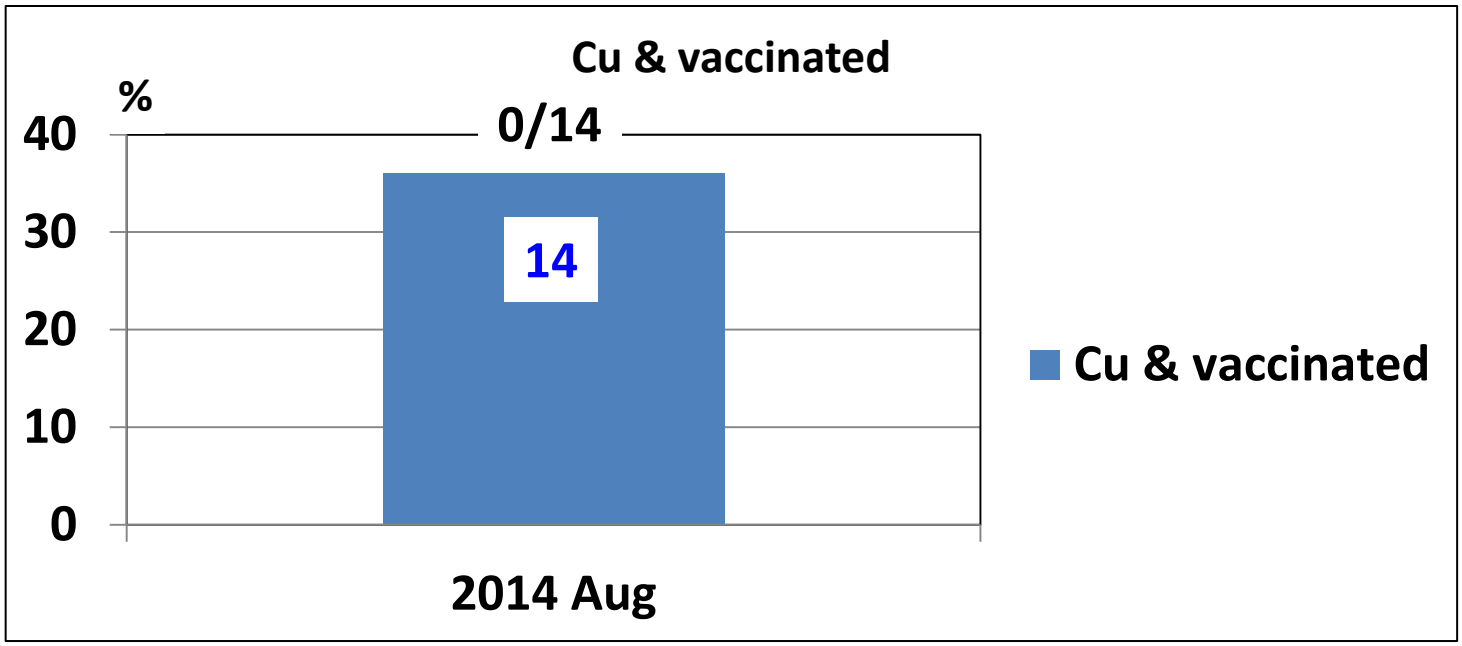
- Fraction of examined fish with melanin spots anywhere in muscle (in black in the uppermost row)
  - Spots were observed in 9 of 91 fish (vaccinated + unvaccinated)
- Percentage of fish (vertical axis) with the described type of change observed in histological sections of the cranial abdominal wall
  - Numbers of fish examined in blue from the vaccinated group and in brown from the unvaccinated group
  - The pathology was observed in 7 of 84 fish (vaccinated + unvaccinated) examined by histology

Pathology in muscle
- salmon fed the Zn supplemented diet



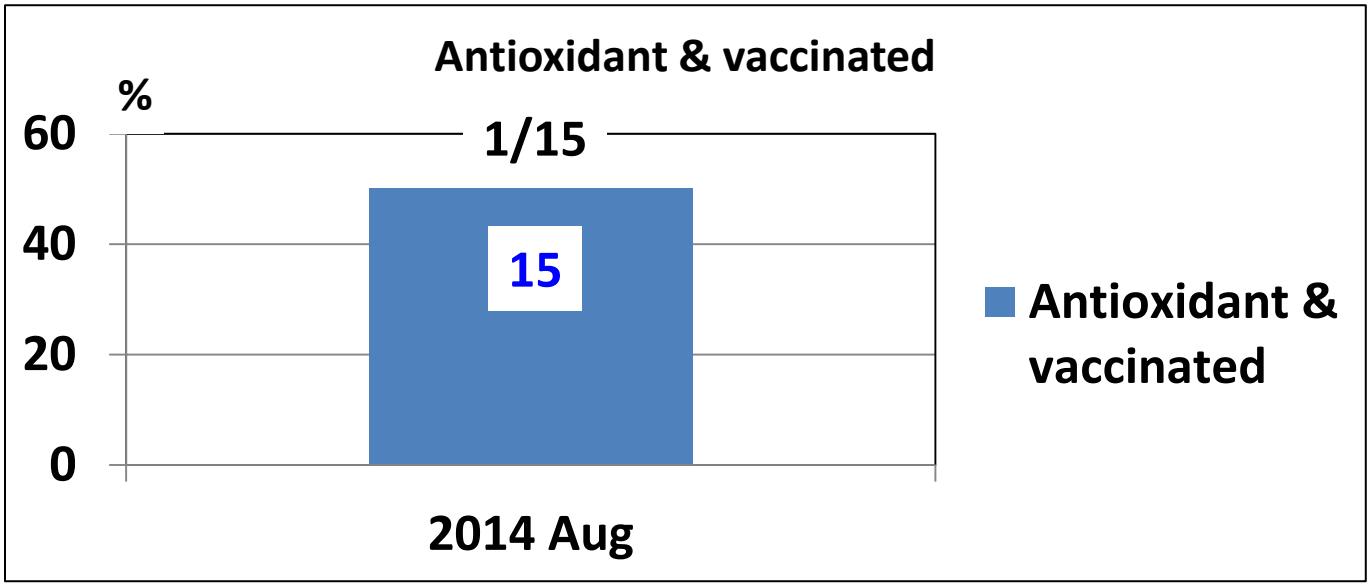
- **Fraction of examined fish with melanin spots anywhere in muscle (in black in the uppermost row)**
- **Percentage of fish (vertical axis) with the described type of change observed in histological sections of the cranial abdominal wall (numbers of fish examined in blue)**

Pathology in muscle
- salmon fed the Cu supplemented diet



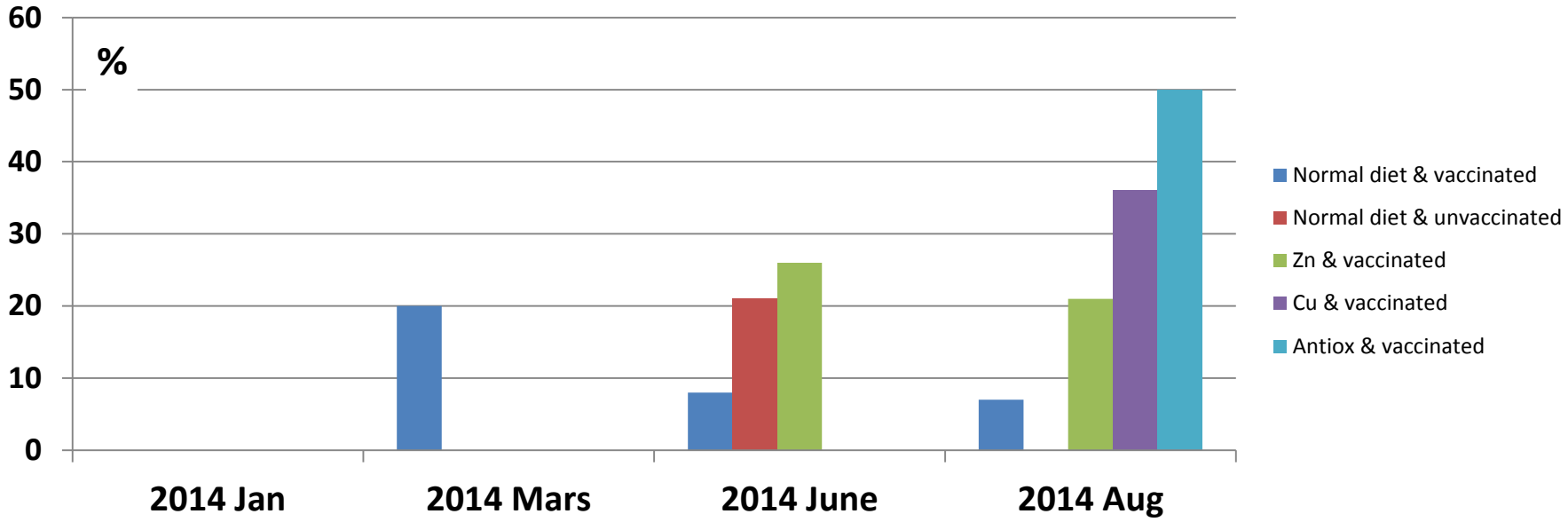
- **Fraction of examined fish with melanin spots anywhere in muscle (in black in the uppermost row)**
- **Percentage of fish (vertical axis) with the described type of change observed in histological sections of the cranial abdominal wall (numbers of fish examined in blue)**

Pathology in muscle
- salmon fed the antioxidant supplemented diet



- **Fraction of examined fish with melanin spots anywhere in muscle (in black in the uppermost row)**
- **Percentage of fish (vertical axis) with the described type of change observed in histological sections of the cranial abdominal wall (numbers of fish examined in blue)**

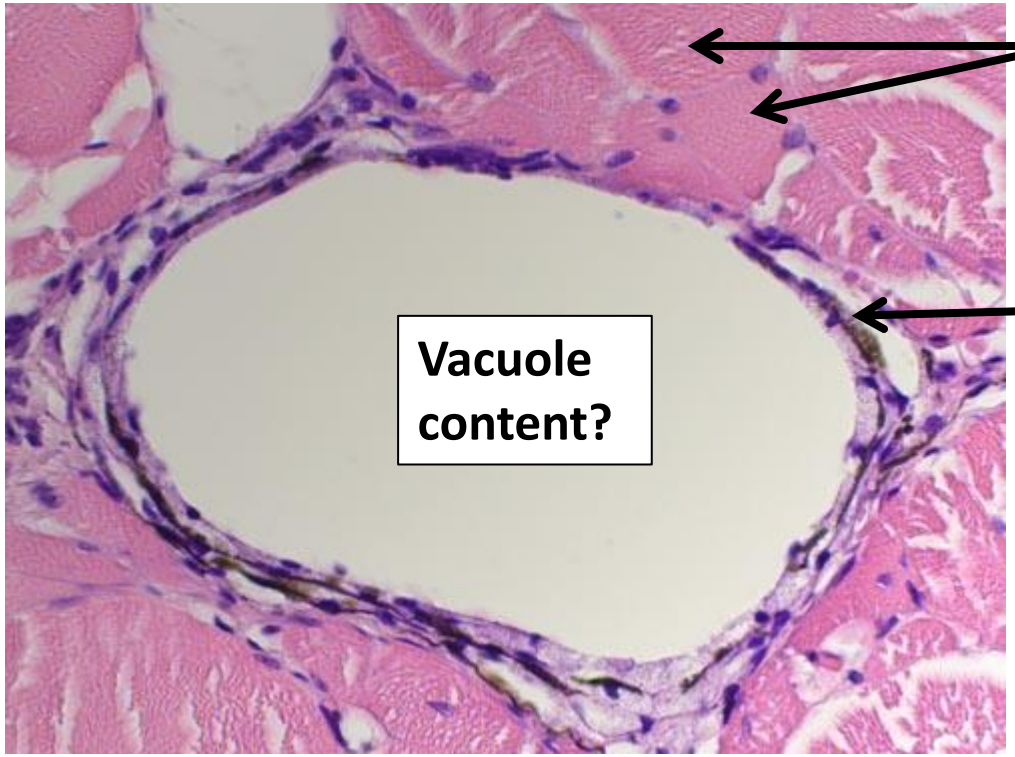
Pathology - summary



- **Melanin spots were in total observed in 24 of 174 salmon**
- **The pathology in the cranial abdominal wall was in total observed in 29 of 163 salmon (figure)**
  - **If the prevalence and diets/vaccination are**
    - **unassociated; the figures indicate an increase from January until August ?**
    - **associated; the figures indicate that increased Zn, Cu and antioxidants may provoke the pathology ?**

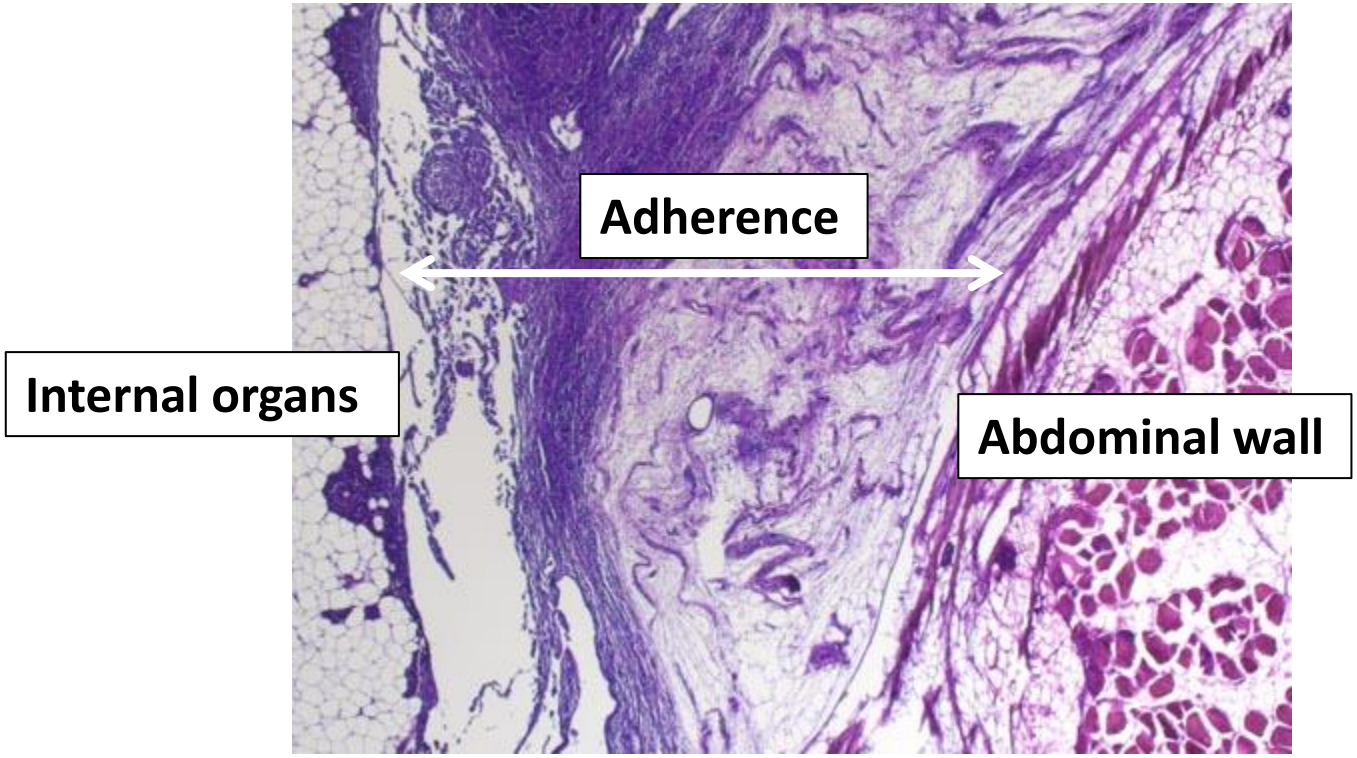
Melanin spot in the abdominal belly

Histology



Melanin-containing cells & inflammatory cells were additionally observed at several sites in the tissue

Adherence (samanveksing) between internal organs and the abdominal wall (bukveggen)



2014 June: Few degenerated white muscle cells in the adjacent abdomianl wall

2014 August: Not found

Conclusion

Pathology has been detected

1) as slight changes in the cranial abdominal wall

It is at present unknown if these represent initial steps in the development of black spots

2) as black spots in different parts of the fillet but mainly the abdominal wall

It is unknown where the pathology starts

Alternatives are muscle, connective tissue, peritoneum, vessels, fatty tissue, nerves, ribs

Internal organs?

Further work

Histologi (and electron microscopy)

- **Routine sections**
- **Special staining methods for specific tissue structures and microorganisms**

Molecular methods

- **Area of the cranial abdominal wall**
- **Melanin spots anywhere in muscle**
- **Internal organs?**