Melanin and melanisation

Erling Olaf Koppang

Norges veterinærhøgskole

Oslo

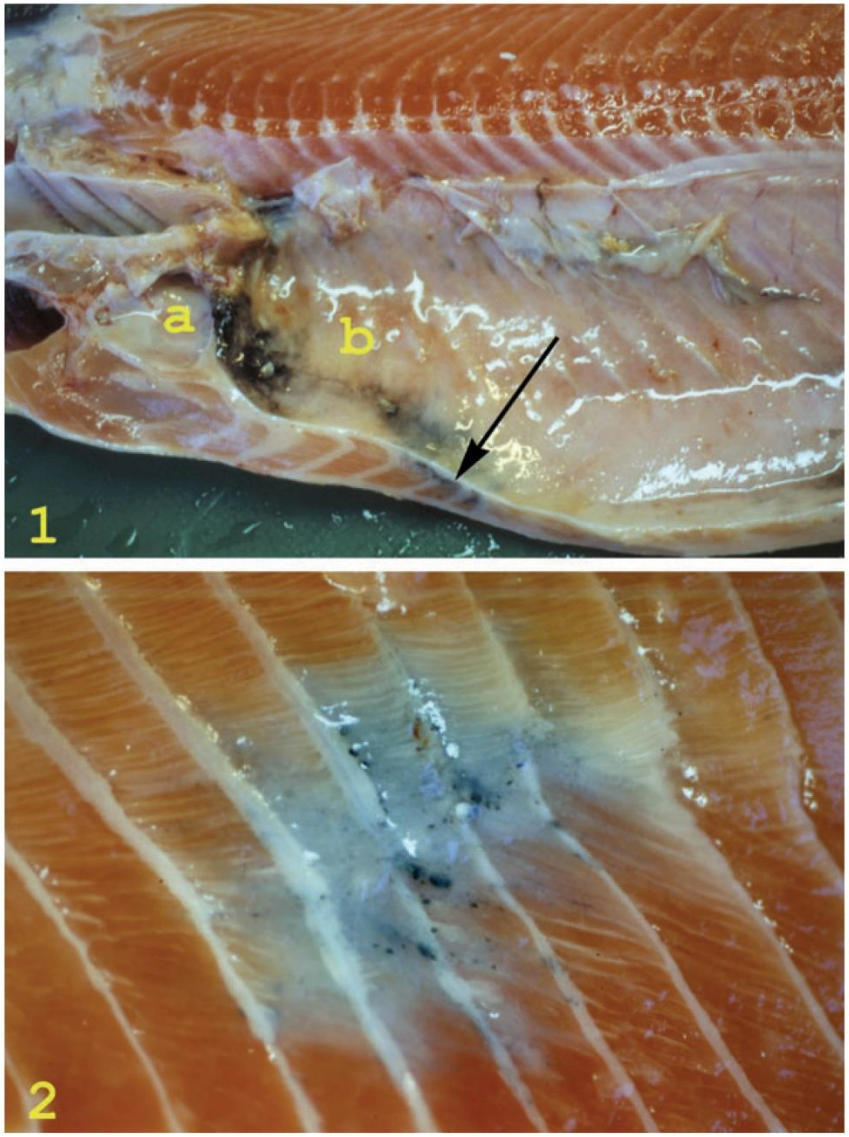
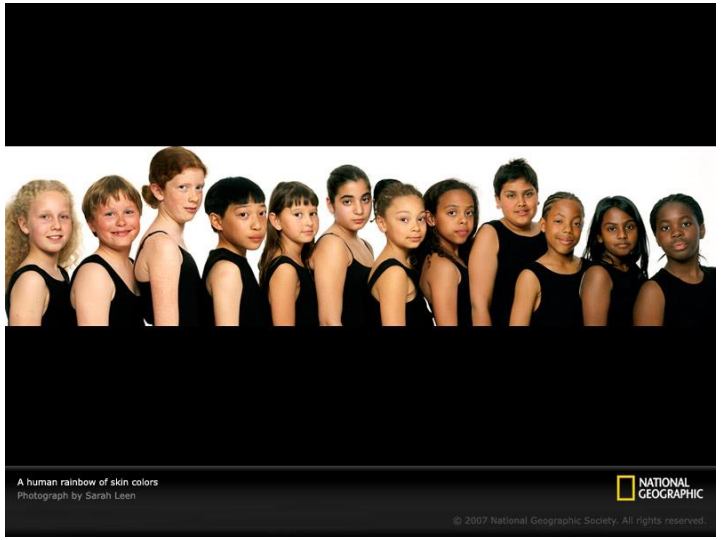


Figure 1 Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).
Figure 2 A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular septa.


A human rainbow of skin colors
Photograph by Sarah Leen
NATIONAL GEOGRAPHIC
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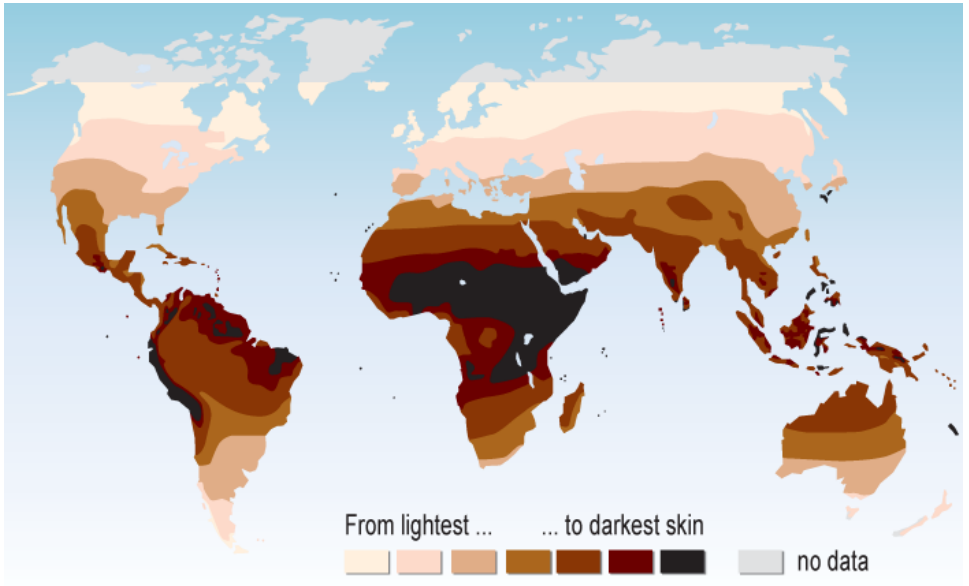
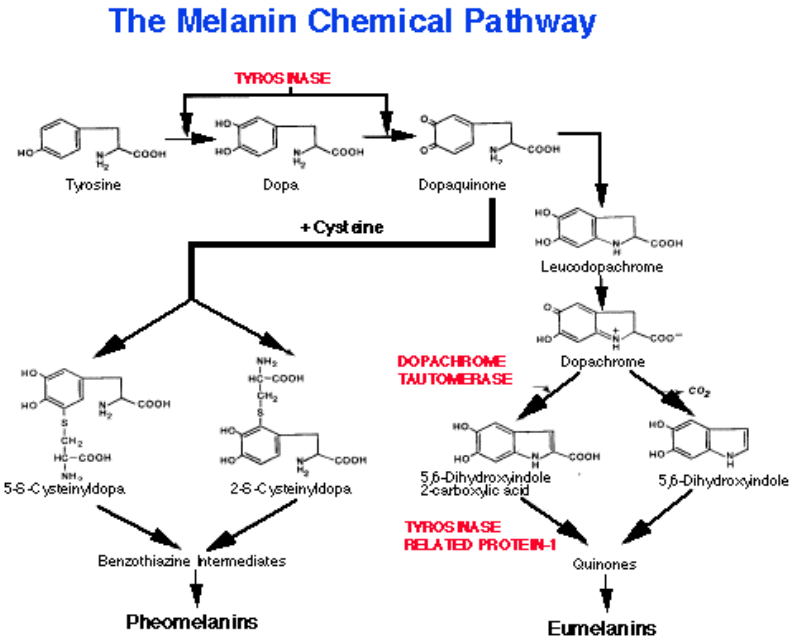
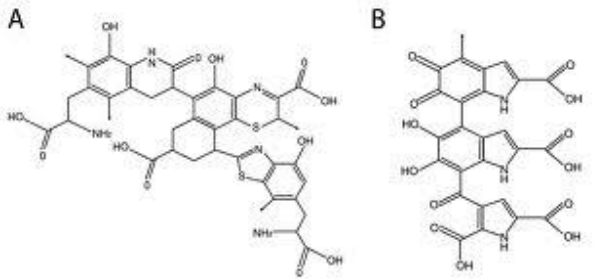
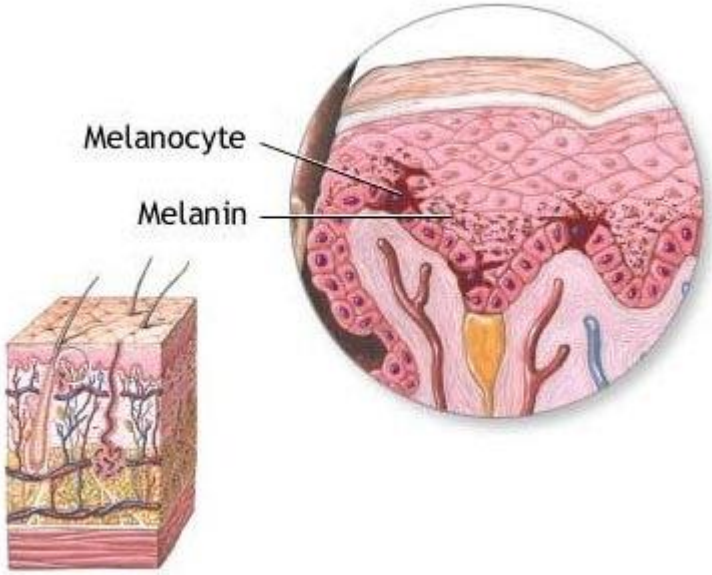

From lightest ...
... to darkest skin
no data

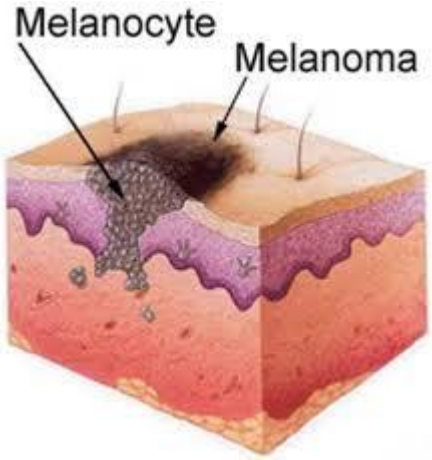
What is melanin?

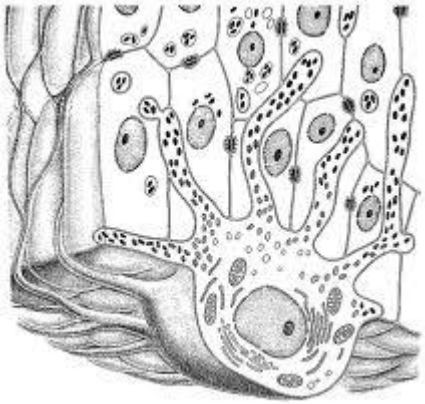




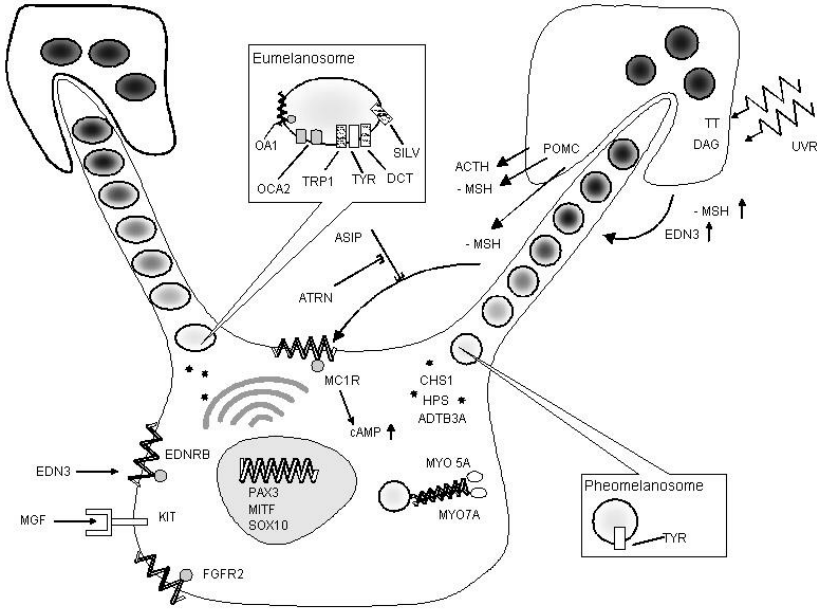
Where is melanin formed?

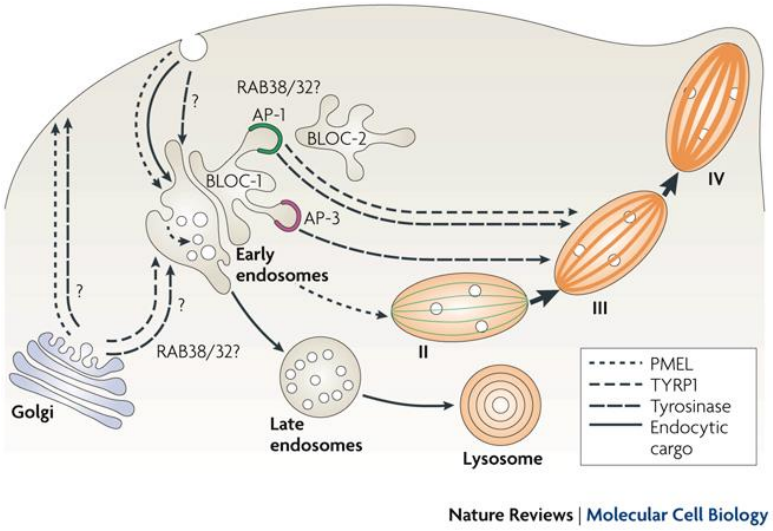






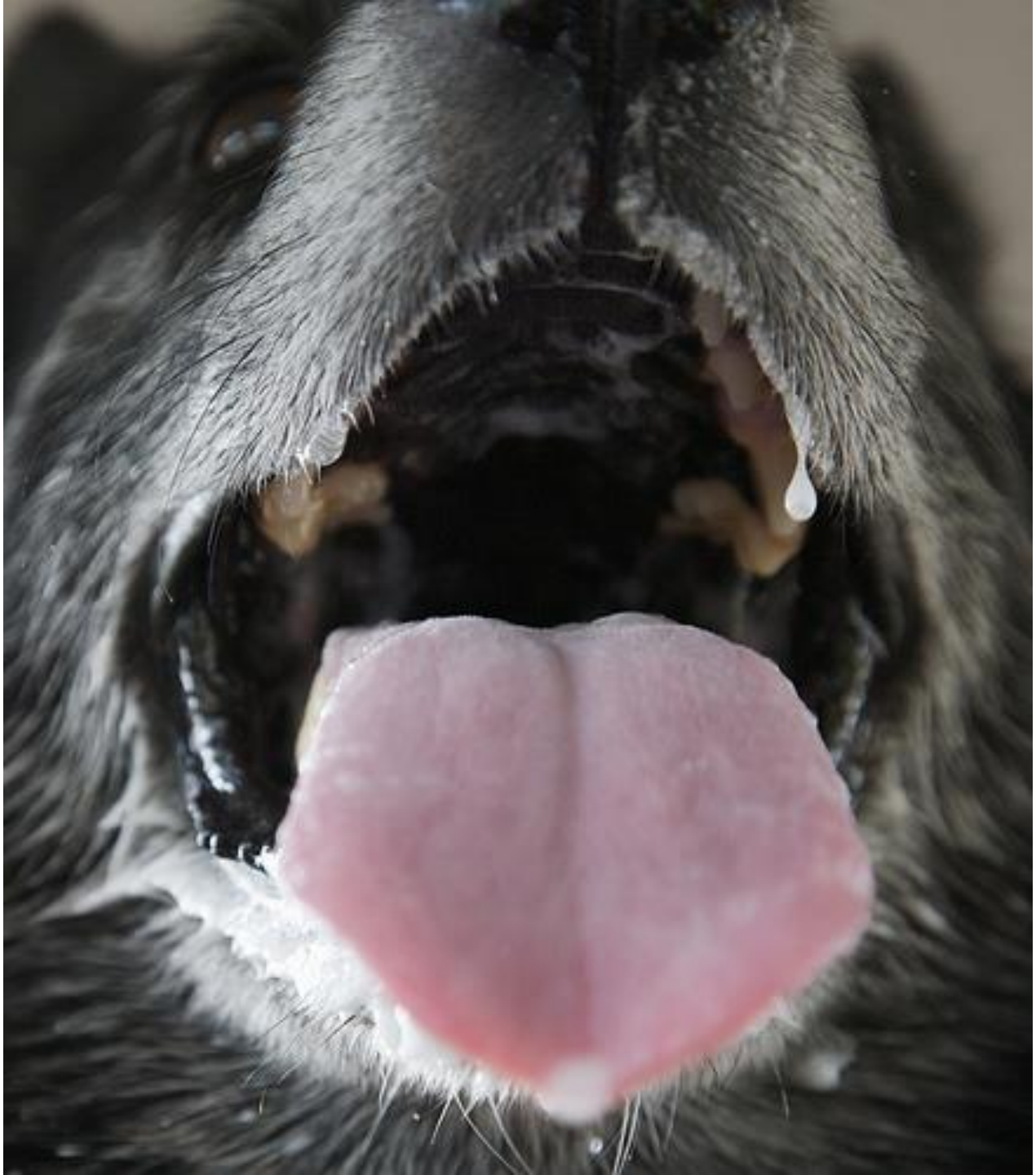
Melanosome: the intracelluar melanin producer





What are the funcions of melanin?

Burkhart & Burkhart. Int. J. Dermatol. 2005:44;340



Giovanni Sichel – Universitetet i Catania


Lung
Liver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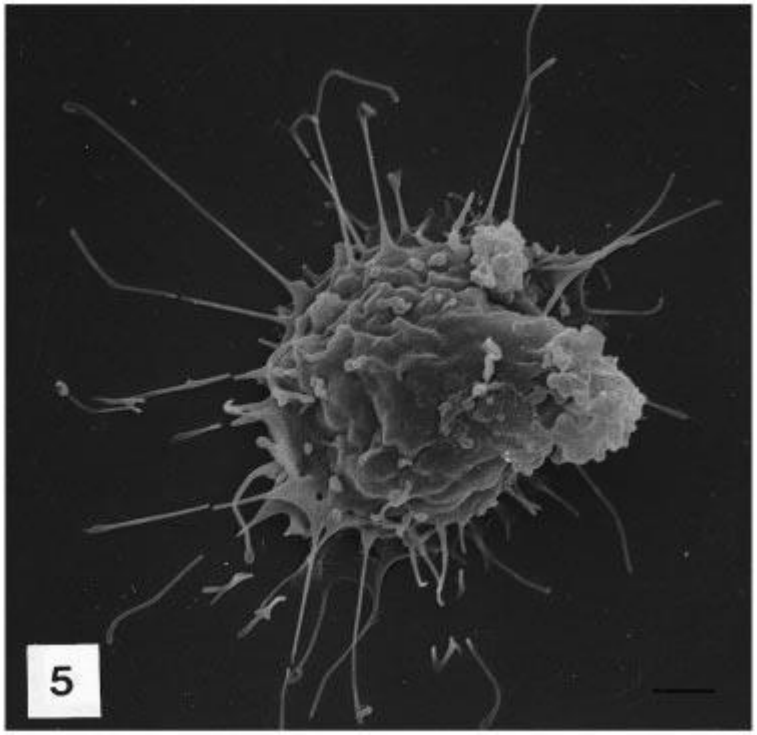
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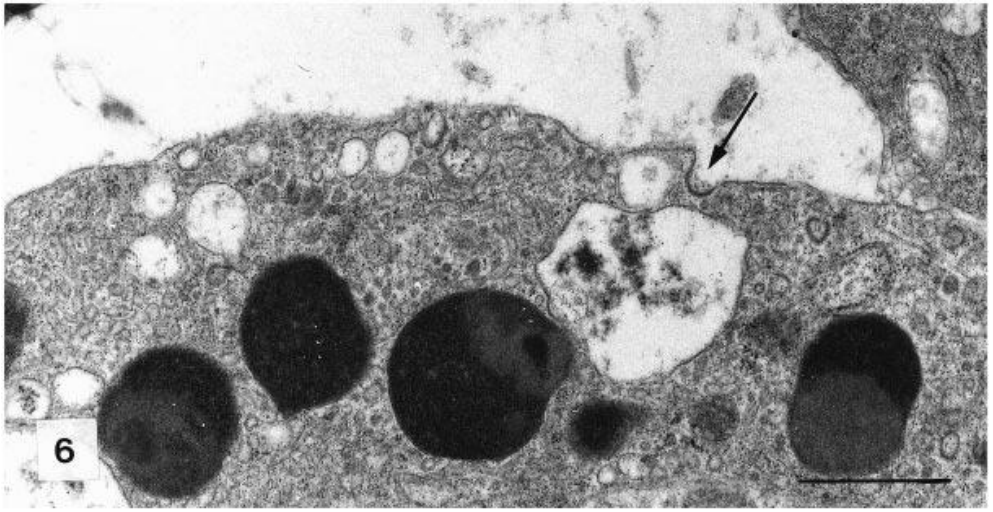
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Giovanni Sichel and co-workers:

- Melanin poduction in vertebrates is not exclusively confined to cells originating from the ectoderm.
- Kupffer cells in amphibian liver my synthesise melanin
- Kupffer cells derive from mesenchyme
- Conclusion: Populations of immune cells may synthesise melanin (contrast to mammals)

Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle

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2 Department of Fisheries and Marine Biology, HiB, University of Bergen, Norway

Abstract

The purpose of this study was to investigate the nature of variably sized pigmented foci encountered in fillets of farmed Atlantic salmon, *Salmo salar* L. The material was sampled on the fillet production line and on salmon farms from fish with an average size of 3 kg from various producers. The fish had been routinely vaccinated by injection. Gross pathology, histology, immunohistochemistry using antisera against major histocompatibility complex (MHC) class II β chain and transmission electron microscopy (TEM) were used to characterize the changes. Macroscopically, melanized foci were seen penetrating from the peritoneum deep into the abdominal wall, sometimes right through to the skin, and also embedded in the caudal musculature. Histological investigation revealed muscle degeneration and necrosis, fibrosis and granulomatous inflammation containing varying numbers of melano-macrophages. Vacuoles, either empty or containing heterogeneous material, were frequently seen. The presence of abundant MHC class II^+ cells indicated an active inflammatory condition. TEM showed large extracellular vacuoles and leucocytes containing homogeneous material of lipid-like appearance. The results showed that the melanized foci in Atlantic salmon fillet resulted from an inflammatory condition probably induced by vaccination. The described condition is not known in wild salmon and in farmed salmon where injection vaccination is not applied.

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Keywords: Atlantic salmon, inflammation, melano-macrophage, major histocompatibility complex class II, mineral oil, vaccine.

Introduction

Various pathological conditions may be associated with abnormal pigmentation in tissues and organs. Such pigments may either be of exogenous or endogenous origin. Endogenous pigments include derivates of lipids, haemoglobin, porphyrins and melanin. The term melanosis is used to describe the presence of melanin in abnormal locations (Thomson 1984). In vertebrates, melanin is synthesized by melanocytes and organized in melanosomes, which are lysosome-related intracellular organelles (Orlow 1995; Raposo, Fevrier, Stoorvogel & Marks 2002). Mammalian melanocytes originate from the embryonic neural tube (Sulaimon & Kitchell 2003) and it has been observed that such cells can migrate into inflamed tissue (Thomson 1984).

Inflammatory reactions and tissue regeneration in salmonids seem similar to those of mammals (Finn & Nielson 1971), but have in addition been associated with the involvement of so-called melano-macrophages (Roberts 1975; Agius & Roberts 2003). The origin of melanosomes in melanin-containing viscerally located cells in fish is not clear (Agius & Roberts 2003), but Sichel, Scalia, Mondio & Corsaro (1997) suggested that melanogenesis in poikilothermic vertebrates may occur in mesenchyme-derived cells of the haematopoietic lineage. Although teleost melano-macrophages have been ascribed macrophage-like properties, their functions and significance are

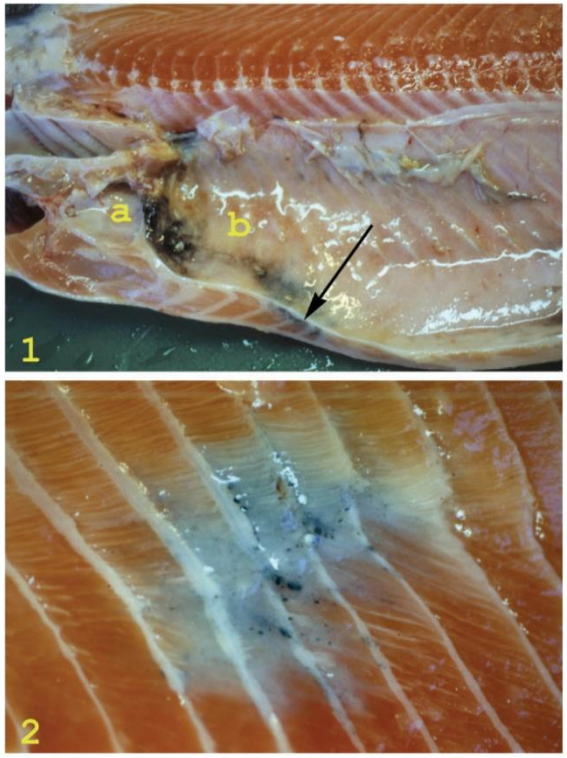


Figure 1 Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).
Figure 2 A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular septa.

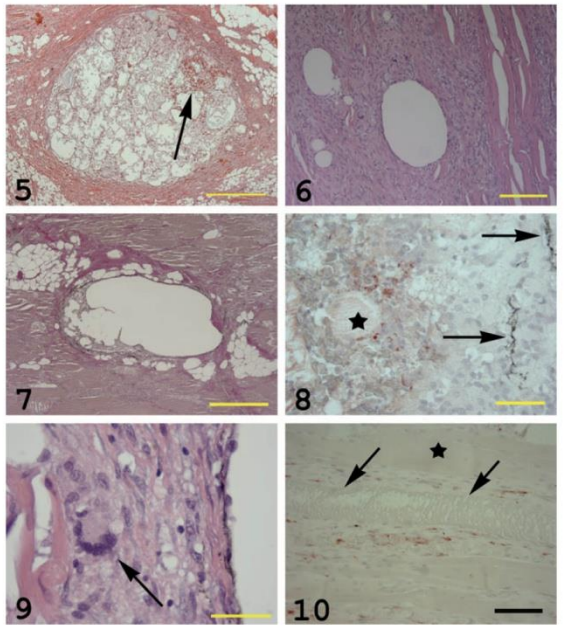
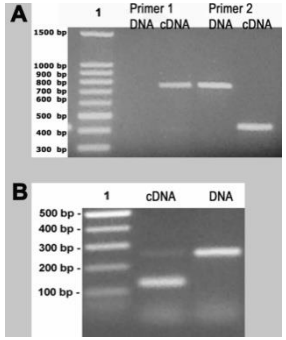


Figure 5 A large vesicle embedded in an intermyotomal septum containing macrophage-like cells, debris and a fresh haemorrhage (arrow) (H&E, bar = 500 μm).
Figure 6 Empty vesicles surrounded by granulomatous tissue embedded in the white musculature. Note adjacent, seemingly unaffected muscle cells (H&E, bar = 200 μm).
Figure 7 Vesicles embedded in the white musculature surrounded by fibrogranulomatous tissue (red staining) (EVG, bar = 500 μm).
Figure 8 Reaction against oil (red staining) in a vesicle as shown in Fig. 5. Homogeneous masses (asterisk) and macrophage-like cells show positive reactions. Note the melano-macrophages in the vesicle wall (arrows) (oil red O, bar = 50 μm).
Figure 9 High magnification of the wall of a vesicle as seen in Fig. 6. The wall contains a multinucleated giant cell (MGC)(arrow), epithelioid-like cells, small vacuoles and is lined towards the lumen of the greater vesicle with melanosome-containing cells, probably swollen melano-macrophages (H&E, bar = 40 μm).
Figure 10 Muscle cells infiltrated with MHC class II^+ cells. One muscle cell is unaffected (asterisk). One fibre shows severe degeneration (arrowhead), whereas one is invaded by MHC class II^+ cells (red reaction) (MHC class II immunostain, haematoxylin counterstain, bar = 100 μm).

- Primer pairs recognising a fish CD83 homolog



doi: 10.1111/j.1600-0749.2006.00297.x

Melanogenesis and evidence for melanosome transport to the plasma membrane in a CD83^{+} teleost leukocyte cell line

Erlend Haugarvoll[1]*, Jim Thorsen[1], Morten Laane[2], Qirong Huang[1] and Erling Olaf Koppang[1]

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Key words: CD83/dendritic cell/endosomal pathway/macrophage/melanogenesis/melanomacrophage/teleost

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Summary

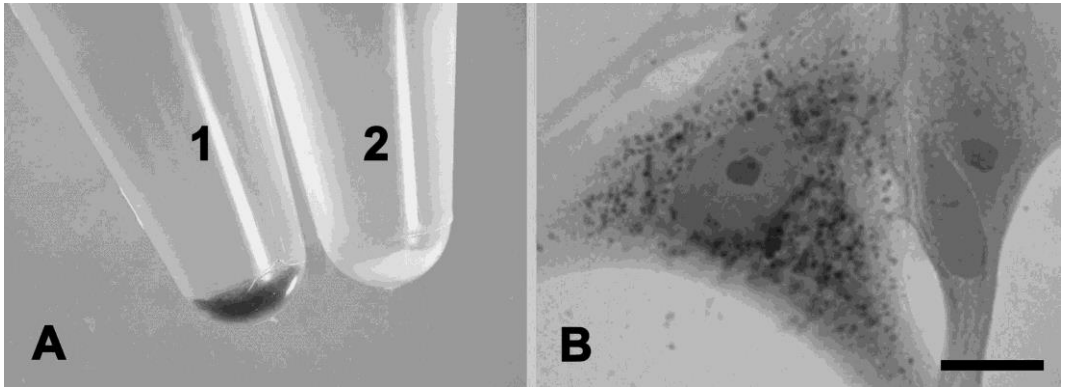
Introduction

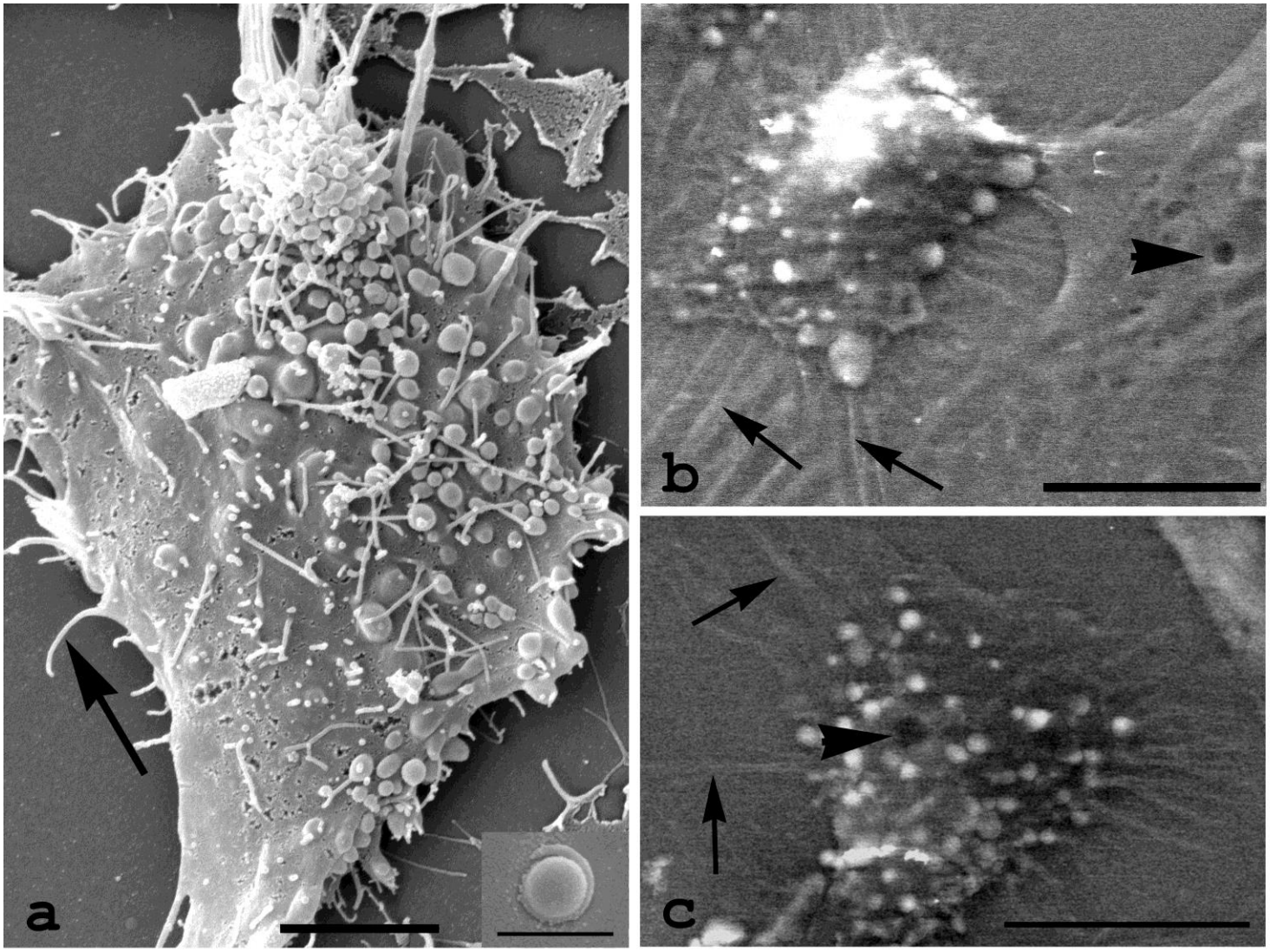
Melanins are complex polymeric pigments, which are formed by a wide variety of living organisms ranging from fungi and bacteria to higher vertebrates (Margalith, 1992; Orlow, 1995; Raposo et al., 2002). Common for

- Primer pair detecting Dct/TRP-2

- A; 0,1 mM PTU inhibit tyrosinase dopachrome production from L-DOPA

- B; a few long cultured cells showed melanin reduction potential

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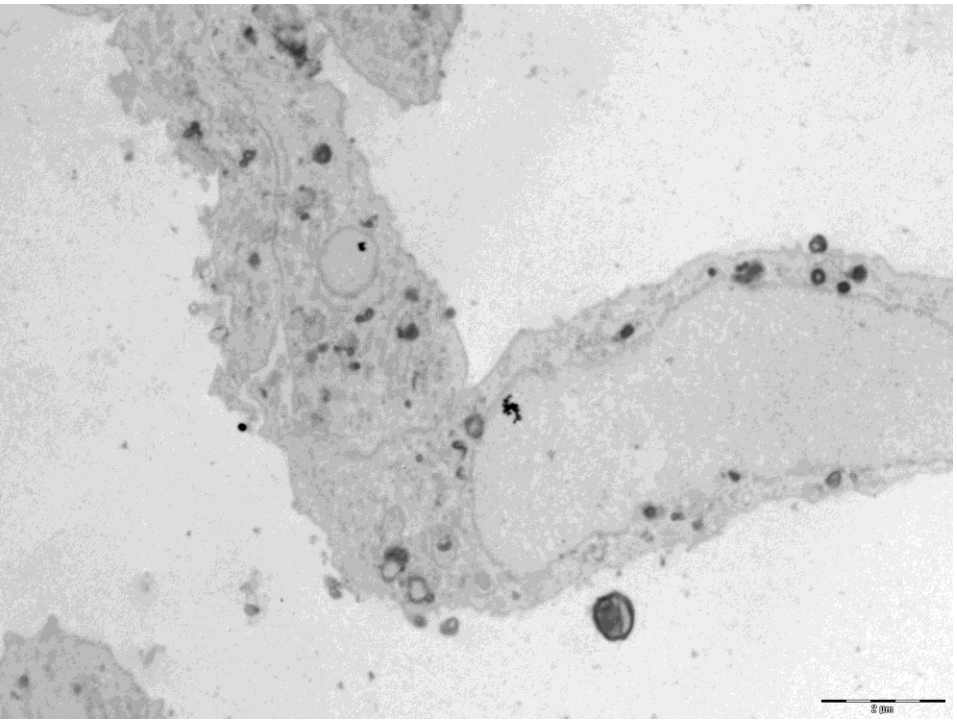
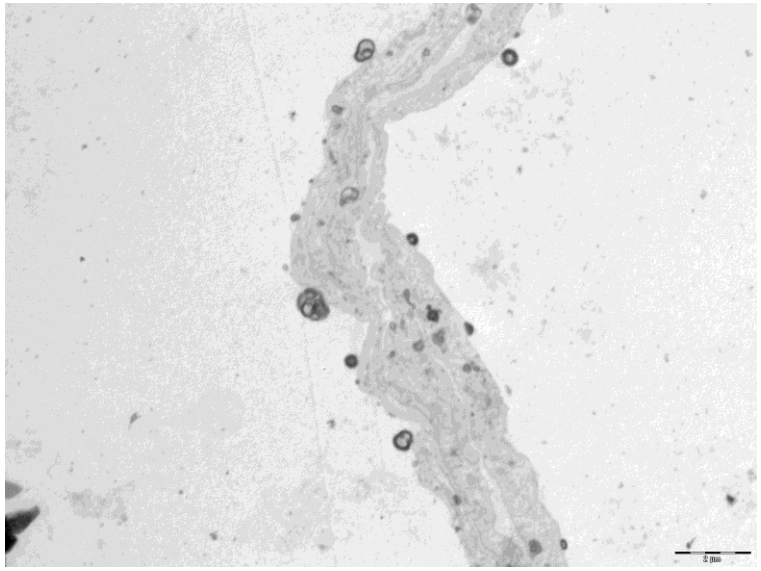


Haugarvoll et al. Pigment Cell Res 2006;19:214-225

Thorsen et al. Pigment cell Res

2006;19:327-336



doi: 10.1111/j.1600-0749.2006.00319.x

Isolation of the Atlantic salmon tyrosinase gene family reveals heterogenous transcripts in a leukocyte cell line

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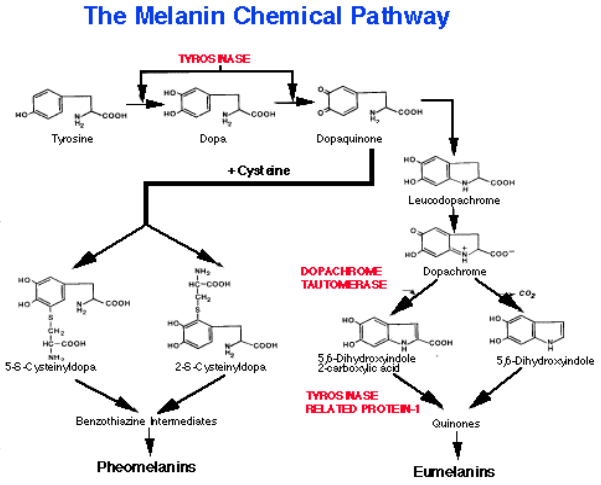
Summary

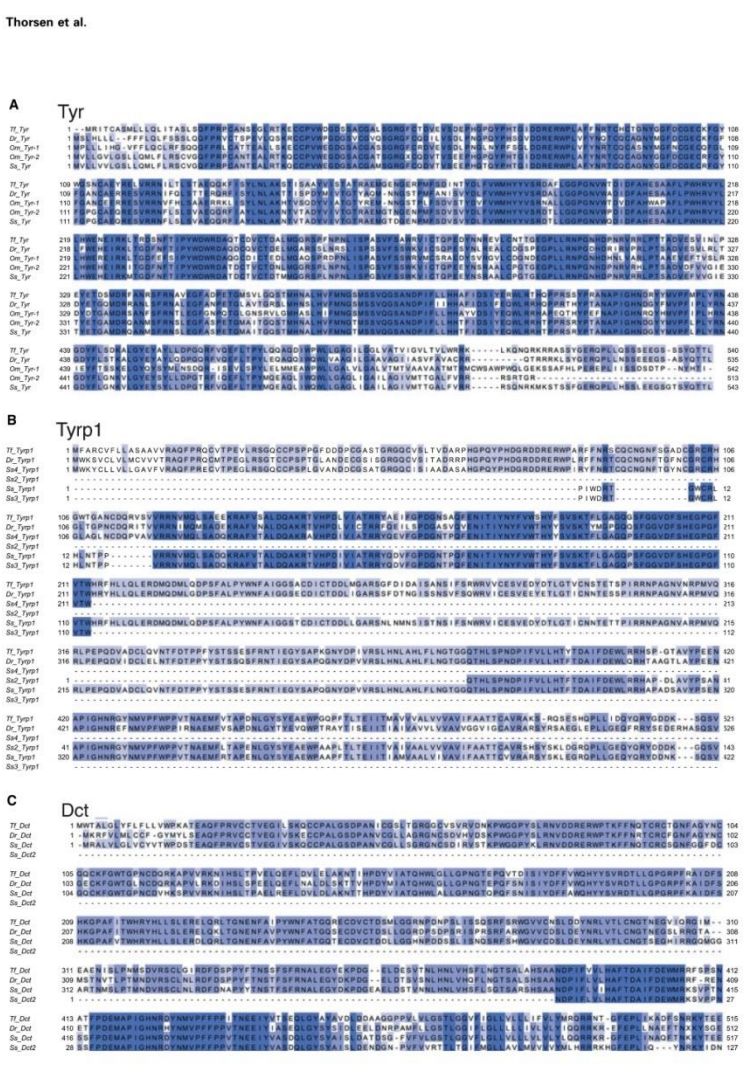
In ectothermic vertebrates, visceral organs harbor melanin-containing cells. Their ability as pigment producers is nevertheless disputed. To address expression of the key genes for melanogenesis in Atlantic salmon (*Salmo salar*), a tyrosinase-positive leukocyte cell line (SHK-1) and skin were used to obtain full-length tyrosinase (Tyr), tyrosinase-like protein-1 (Tyrp1), and dopachrome tautomerase (Dct) mRNA transcripts. In the SHK-1 cells, two different Tyrp1 transcripts were identified, one lacking exon 1. However, only the full-length version of Tyrp1 was identified in the skin. Sequen-

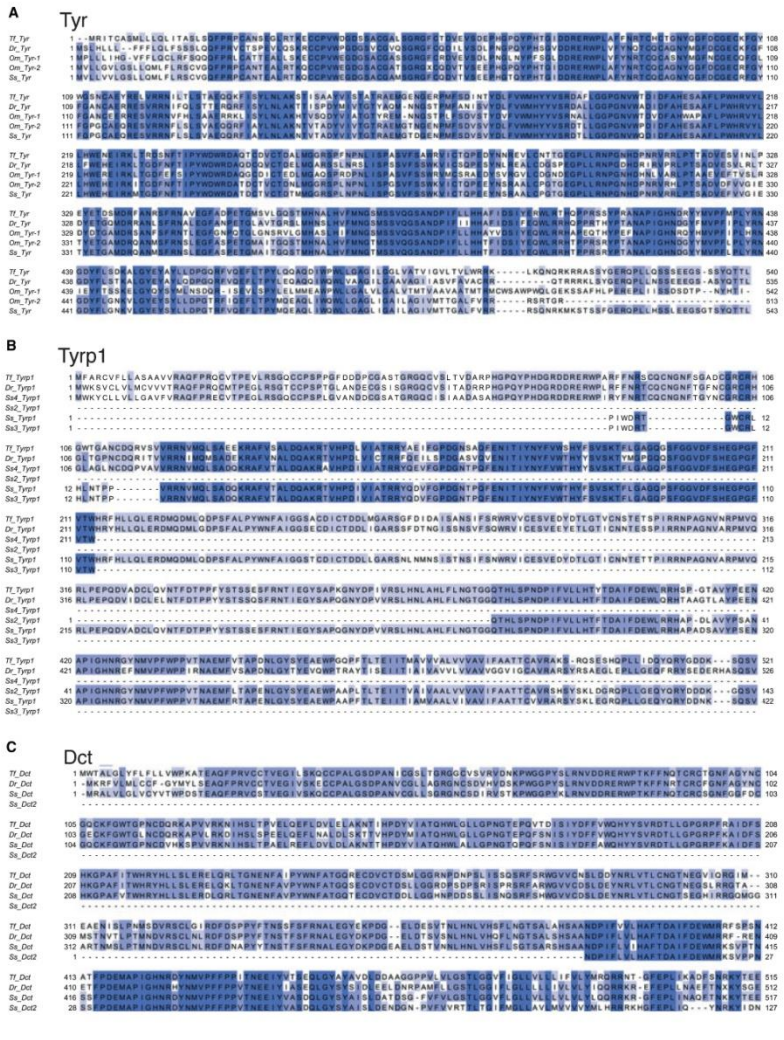
Introduction

The pigmentary system of vertebrates has predominantly been addressed in cells derived from the embryonic neural tube ectoderm (Boissy and Nordlund, 1997). Pigmented cells or melanocytes in the normal adult mammal occur in the skin, uvea, retina, meninges, the inner ear, and the Harderian gland (Boissy, 1998). Such cells are characterized by their ability to synthesize melanin, a process confined within discrete organelles termed melanosomes. Melanosomes share several properties with lysosomes (Orlow, 1995; Raposo et al., 2002), and any melanocyte precursor is defined as a melanoblast (Fitzpatrick et al., 1966). The functions of melanin are not only restricted to absorb, scatter and reflect light, but also include binding of metal ions and organic cations, and acting as antioxidants and scavengers of free reactive radicals (Margalith, 1992; Sarna and Swartz, 1998). Interestingly, increasing information link the functions of the pigmentary and immune systems (Mackintosh, 2001; Raposo et al., 2002).

The principal genes encoding the enzymes involved in



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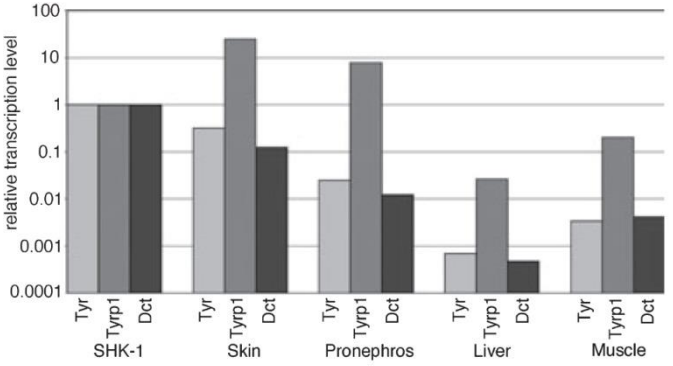
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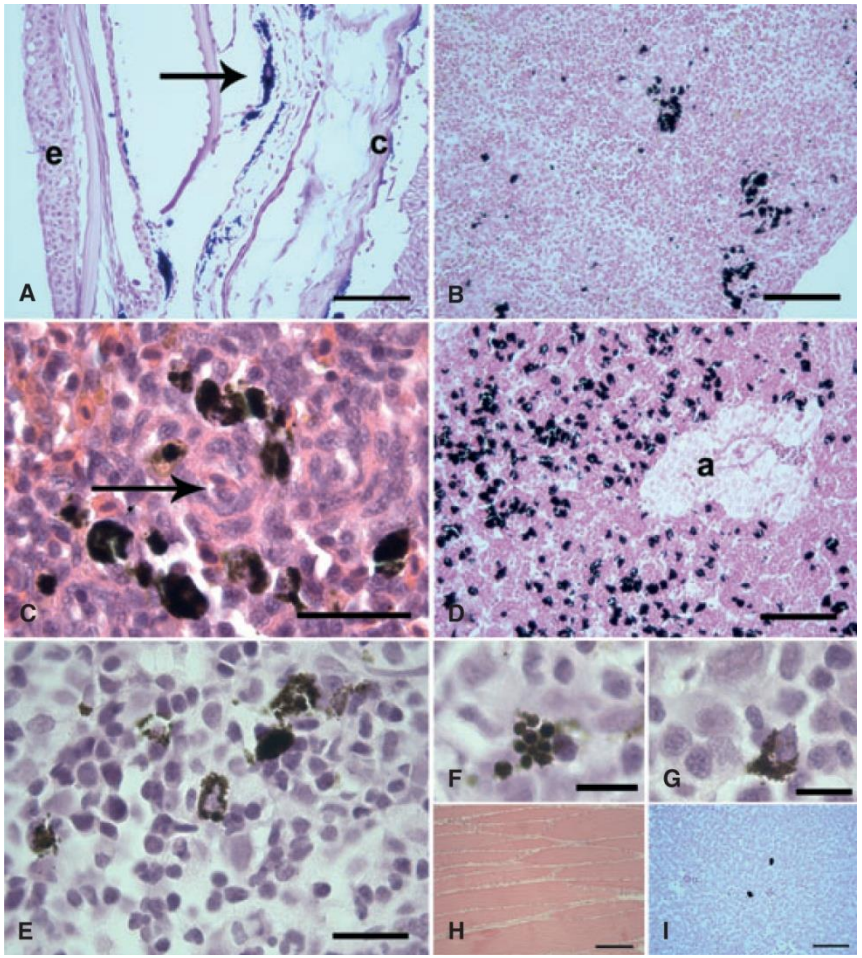
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Biochem Cell Biol. 2012 Dec;90(6):769-78. doi: 10.1139/o2012-033. Epub 2012 Nov 20.

Melanogenesis in visceral tissues of Salmo salar. A link between immunity and pigment production?

Arciuli M, Fiocco D, Cicero R, Maida I, Zanna PT, Guida G, Horsberg TE, Koppang EO, Gallone A.

Source

Sezione di Biologia Medica, Dipartimento di Scienze Mediche di Base, Neuroscienze ed Organi di Senso, Università degli Studi di Bari, Policlinico-Piazza Giulio Cesare, 70124 Bari, Italy.

Fish & Shellfish Immunology 33 (2012) 277–285

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Pigment-producing granulomatous myopathy in Atlantic salmon: A novel inflammatory response

Hilde A.S. Larsen [a], Lars Austbø [b], Turid Mørkøre [c], Jim Thorsen [d], Ivar Hordvik [e], Uwe Fischer [f], Emilio Jirillo [g], Espen Rimstad [h], Erling O. Koppang [a,*]

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[b] Section of Genetics, Department of Basic Science and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway
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[e] Department of Biology, University of Bergen, Bergen, Norway
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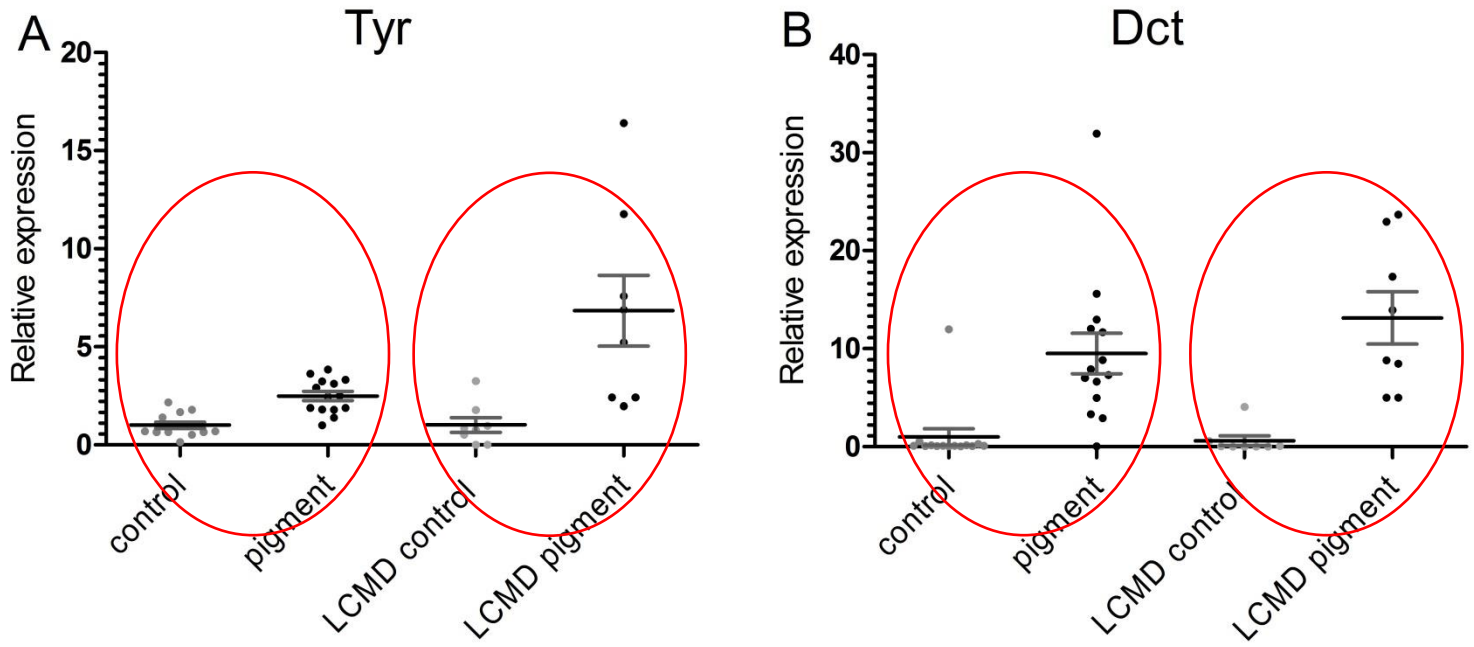
Up-regulation of the tyrosinase gene family in the black spots



Journal of Fish Diseases 2013 doi:10.1111/jfd.12106

The effect of vaccination, ploidy and smolt production regime on pathological melanin depositions in muscle tissue of Atlantic salmon, *Salmo salar* L.

H A S Larsen[1], L Austbø[2], A Nødtvedt[3], T W K Fraser[3], E Rimstad[4], P G Fjelldal[5], T Hansen[5] and E O Koppang[1]

1 Department of Basic Science and Aquatic Medicine, Section of Anatomy and Pathology, Norwegian School of Veterinary Science, Oslo, Norway
2 Department of Basic Science and Aquatic Medicine, Section of Genetics, Norwegian School of Veterinary Science, Oslo, Norway
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4 Department of Food Safety and Infection Biology, Section of Microbiology, Immunology and Parasitology, Norwegian School of Veterinary Science, Oslo, Norway
5 Institute of Marine Research, Matre Research Station, Matredal, Norway

Paper II

Developmental and Comparative Immunology 41 (2013) 50–58

Contents lists available at SciVerse ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Transcription of the tyrosinase gene family in an Atlantic salmon leukocyte cell line (SHK-1) is influenced by temperature, but not by virus infection or bacterin stimulation

Hilde A.S. Larsen [a], Lars Austbø [b], Melanie König [b], Henning Sørum [c], Espen Rimstad [c], Erling O. Koppang [a,*]

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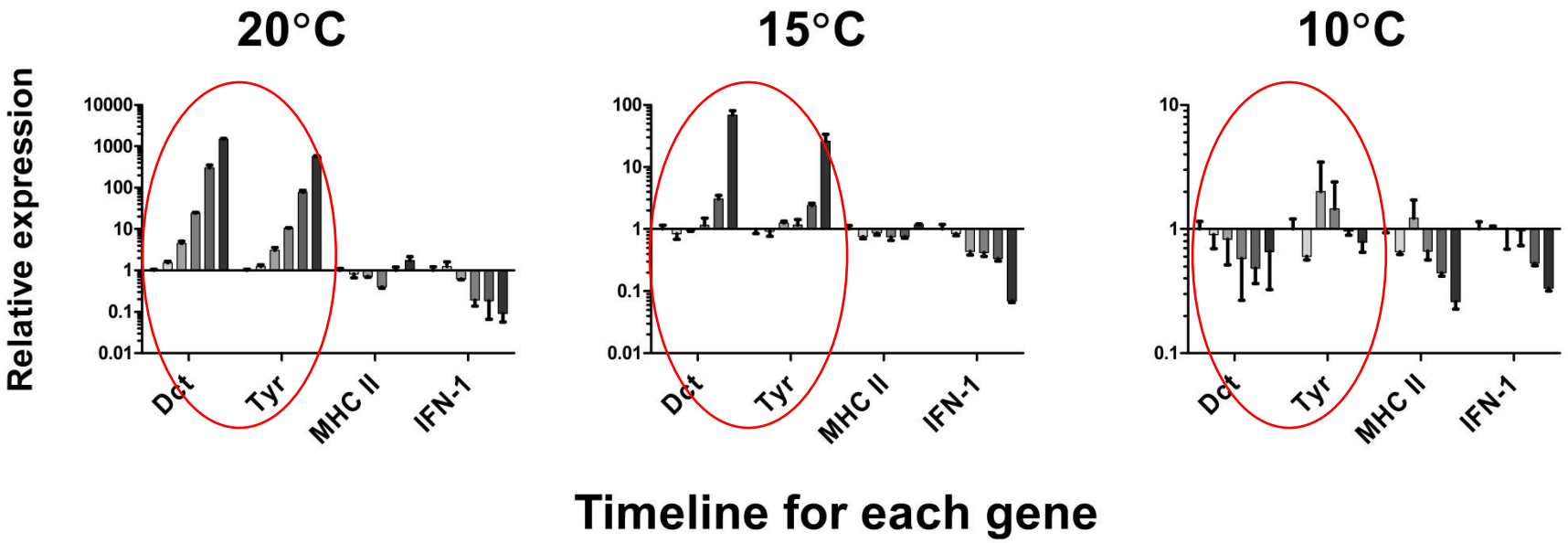
[b] Section of Genetics, Department of Basic Science and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway
[c] Section of Microbiology, Immunology and Parasitology, Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Oslo, Norway

Paper III

Up-regulation of the tyrosinase gene family by temperature stimulation *in vitro*



Paper III

Pathological pigmentation in the hearts of Atlantic salmon (*Salmo salar* L.) with cardiomyopathy syndrome

Hilde A. S. Fagerland[1], Lars Austbø[2], Camilla Fritsvold[3], Marta Alarcon[3], Espen Rimstad[4], Knut Falk[3], Torunn Taksdal[3] and Erling O. Koppang[1,5]

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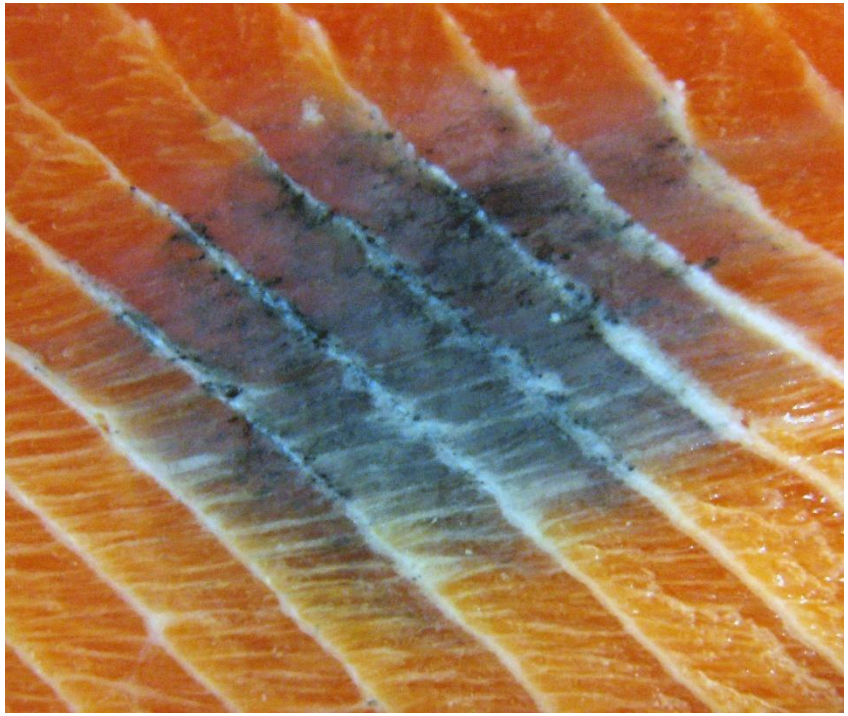
Accepted for publication in Veterinary Research

Is melanin always melanin?




Australia
Melbourne
Devonport
Strahan
Swansea
Hobart

Melanin-containing cells in normal and inflamed tissues

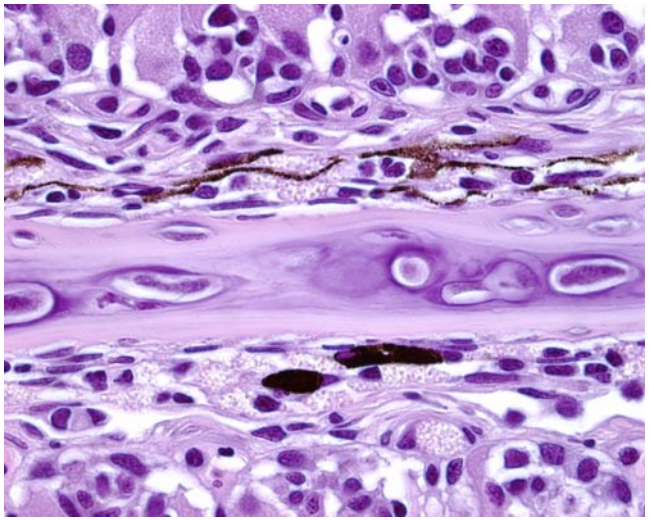
Agnar Kvellestad

Norwegian School of Veterinary Science
Department of Basic Sciences and Aquatic Medicine

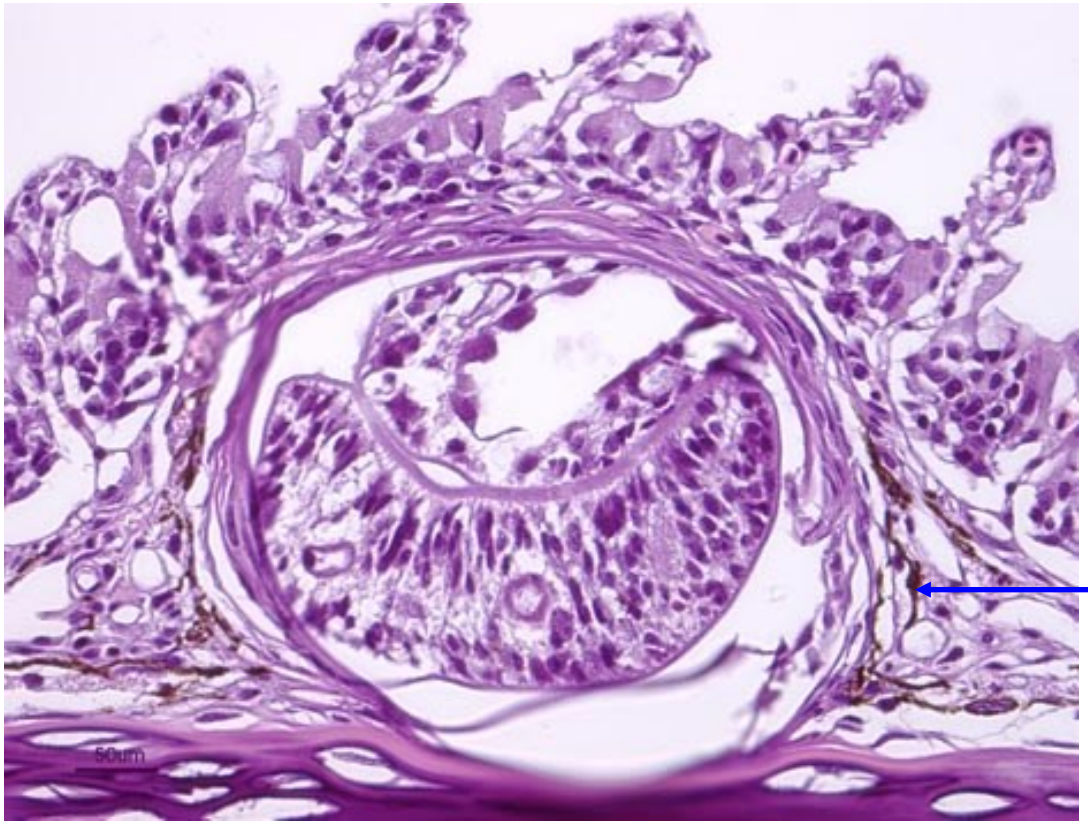
Melanin-containing cells

Normally present in the septum of gills

Occasionally seen in gill filaments – pathology?

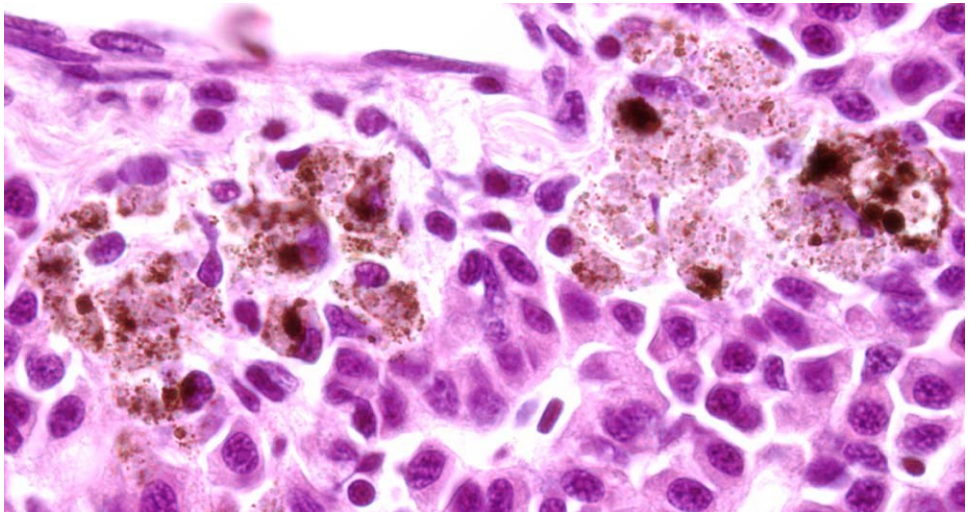


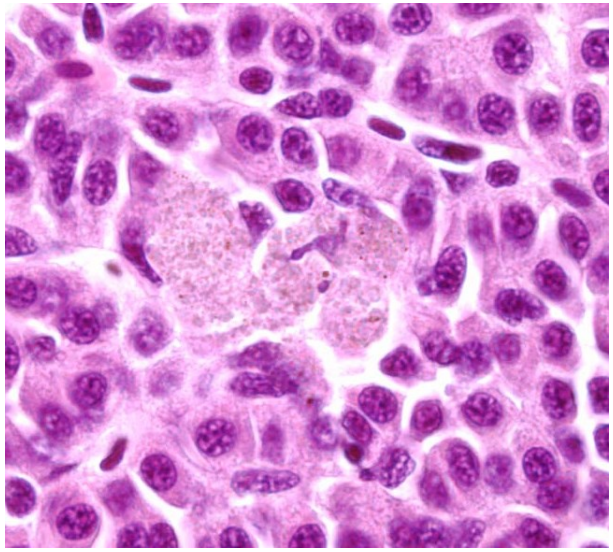
Immune and pigment systems

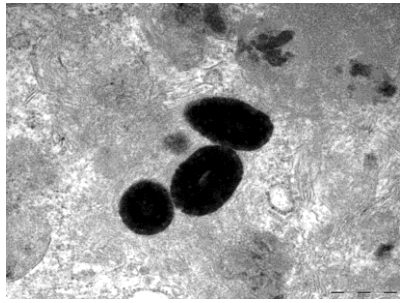


Encapsulated metacerkariae of *Cryptocotyle lingua*, visible as black spots in gills and skin of salmon and marine fish

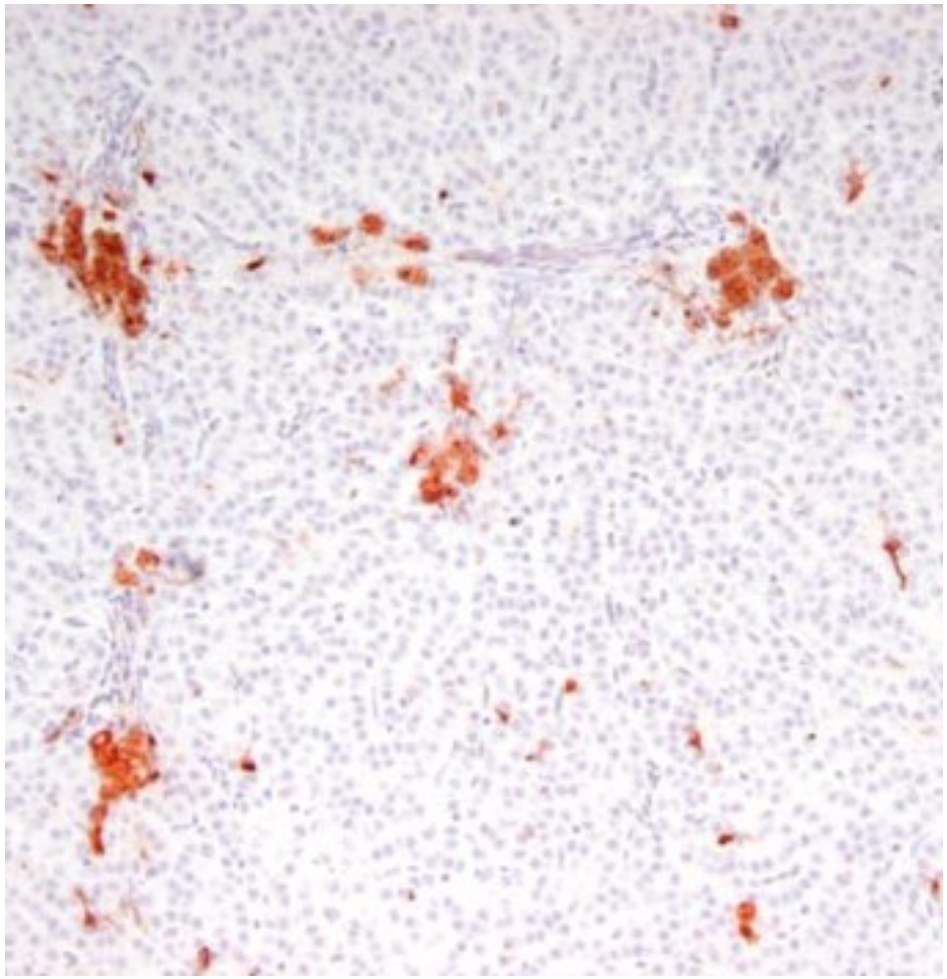
Hepatitis:
Macrophage-like cells may contain melanin-granules
De novo synthesis?

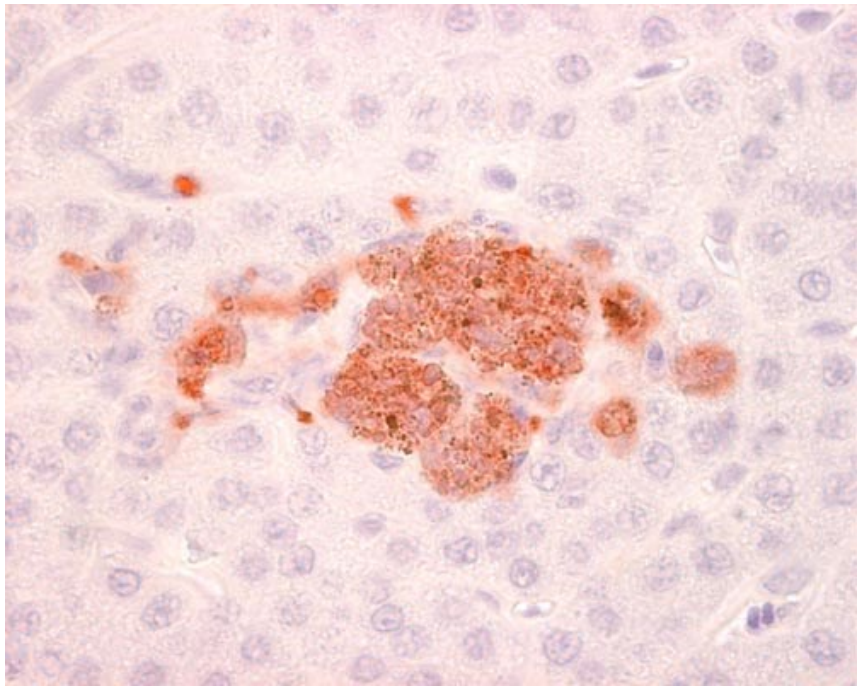






MHC class II molecules in macro-phage like cells

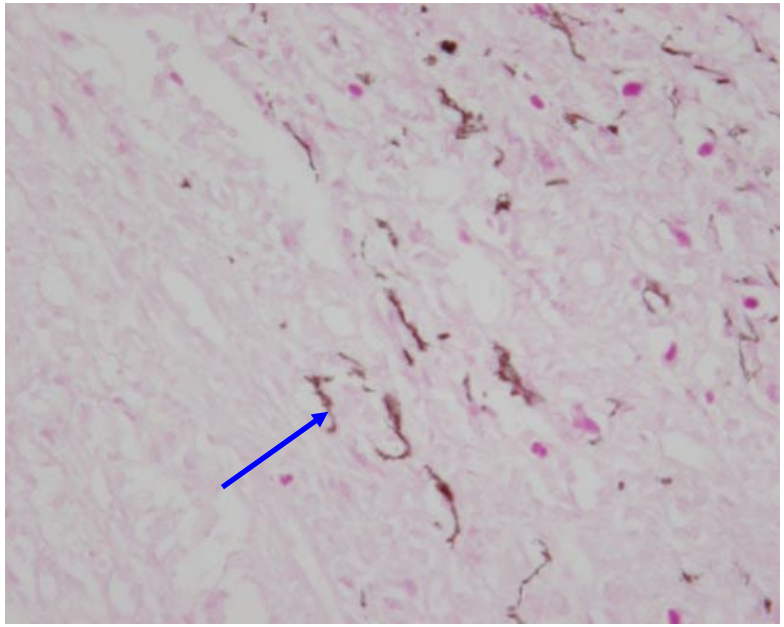




Kvellestad & Koppang

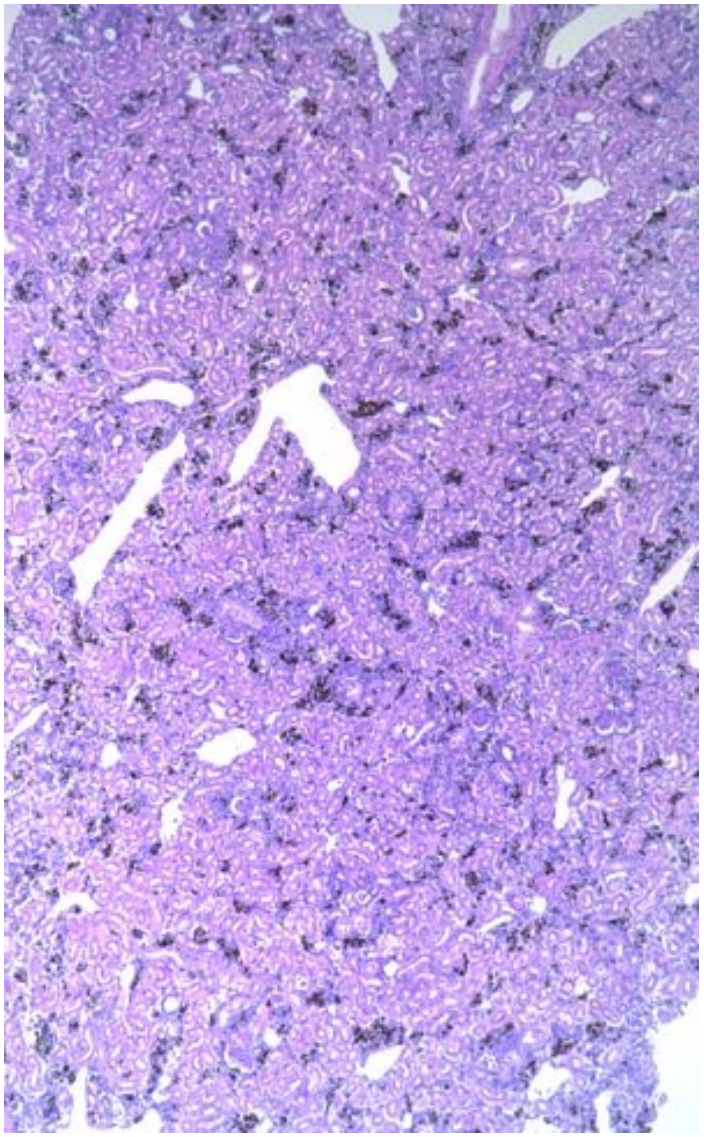
Chronic peritonitis following i.p. Injection of oil-adjuvanted vaccines

Poppe & Breck (1997): Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil-adjuvanted vaccine. A case report. DAO 29: 219-226.



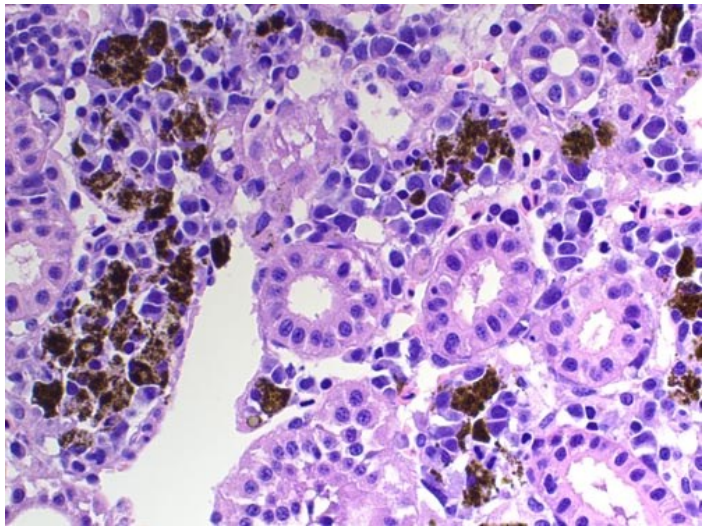
Elongated/flattened cells.
PAS stain. Photo: Agnar Kvellestad

Emaciated post-smolt



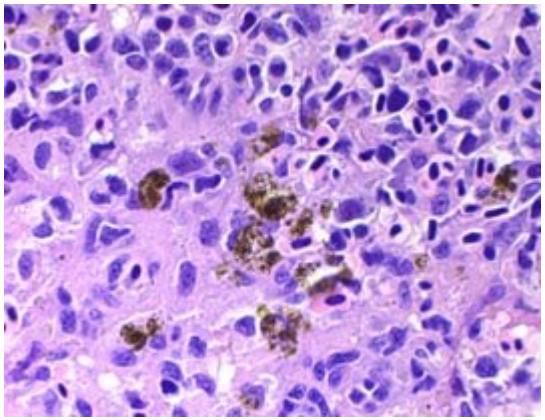
2005-09-0476-L6R-40x-Zeiss Axio 2013 NVH-nyre m rikeleg melanin

Kidney

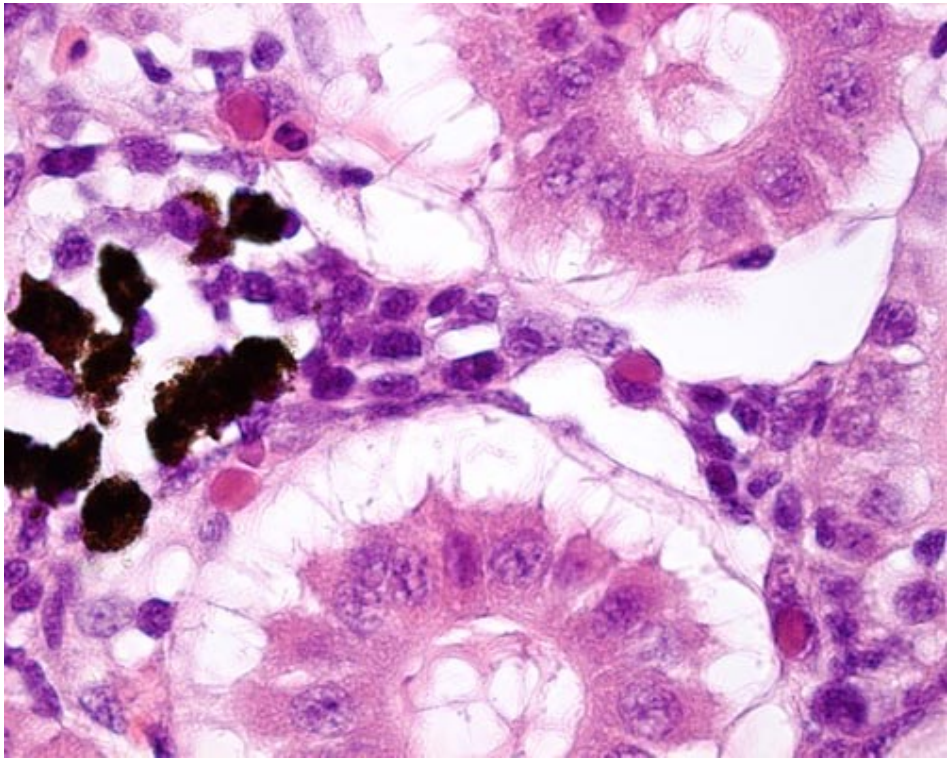


2005-09-0476-L6R-2komma5x-Zeiss Axio 2013 NVH-nyre m rikeleg melanin crop mm

Spleen



Kidney with degradation of erythrocytes



Anatomy of muscle

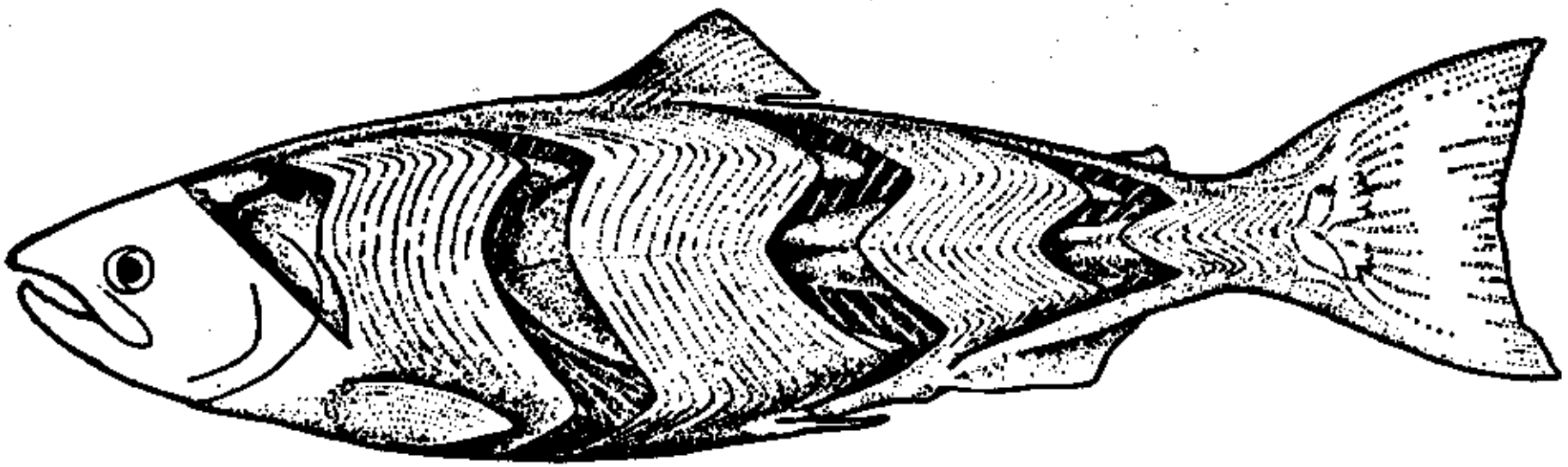
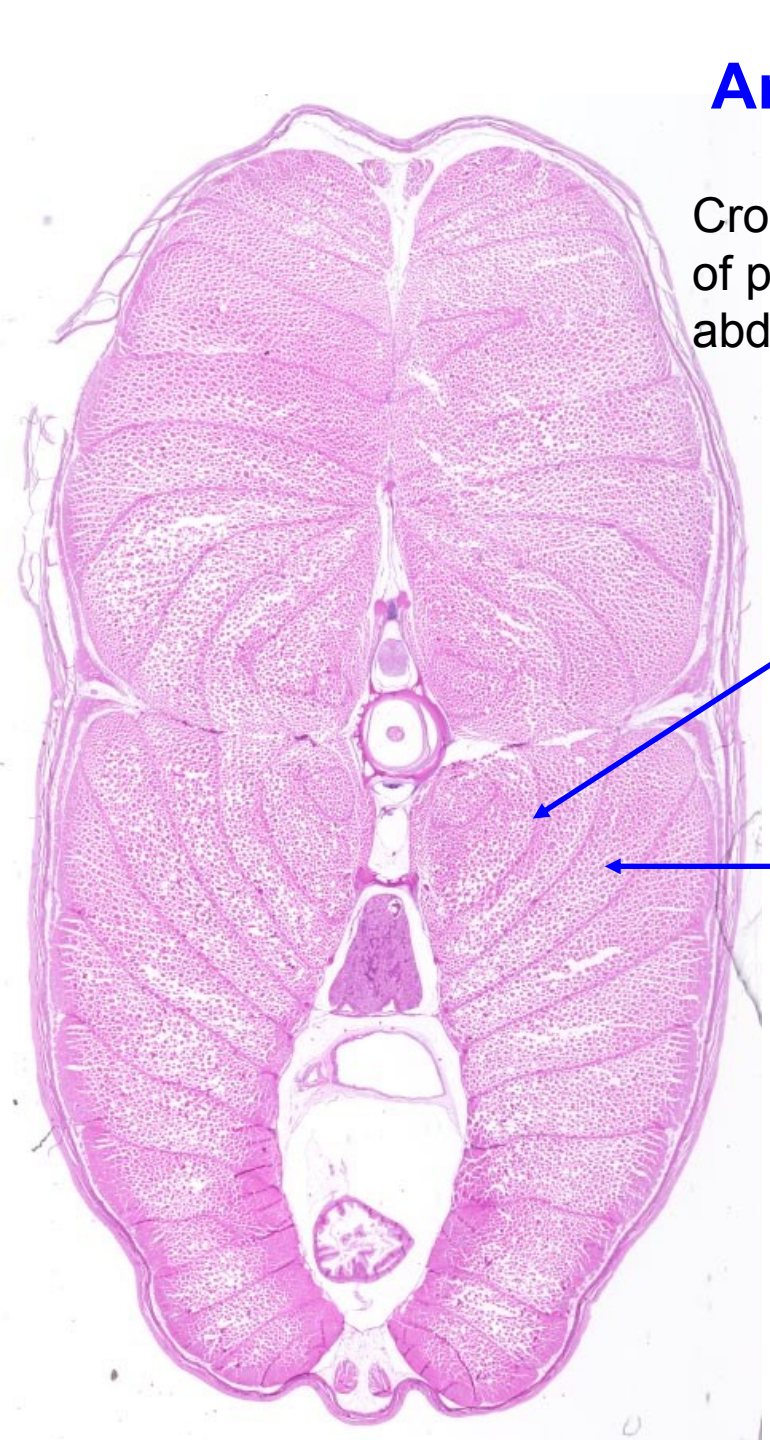


Fig. 83. The axial musculature of a salmon. In four places a series of myomeres has been removed to show the complicated internal folding of these segmental structures. Within the body each W projects farther anteriorly or posteriorly than it does at the surface. The myoseptum horizontale is cutting the main, anterior-pointing W. (After Greene from Romer.) To p. 97, 98.

Harder 1975 Fig 83 Atlantic salmon-axial musculature-tekst-1000pix.tif

Anatomy of muscle

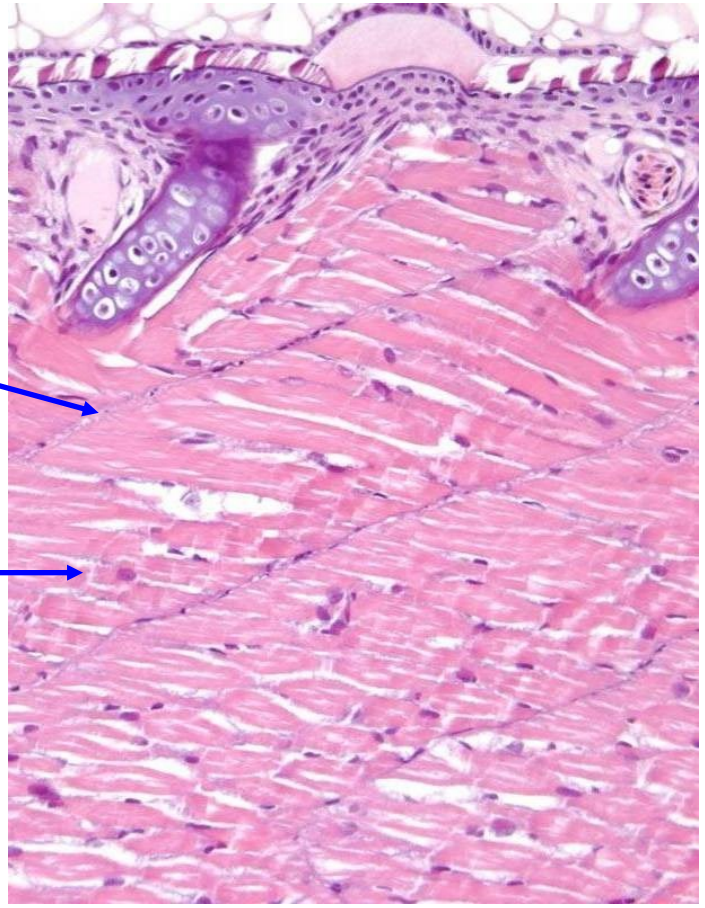


Cross section of posterior abdomen

Myoseptum

Myomere

Longitudinal section

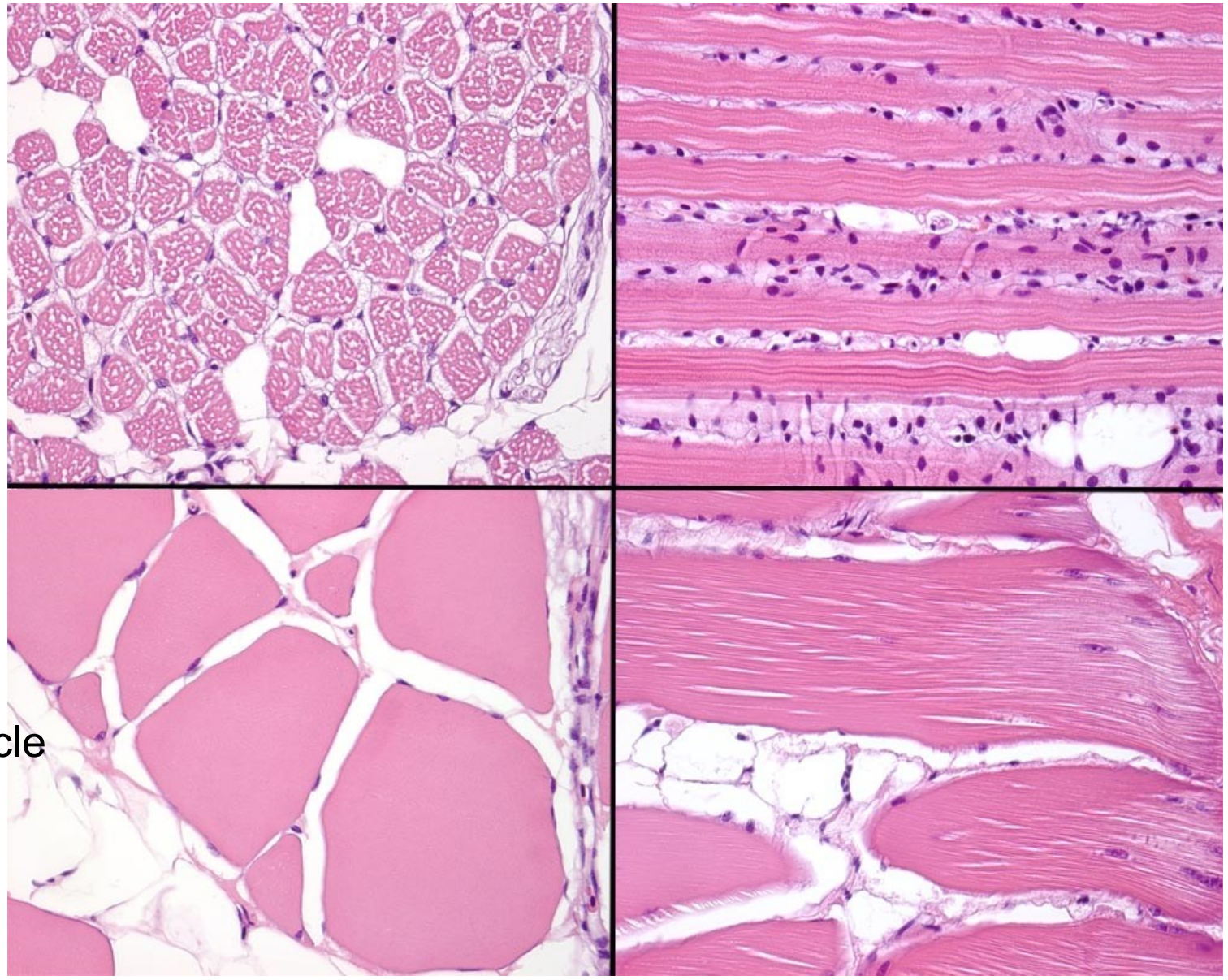


Cross section

Longitudinal section

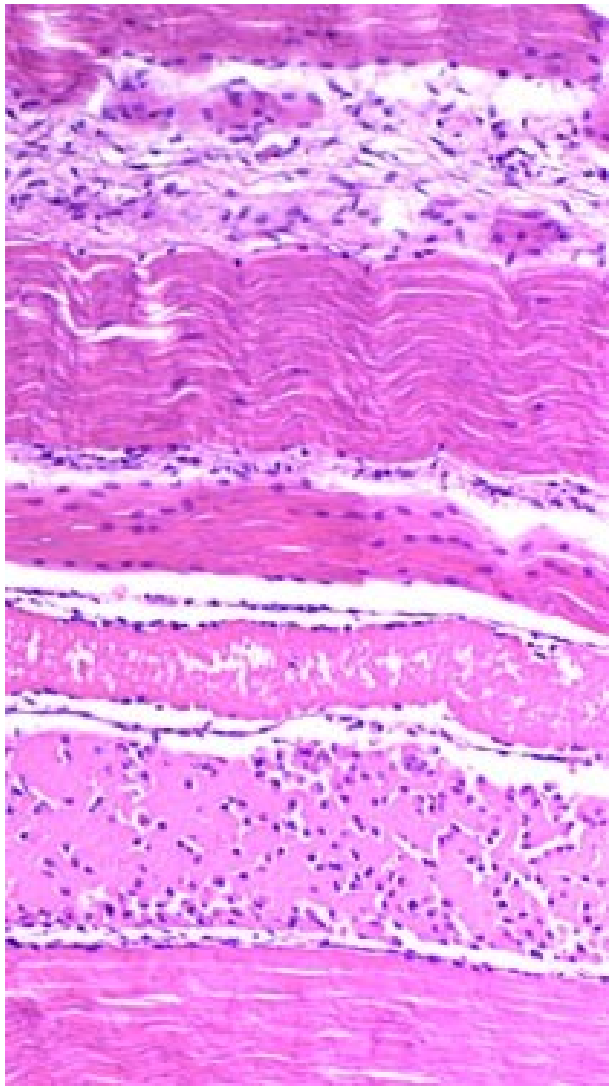
Red muscle

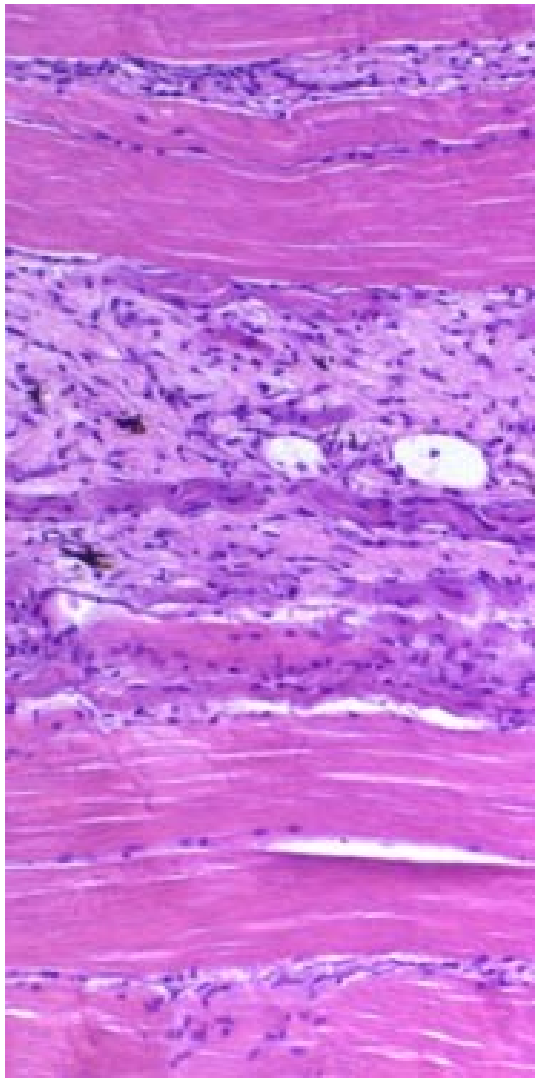
White muscle



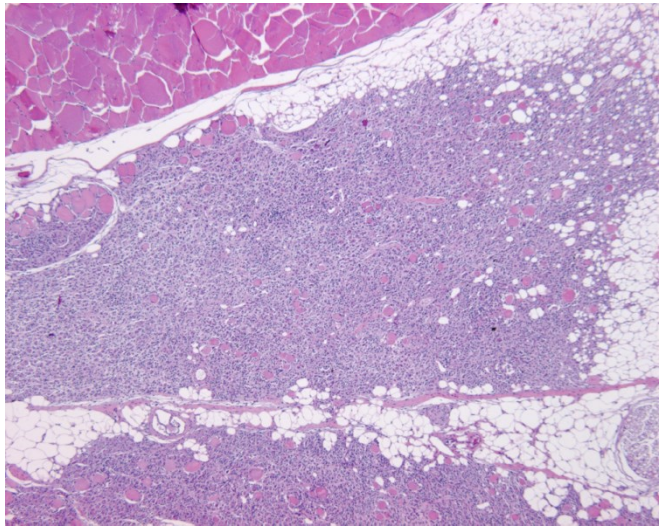
Farm with high prevalence and extensive changes

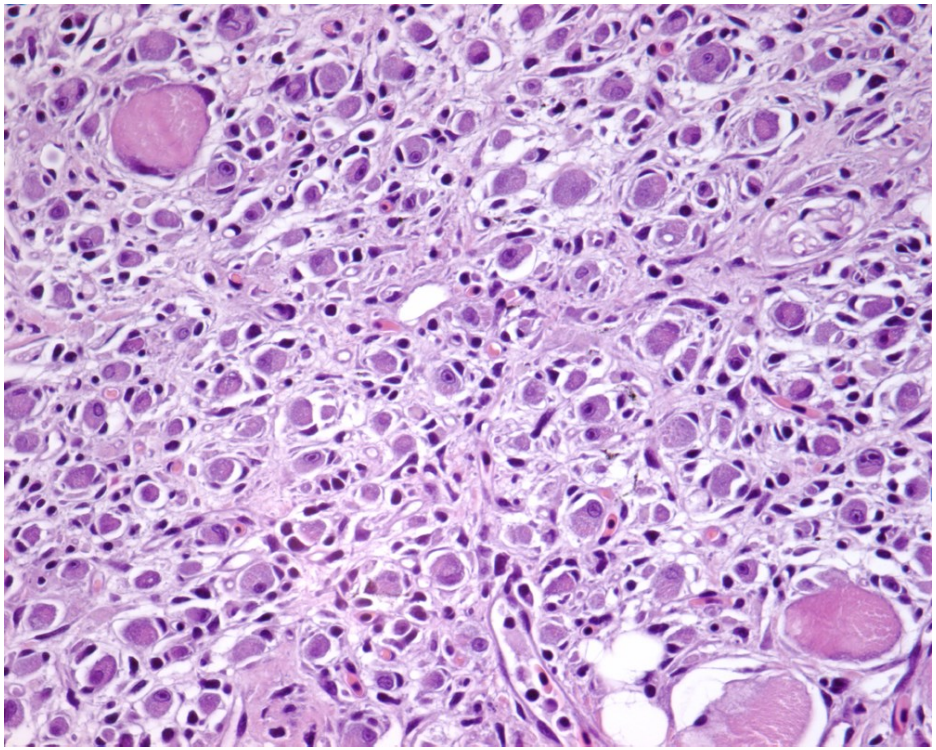
Photo: Local Fish Health Service

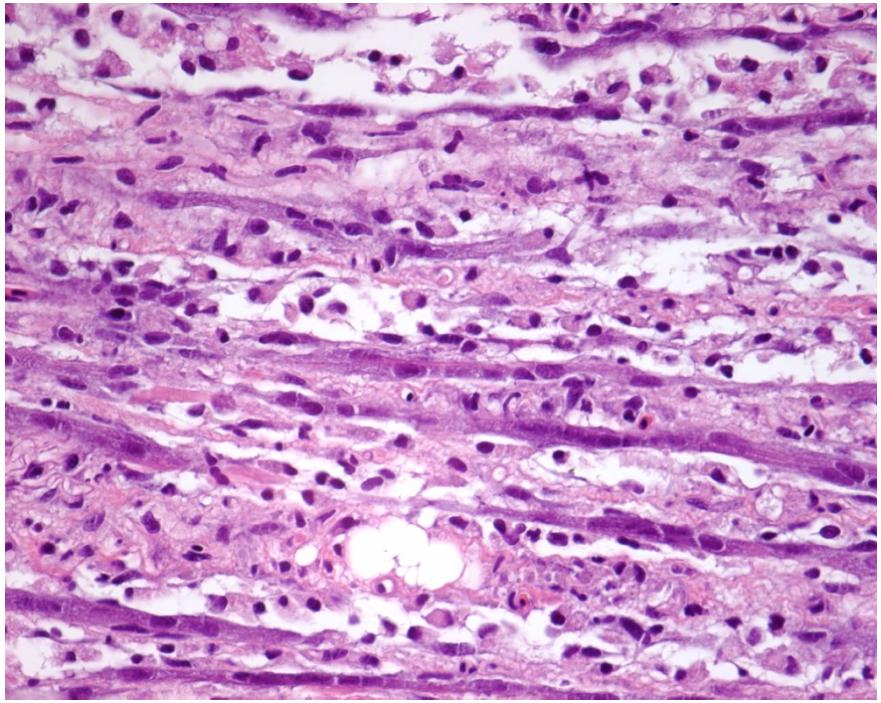




Pancreas disease







Aetiology?

- Multifactorial
    - Alternatives:
    - Nutritional
    - Toxicity
    - Infectious
    - Immune-mediated

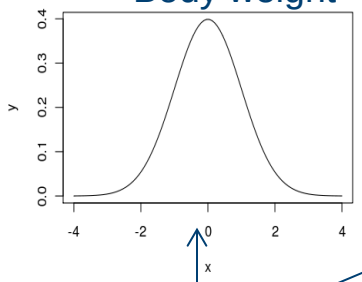
Quantitative genetics of vaccine-induced side effects in Atlantic salmon

Bjarne Gjerde
Tale Marie Drangsholt, Jørgen Ødegård, Hans B. Bentsen, Frode Fridell, Øystein Evensen

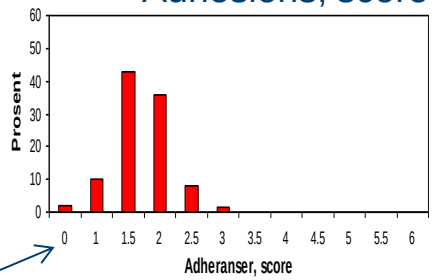
Quantitative genetics

The study of the effects a large number of unknown genes
and unknown environment factors have on phenotypic variation of traits

Body weight

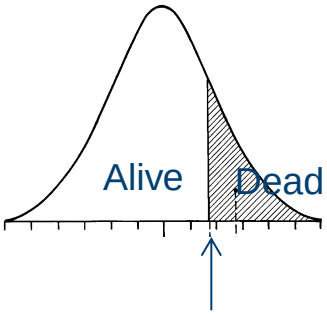


Adhesions, score



Phenotype (P) = Genotype (G) + Environment (E)

Survival



Sire + Dam + Mendelian sampling

½ V_G ½ V_G

$V_P = V_G + V_E$

Heritability, $h^2 = V_G/V_P$

Background

- **Vaccination**
  - Negative side effects (adhesions, melanin, reduced growth)

- **Adhesions and melanin deposits heritable traits?**
  - and thus be reduced through selective breeding

- **Magnitude of genetic correlations between traits**
  presently selected for (e.g. growth, disease resistance) and adhesions and/or melanin deposits?

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Genetic correlation, r_G

Adhesions, score

$$P_1 = G_1 + E_1$$

r_G

To what degree individuals / families rank similar / different with respect to the true genetic (breeding) value of two trait

Melanin, score

$$P_2 = G_2 + E_2$$

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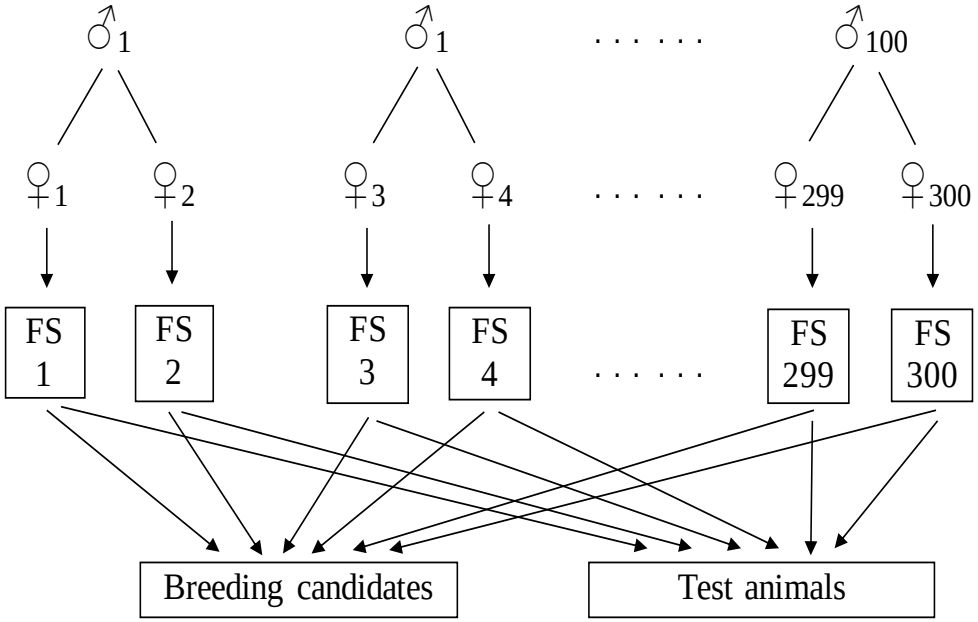


- Disease challenge tests
- Carcass quality traits
- Vaccine induced side effects

Traits that cannot/difficult to record on the live breeding candidates

YC 2007 from SalmoBreed

150 families from SalmoBreed

15 fish/family/experimental group

Vaccine

- Standard 6-comp. (SV), 0.1 ml/fish
- Experimental 6-comp. (EV), 0.05ml/fish
- Unvaccinated control, saline water (Co)

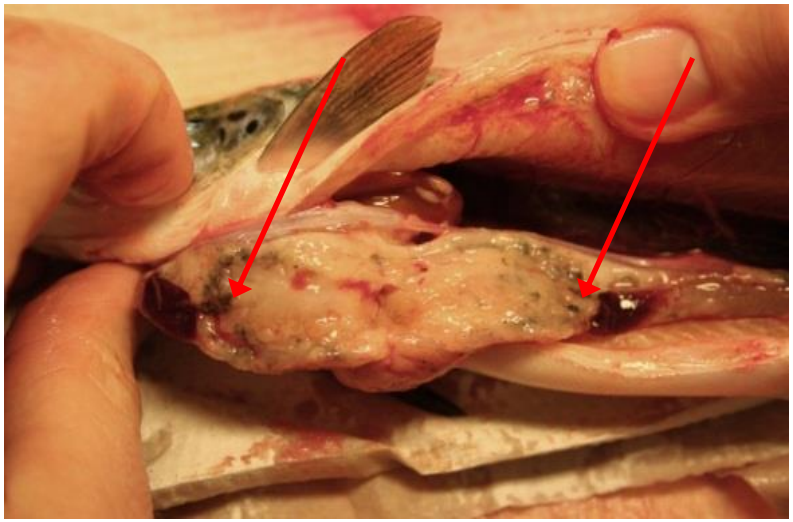
Vaccine induced side effects

- "Fresh Water" (**FW)**
  - recorded after 3 months at 17 ºC at Nofima, Sunndalsøra

- "Sea Water"
  - recorded after 6 (**SW6**) and 12 (**SW12**) months in a net cage in the sea at Nofima, Averøy

Recordings of vaccine induced side effects

Adhesions

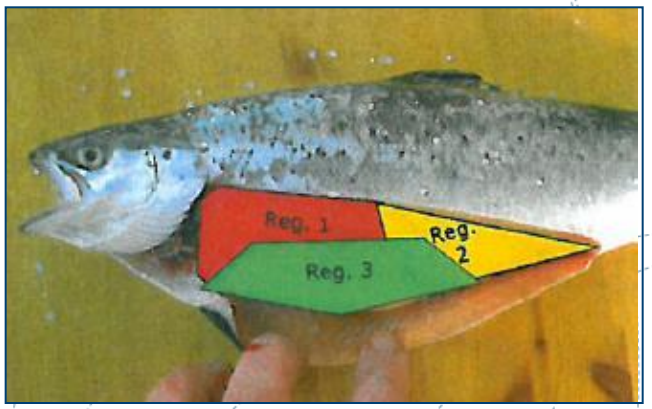


Melanin deposits

Scored by trained persons from PHARMAQ

Adhesions: scale 0-6
- in three regions of the abdominal cavity

Melanin deposits: scale 0-3
- internal organs and abdominal wall



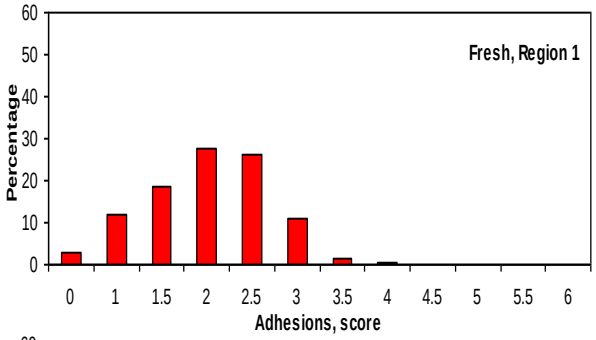
Adhesions – in different regions of abdominal cavity

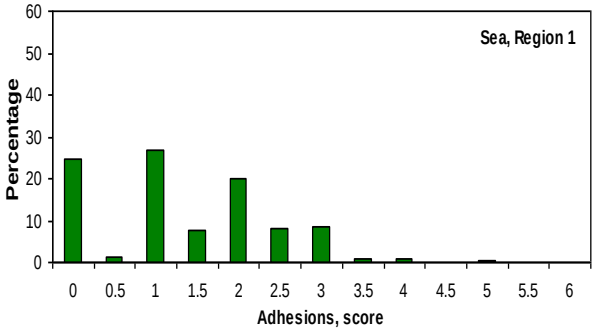
FW

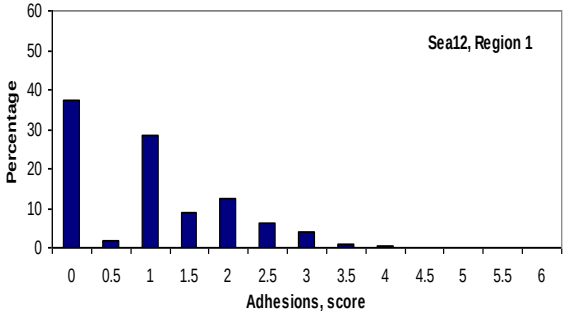
SW6

SW12

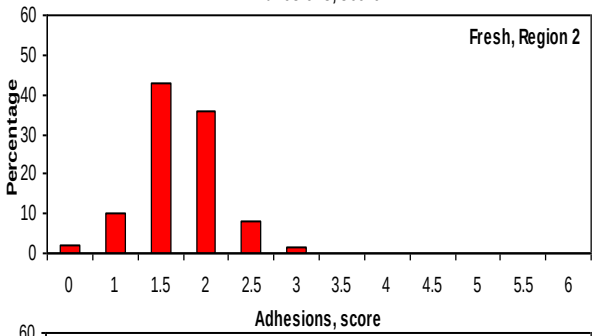
Region 1

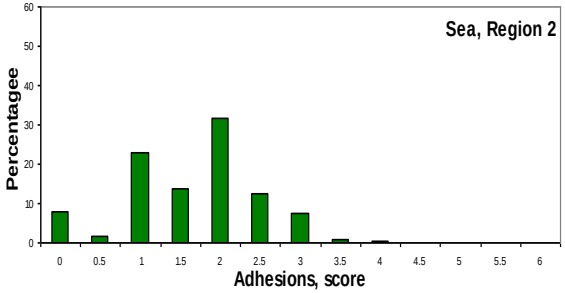


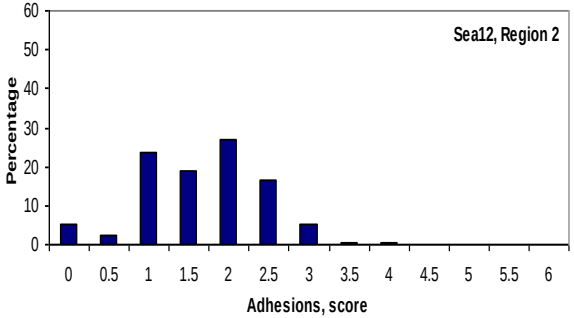




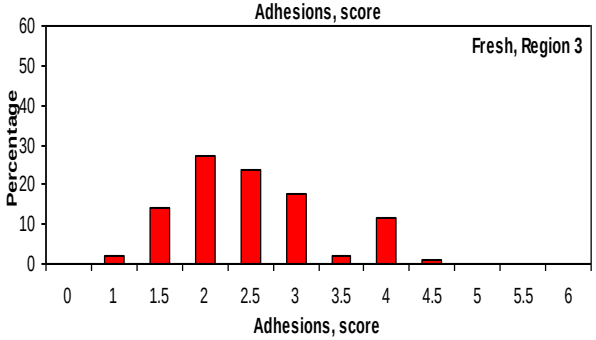
Region 2

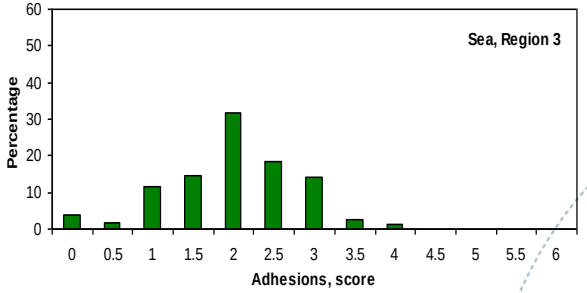


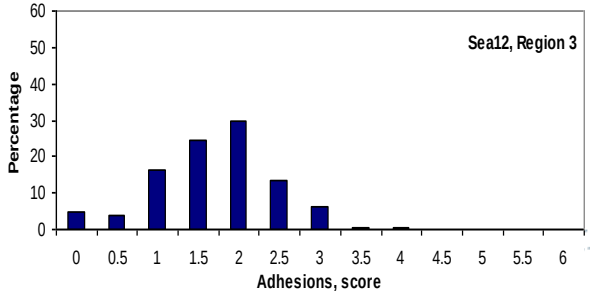




Region 3







X=**2.06; SD=0.49**

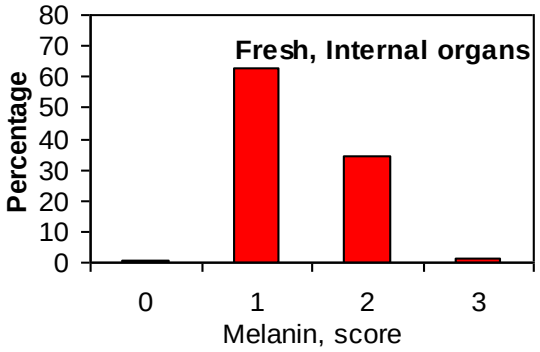
X=**1.68; SD=0.65**

X=**1.46; SD=0.56**

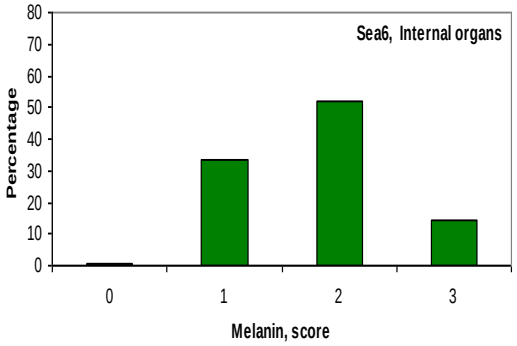
Melanin score - intern organs and abdominal wall

Internal organs

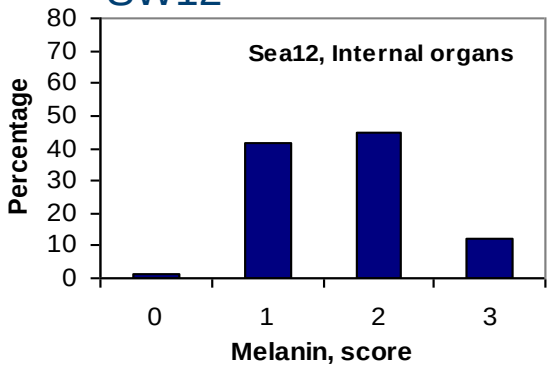
FW



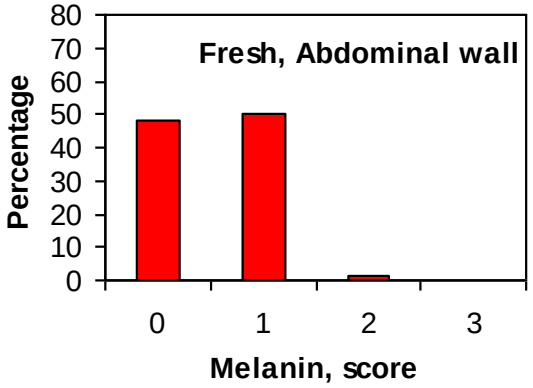
SW6

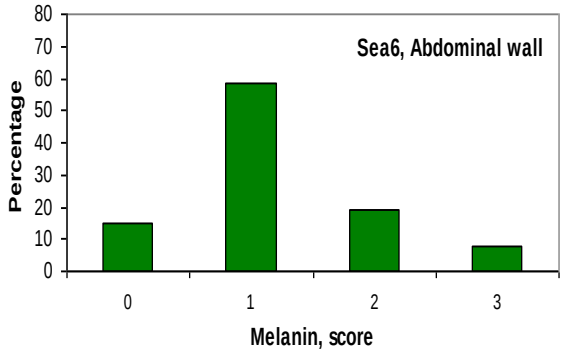


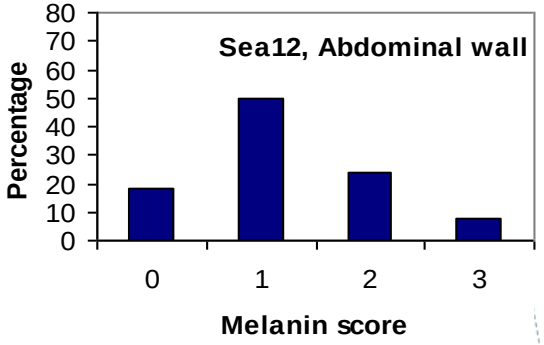
SW12



Abdominal wall







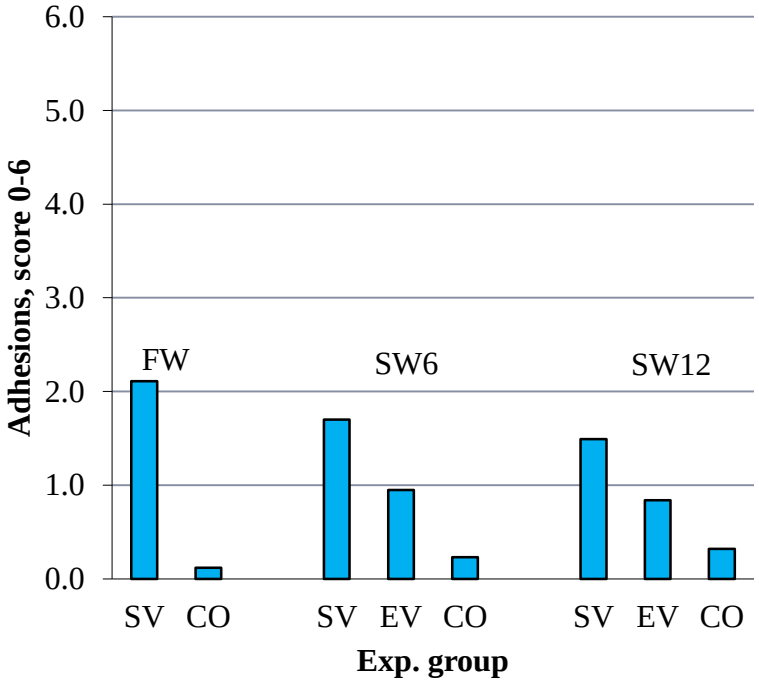
X=**0.95; SD=0.44**

X=**1.49; SD=0.62**

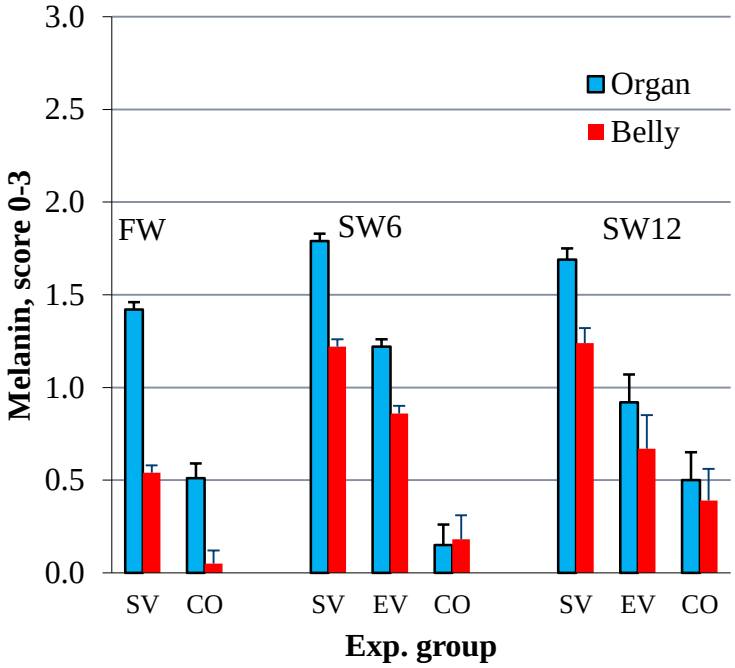
X=**1.45; SD=0.69**

Effects of different Vaccines

Adhesions, score 0-6



Melanin, score 0-3



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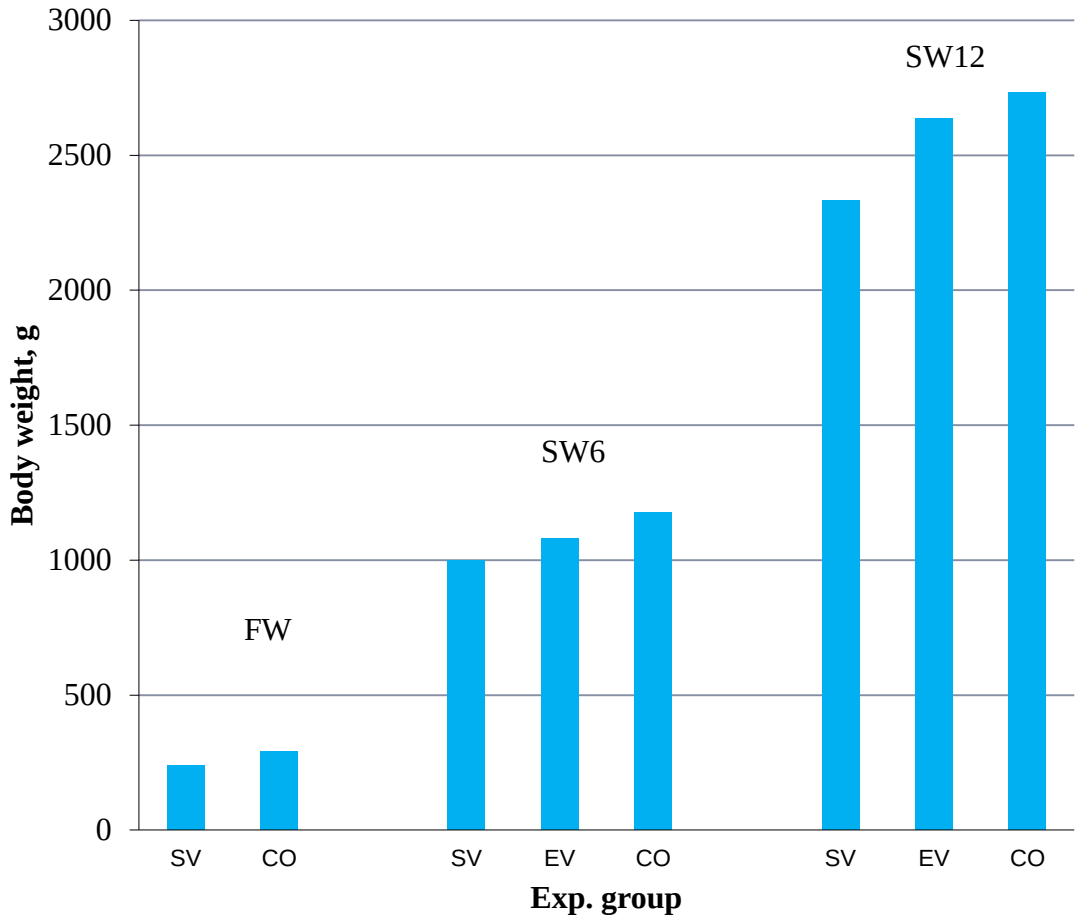
Growth


3000
2500
2000
1500
1000
500
0
Body weight, g
FW
SW6
SW12
SV
CO
SV
EV
CO
SV
EV
CO
Exp. group

Heritabilities and genetic correlations

	Heritability		Genetic corr.
Exp. group	**Adhesions**	**Melanin**	**Adh - Mel**
FW	0.31 ±0.05	0.27±0.05	0.52 ±0.11
SW6-SV	0.19 ±0.04	0.28±0.05	0.89 ±0.06
SW12-SV	0.16 ±0.04	0.30±0.05	0.87 ±0.06
SW6-EV	0.08 ±0.03	0.11 ±0.03	0.76 ±0.14

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Genetic correlations between exp. groups

	Adhesions	Melanin
FW vs. SW12	0.48 ± 0.14	0.61 ± 0.11
SW6 vs. SW12	0.92 ± 0.11	0.89 ± 0.07

Conclusions

- Adhesions and Melanin deposits are heritable traits,
  - can thus be reduced through selective breeding
- Highly genetic correlated traits in the seawater period
  - thus to a larges extent caused by the same genes
- Substantial re-ranking of families for these traits in freshwater and seawater – test of families for these traits should be performed based on data recorded in seawater
- Vaccine induced side effects can be reduced through the development of improved vaccines
  - thus leaving higher scope for genetic gain for other traits

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Genetic correlations between resistance to furunculosis and vaccine induced side effects

	Adhesions	Melanin
Resist, to fur. vaccinated fish	0.06 ± 0.16	0.03 ± 0.15
Resist. to fur. unvaccinated fish	0.24 ± 0.16	0.00 ± 0.15
Growth, SW12	0.08 ± 0.15	-0.04 ± 0.14

All correlations not significantly different from zero

Nofima

Conclusions

Selection for increased resistance to furunculosis or increased growth is not expected to give any positive or negative correlated responses in vaccine induced side effects

This indicates that adhesions and melanin deposits are caused by other components of the immune system than those responsible for the genetic variation in both innate and acquired resistance to furunculosis

Nofima

For more details see:

Heredity (2011), 1–7

www.nature.com/hdy

ORIGINAL ARTICLE

Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.)

TMK Drangsholt[1,2], B Gjerde[1,2], J Ødegård[1,2], F Finne-Fridell[3], Ø Evensen[4] and HB Bentsen[2]

[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (UMB), Ås, Norway;* [2]*Nofima Marin, Ås, Norway;* [3]*PHARMAQ AS, Oslo, Norway and* [4]*Norwegian School of Veterinary Science, Oslo, Norway*

Aquaculture 318 (2011) 316–324

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Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*)

Tale Marie K. Drangsholt [a,b,*], Bjarne Gjerde [a,b], J. Ødegård [a,b], F. Fridell [c], Hans B. Bentsen [b]

[a] *Norwegian University of Life Sciences (UMB), PO Box 5003, 1432 Ås, Norway*
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Aquaculture 324–325 (2012) 312–314

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Short communication

Genetic correlations between disease resistance, vaccine-induced side effects and harvest body weight in Atlantic salmon (*Salmo salar*)

Tale Marie Karlsson Drangsholt [a,b,*], Bjarne Gjerde [a,b], Jørgen Ødegård [a,b], Frode Finne-Fridell [c], Øystein Evensen [d], Hans Bernhard Bentsen [a]

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Aquaculture 287 (2009) 52–58

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journal homepage: www.elsevier.com/locate/aqua-online

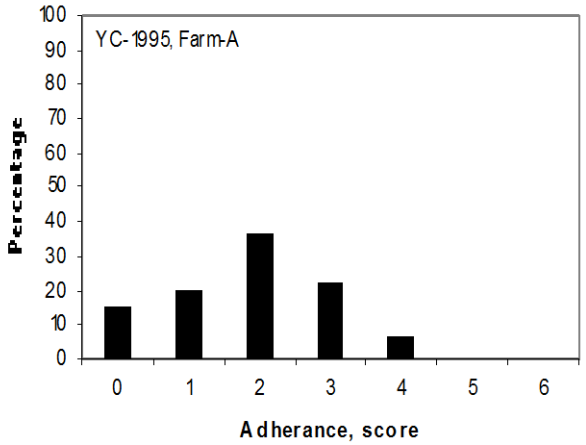
Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*)

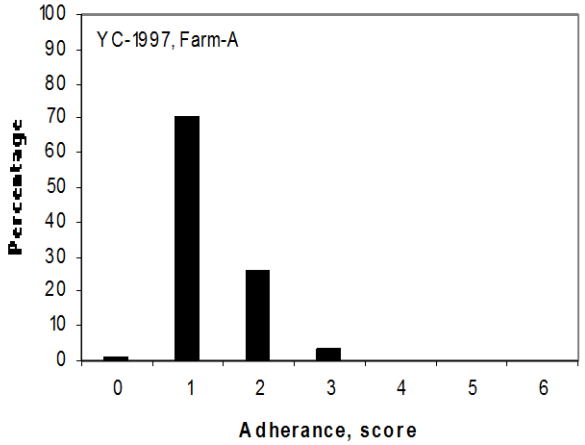
Bjarne Gjerde [a,*], Øystein Evensen [b], Hans B. Bentsen [a], Arne Storset [c]

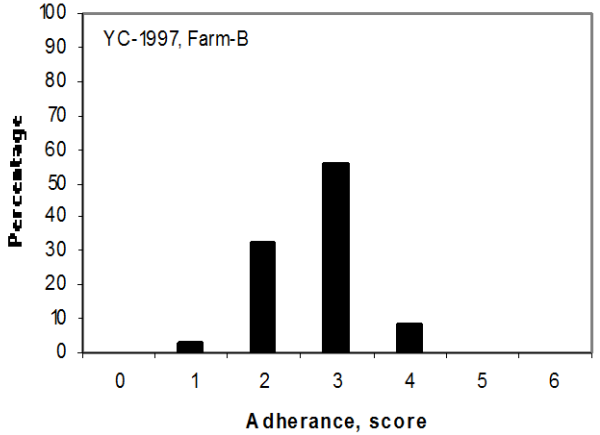
[a] *Nofima Marine, P.O. Box 5010, N-1432 Ås, Norway*
[b] *Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, PO. Box 8146 Dep Oslo 0033, Norway*
[c] *Aqua Gen AS, P.O. Box 1240, Pir-Senteret, N-7462 Trondheim, Norway*

YC 1995 and 1997 from AquaGen

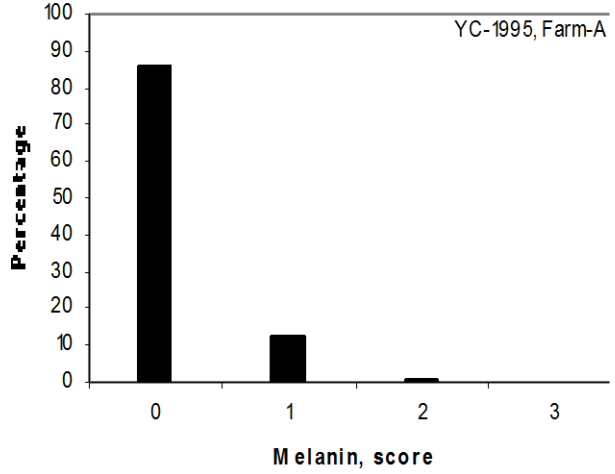
Adhesions

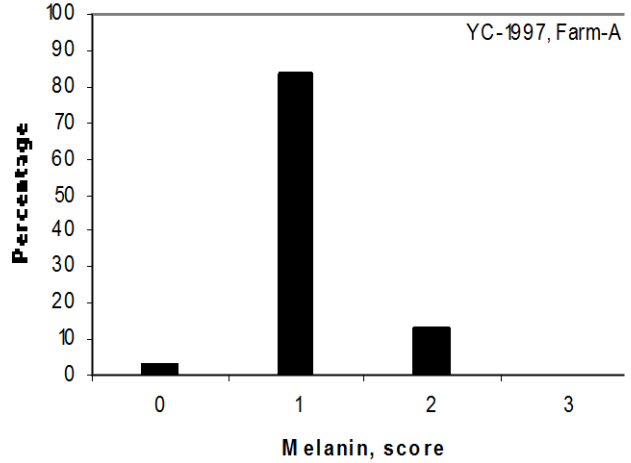


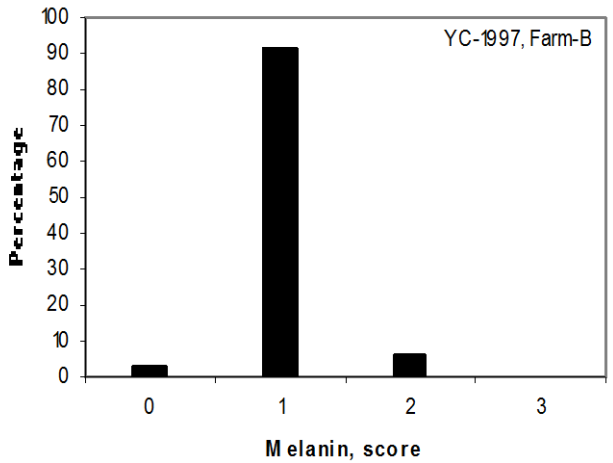




Melanin

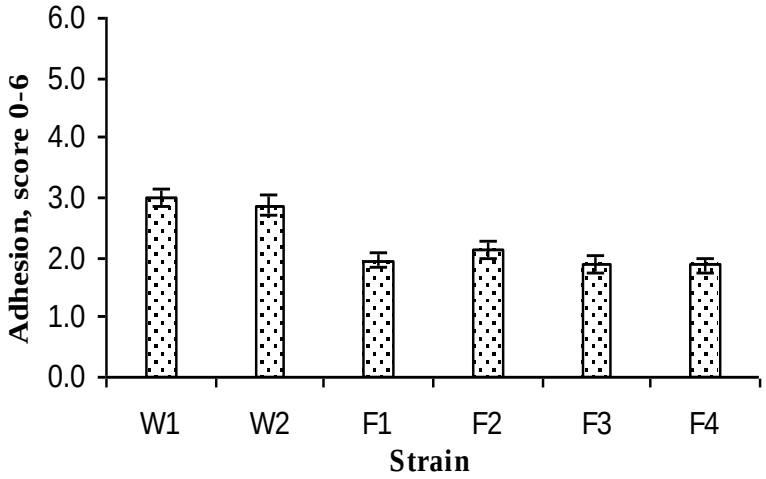


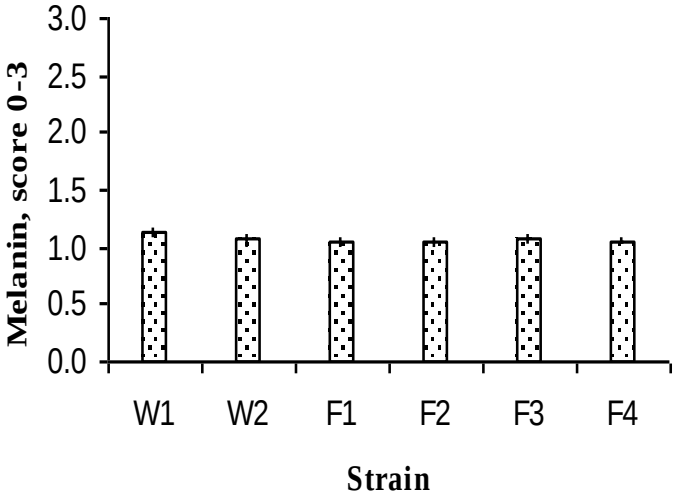




AquaGen YC 1995 and 1997

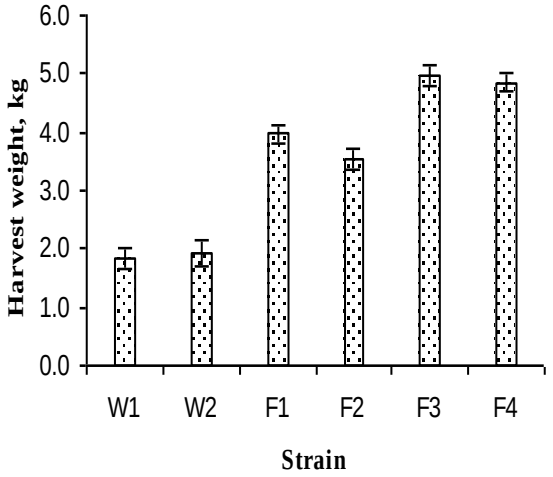
Gjerde, B., Evensen, Ø., Bentsen, H.B., Storset, A. 2009. Aquaculture 287, 52-58.



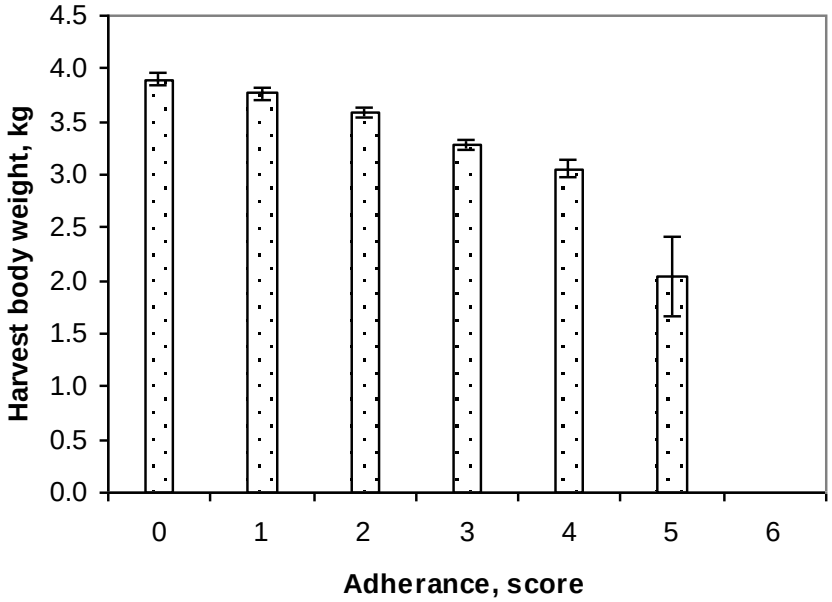


	h2 ± se
Adhesion, score	0.19 ± 0.03
Melanin, score	0.19 ± 0.03

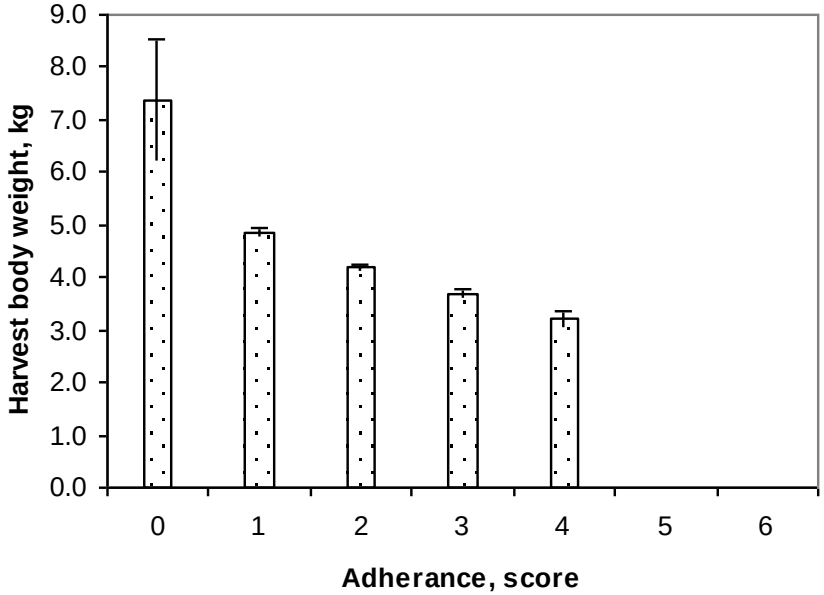
	Weight	Adhesion	Melanin
Harvest weight	-	-0.43±0.11	-0.21 ± 0.12
Adhesions, score	-0.26±0.02	-	0.74 ± 0.08
Melanin, score	-0.17±0.02	0.38±0.02	-



YC-1995



YC-1997



Dark spots in salmon fillets

statistics and status on causalities

Turid Mørkøre

Nofima

DARK SPOTS IN SALMON FILLETS
Workshop , BEST WESTERN Oslo Airport Hotel
November 5th 2013

Common goal

Sustainable and cost efficient production of high-quality food

We are all involved in food production

Nofima

Starting point - 2009

- Dark stained fillets are a costly problem for the aquaculture industry
- Dark spots are due to melanin deposition
- The cause is related to vaccine / vaccination
- Statistics missing

FHF project on quality deviations – conclusion 2012

- Dark spots due to different causes
  - NOT ONLY VACCINATION

- The impact of vaccine/vaccine regime is, however, still relevant to study

Mørke flekker i laksefilet 2012-2015

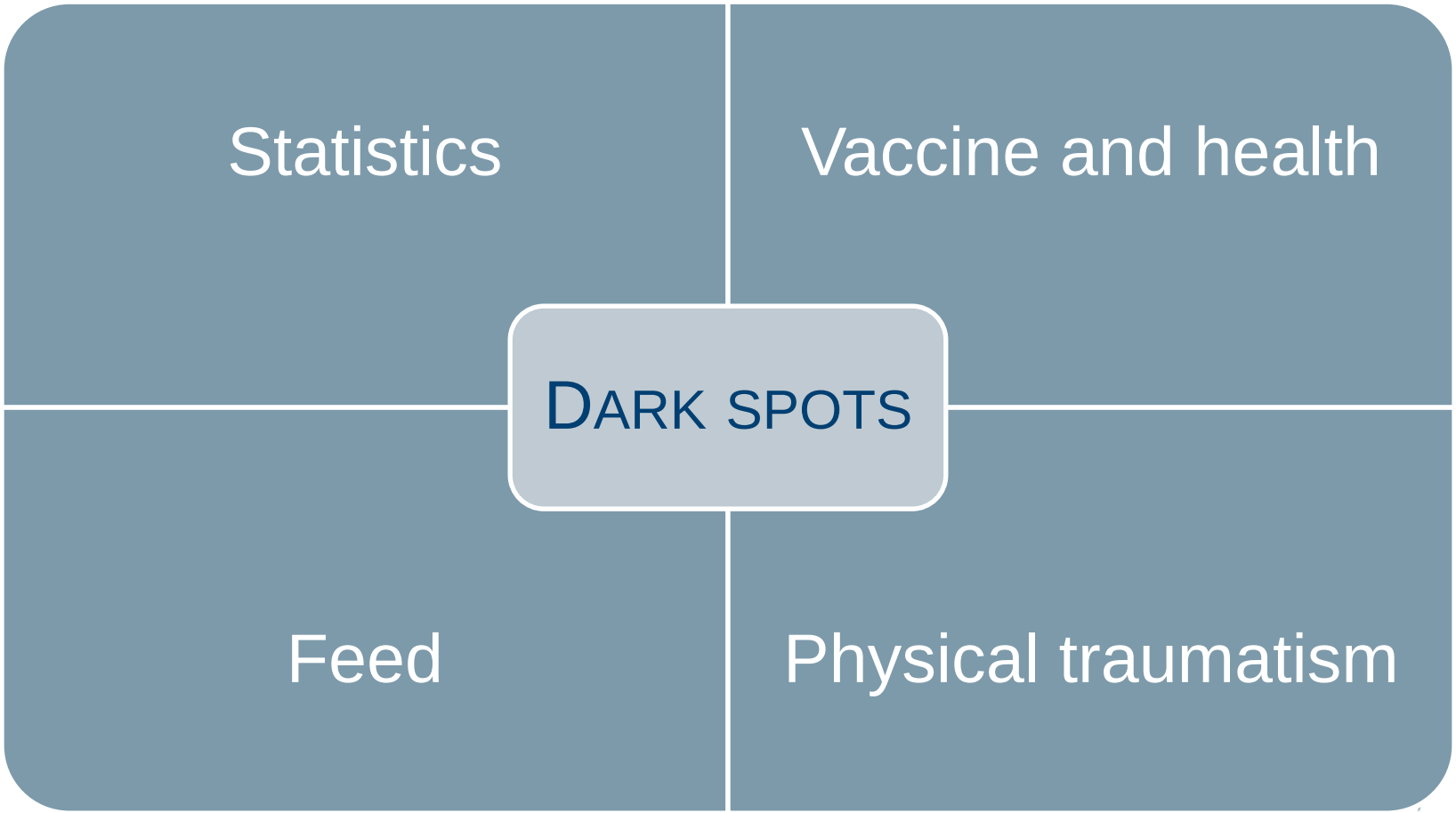
-Årsaker til forekomst og forebyggende tiltak

Prosjektbeskrivelse

Sammendrag:	*Går til:*
Det overordnede målet er å forhindre dannelse av mørke flekker i laksefilet. I dette ligger en søken etter årsaker til at flekkene oppstår for at kunne anbefale tiltak som kan bidra til å løse problemet. Aktivitetene i prosjektet er delt i fire arbeidspakker (AP): [1]Kartlegging, [2]Vaksine og helse, [3]Fôr og [4]Sortering og skade. Det vil være et nært samarbeid mellom AP1-4, som vil gå parallelt i perioden 2012 og ut 2014.	Fiskeri og havbruksnæringens forskningsfond

Rutinemessig kartlegging av forekomst av mørke filetflekker utføres av kvalitetskontrollører ved filetanlegg med geografisk spredning. Registreringene danner grunnlag for etterrettelig statistikk samt dybdeanalyse for å avdekke årsakssammenhenger. To basispopulasjoner med PIT-tag merket uvaksinert og vaksinert (ulike regimer) laks produseres: mullårssmolt (**BP0+**) og ettårssmolt (**BP1+**). Etter vaksinering undersøkes laksen jevnlig for mørke filetpigmenter frem til slakt. Produksjonsparametere, morfometri og blod analyses også. Mørke filetflekker undersøkes ved avbildende spektroskopi, foto, histologi, sammensetning og genuttrykk. Øvrige kvalitetsegenskaper undersøkes av utvalgt fisk. **BP0+** vil i en 3 måneders periode før slakt få et sluttfôr med og uten forhøyet sink, vitamin E eller fôrtoksiner (ulike vaksineregimer blandet i merder). **BP1+** vil undersøkes mht effekt av lavt sinknivå frem til vaksinering samt fra sjøutsett til slakt. I

Project topics



Parameters being studied in the project

Reliable and updatet statistics

Genetic background

Environment conditions

Vaccine

Health status / disease

Farming conditions

Dietary compostion

Slaughter procedure / stress

Organisation of the project

→ obtain broad information

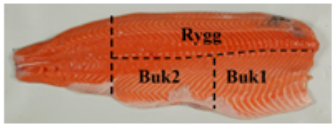
→ Define causes

Nofima

Registrations

Statistics

MELANIN I FILET (poeng)			
Ingen misfarging	0	Flekk 3 - 6cm	4
Diffus flekk, alle størrelser	1	Område større enn 6cm	8
Flekk mindre enn 3cm	2	Gjennomsnitt	0.00



Skjema sendes til thomas.larsson@nofima.no

Kommentarer	
	F. eks. om det er fisk med flere flekker innenfor samme område eller avvik for partiet (bløt filet, brusk, deformiteter)

Frivillige registreringer	
Utført: JA	
NEI	

		FLEKKER I FILET (poeng) flere flekker i samme område summeres			Blek, 0/1 (se fanen "Forklaring")	Gaping, 0/1 (se fanen "Forklaring")
Filet	Total poeng	Buk 1	Buk 2	Rygg		
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0

SPØRSMÅL SOM BESVARES FOR HVER GRUPPE FISK SOM BEDØMMES

Sett gjerne ? der informasjon mangler

Prosessanlegg navn/nr:				
Navn på bedømmer:				
Lokalitetsnr (navn):				*Kvalitetsklasse:*
Dato ved slakt:		Sjøtemp:		Superior
Dato ved måling:		Merd nr:		Ordinær
Vektklasse:		Lot nr:		Produksjon

TILLEGGSINFORMASJON

Smolt					
Leverandør:					
Utsett *måned:*		*år:*		Stamme:	
Maskinvaksinert: JA				Vaksinetype:	
NEI				Vaksineringstemp:	

Forklaring | Registreringsskjema

Klar

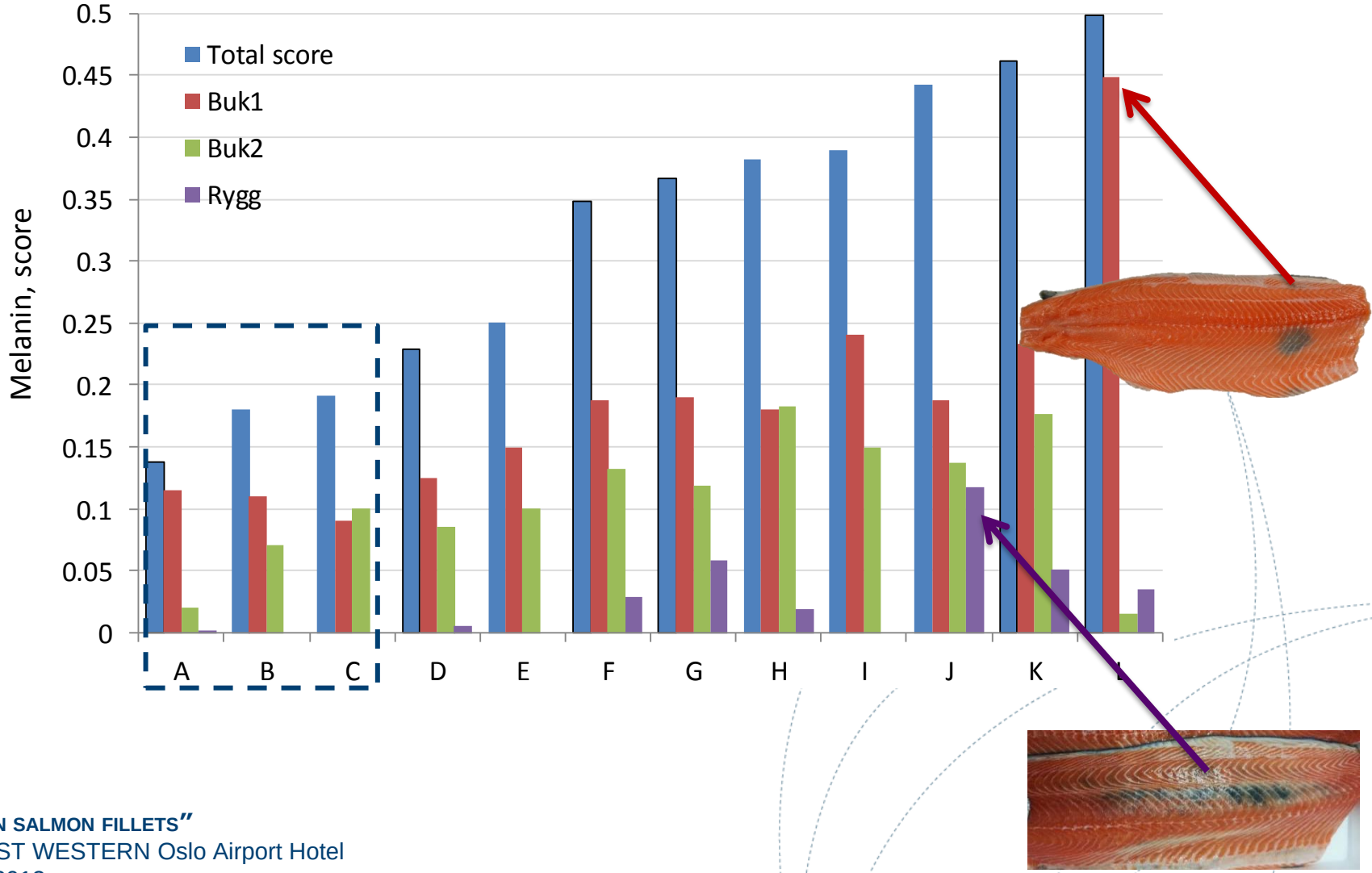
Seawater farms
- Updated June 2013

Statistics

All background data are anonymised

Nofima

Smolt producer – Mid-Norway



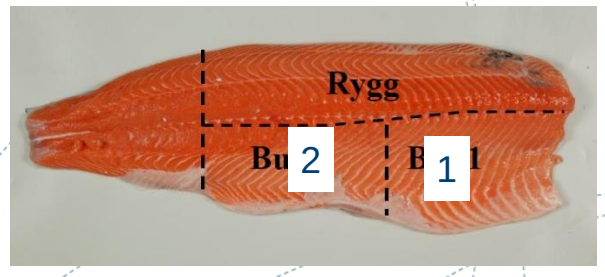
Dark spots - Development

Statistics

	Frequency	Belly flap	Dorsal muscle	Number reg.
2011	13.4%	12.6%	0.8%	35.000
2012	16.1%	15.3%	0.8%	25.000
2013 (- okt)	17.9%	16.1%	1.8%	36.000

Mid- and South: 17-20% (June 2013)
Belly flap **1** + 2

North 13% (June 2013)
Belly flap 1
- but frequency of dark pigmented dorsal muscle increasing in 2013



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Total score
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
1
0
1
2012
2013
0+ smolts higher than 1+smolts
Dorsal muscle

Spots

Statistics

More frequent
in South-Mid Norway

Most frequent
(70% of all spots)

Causes largest problems

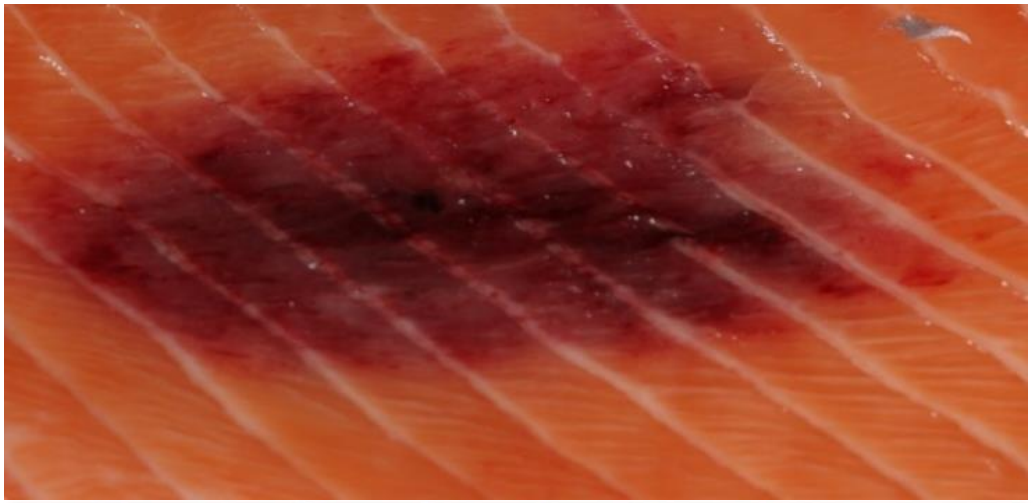
Nofima

Spots

Nofima

Dark spots

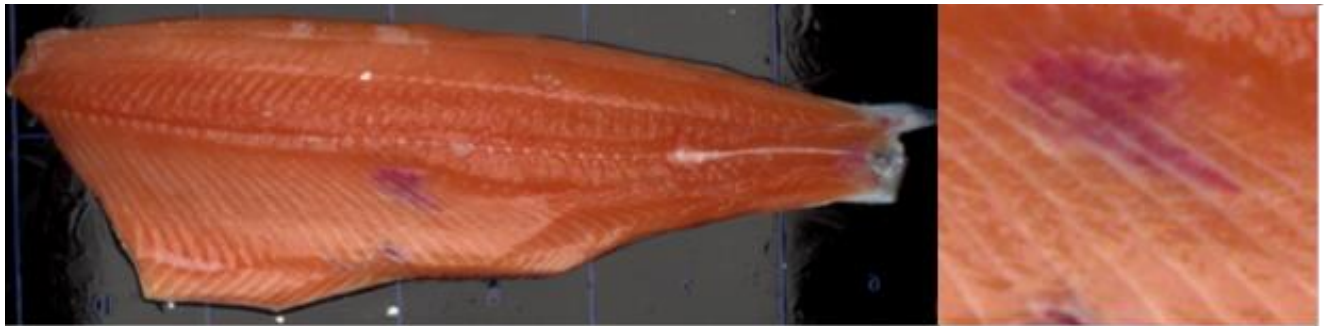
May be difficult to distinguish between blood and melanin



«Red spot»

«Black spot»

Development ?

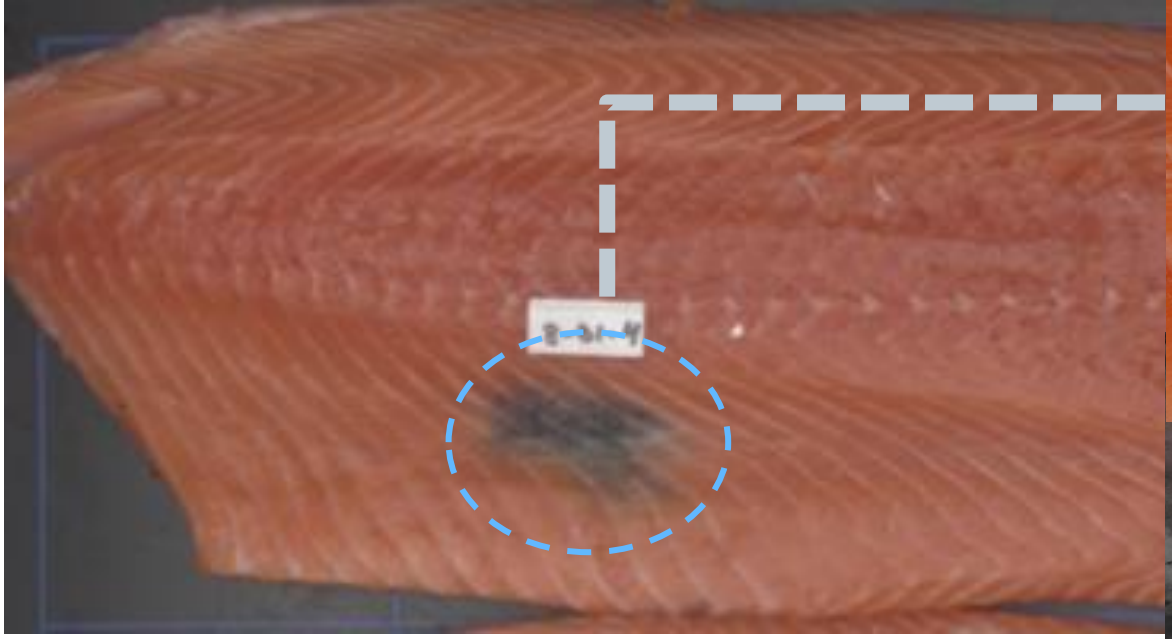


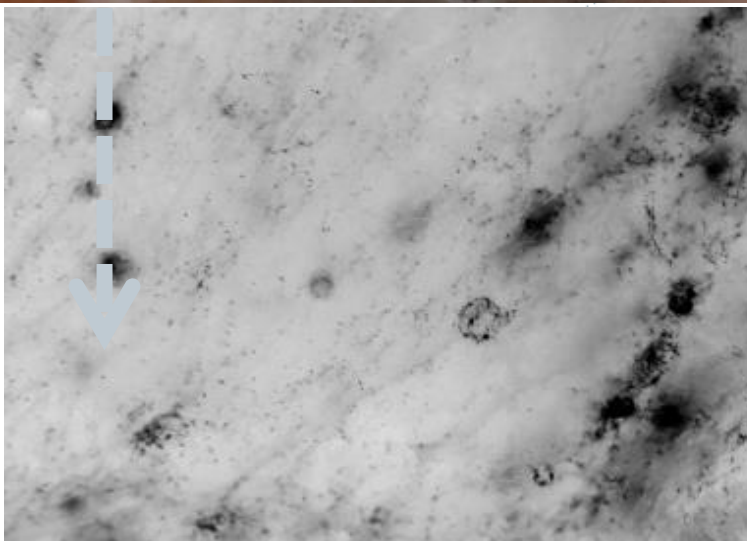
Blood

Melanin

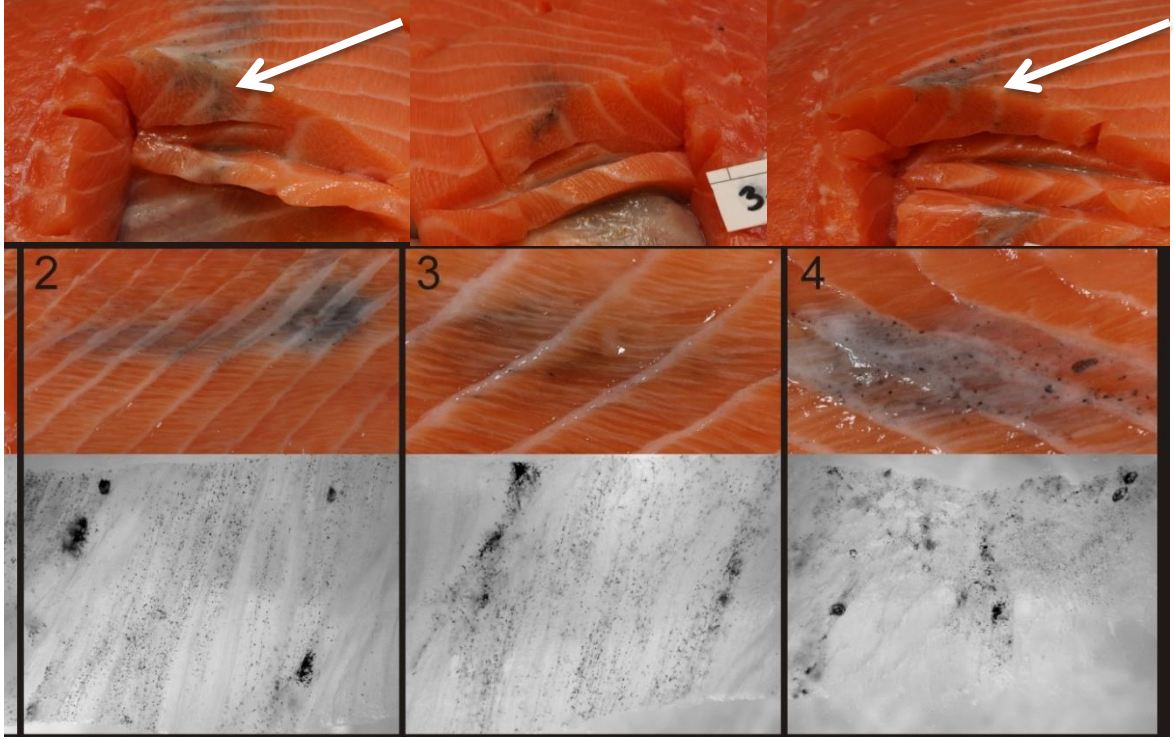
Scar tissue

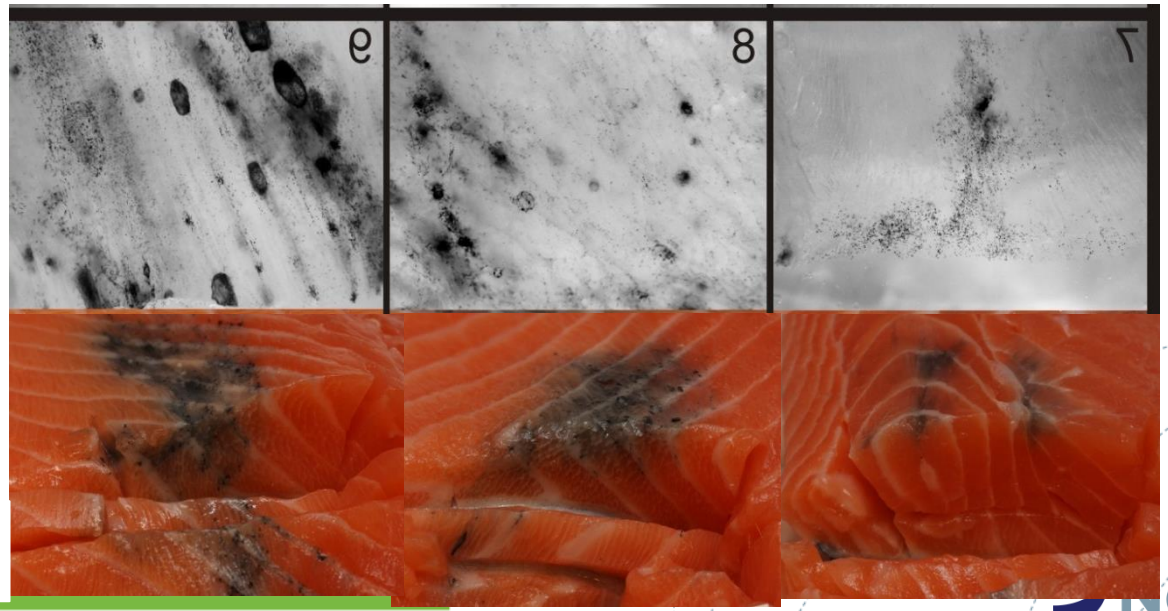
Nofima





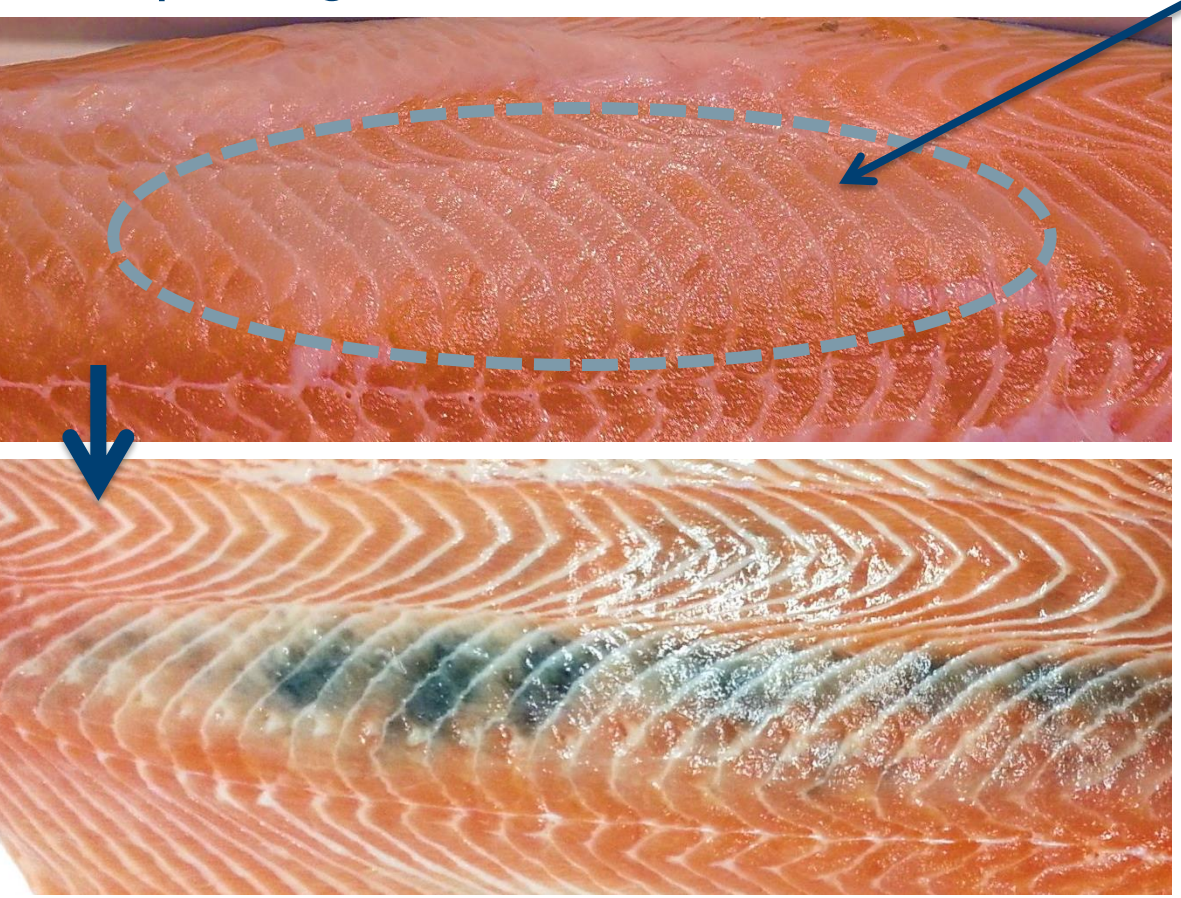
Magnification

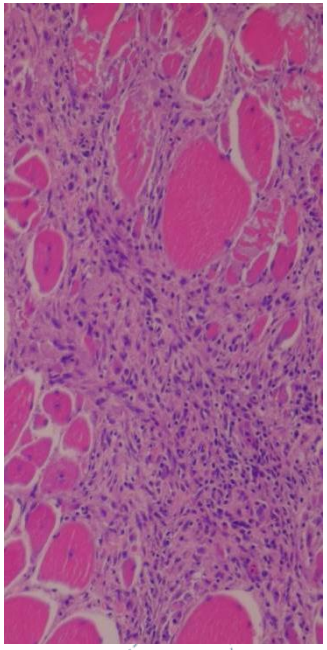

3
2
3
4


9
8
7

Inflammatory condition, dorsal muscle
- no pathogens detected

Pale, gelly like





Increasing frequency coinciding with abrupt temperature rise
Similar conditions seen after PD outbreak

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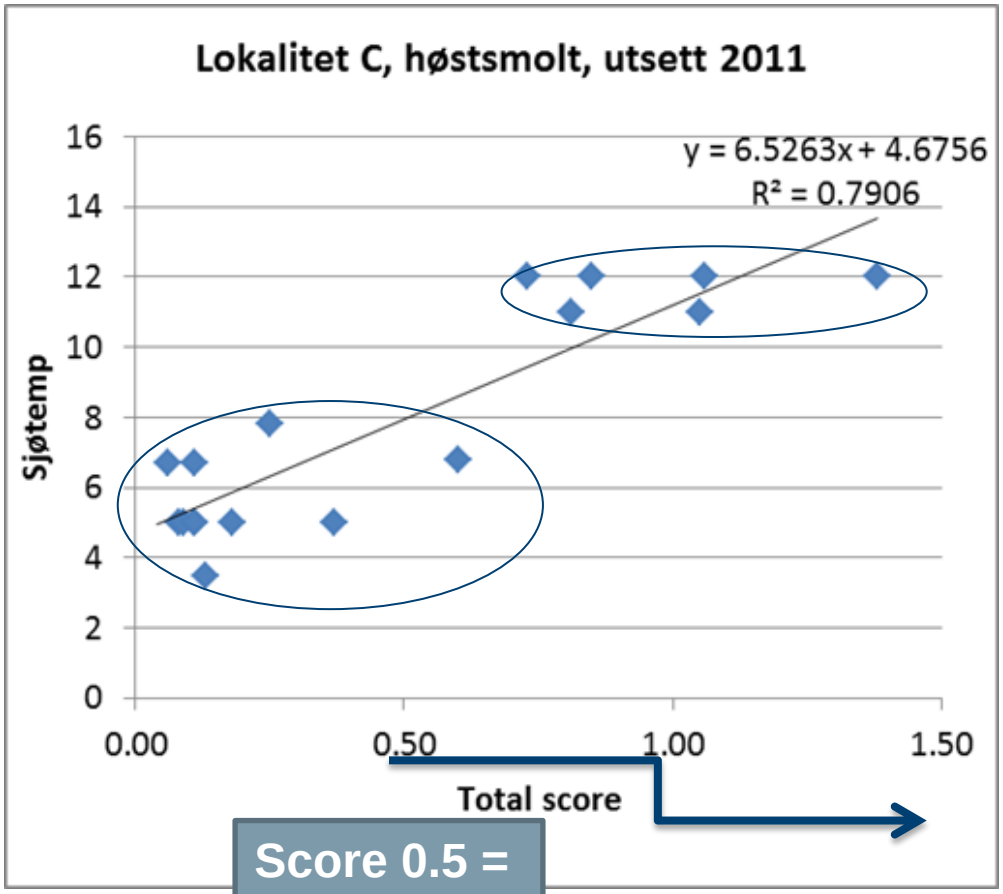
Seawater temperature



Score 0.5 = very high

Temperature induced stress?

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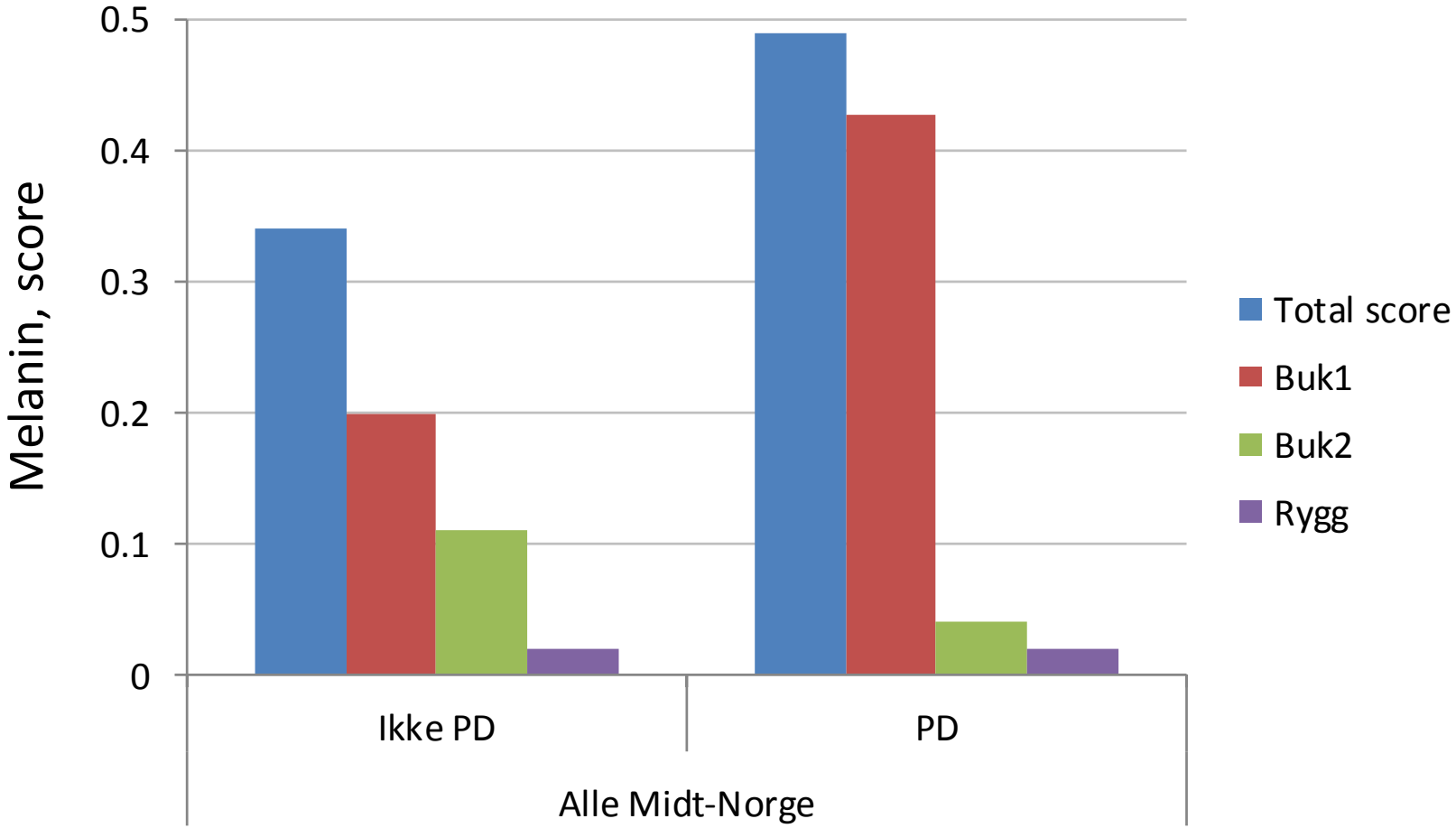
PD
Other diseases?



- **The impact of health status / disease will be studied further**
- **VI has the responsibility of the diagnostics of selected farms in the project**


- Bleeding in the internal musculature afture external physical traumatism, mimicing pumping, but only minor bleeding in the muscle under the skin. The depth of the haemorrhages varied
- Follow up on the impact of stress and mechanical injuries/ physical traumatism (focus on the impact of sorting fish)

Stress

- **Mechanical**: sorting, injury
  - Does severe muscle contractions cause bleeding→pigment deposition
- **Disease**: stress may induce over-growth of «natural» bacteria.
  - Shown for e.g.aeromonas in carp, causing bleeding (hematoidin deposition)
  - Increased growth of such bacteria at higher temperatures
- **Environment**: oxygen level, temperature variations, salinity
- **Feed**: pro-oxidants etc may leed to increased level of haemociderin, lipofuscin,melanin
  - Macrofages from the head kidny may cause severe deposition when «cleaning up» affected areas
  - Feeding study: will be possible to study interaction between vaccine/feed/stress

"Studies on stress and innate immunity in relation to infectious pancreatic necrosis virus in Atlantic salmon" 2012

Norges veterinærhøgskole

OPEN ACCESS Freely available online

PLOS ONE

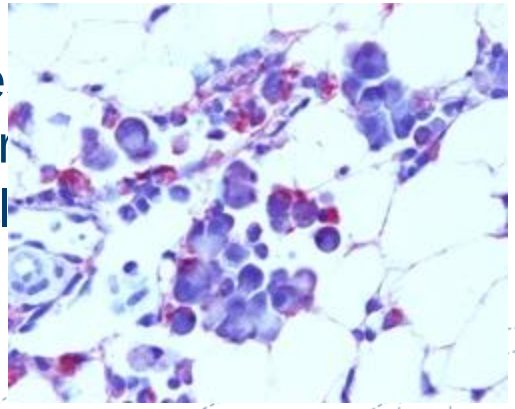
Stress-Induced Reversion to Virulence of Infectious Pancreatic Necrosis Virus in Naïve Fry of Atlantic Salmon (*Salmo salar* L.)

Koestan Gadan[1☯], Ane Sandtrø[1☯], Inderjit S. Marjara[1], Nina Santi[2], Hetron M. Munang'andu[1], Øystein Evensen[1*]

1 Norwegian School of Veterinary Science, Oslo, Norway, 2 Aqua Gen AS, Trondheim, Norway

yt kortisolnivå over
ormering av virus
gså vist at
l resulterer i at

ellers ufarlige varianter av IPN-virus endres til sykdomsfremkallende virus. Med andre ord: stre immunforsvaret, øker «produksjonen» av IPN-vi indre organer og kan føre til «overgang» fra "snil varianter av virus.



Aeromonads, Mycobacteriosis +++ .are other examples

Dietary impacts

- Bleeding / coagulation
  - Nutritional imbalance
  - Fatty livers→ the fish more vulnerable to stress?
  - Relevant minerals: Zn, Cu, Fe (coagulation factors)
  - Need more information on vascularisation
    - Why diferences between the belly part and the tail

Ungoing project

40 grams smolt before vaccination

Summary

The problem is increasing

Several causes to dark pigmentation of salmon fillets

- **Inflammation**
  - PD (SAV3 vs SAV2?)
  - Vaccine/ vaccination
  - Stress induced (e.g.temperature?)
- **Bleeding**

Geographic variation

Severe variation between farms

Higher in 0+ than 1+ salmon

Not found systematic differences

  - Feeding companies
  - Breeding companies
  - Vaccine producers

Relationship between melanin deposition in various tissues: organs, peritoneum, fillet

Jens Erik Dessen & Thomas Larsson, Nofima

Workshop - Dark spots in salmon fillets

BEST WESTERN Oslo Airport Hotel, Gardermoen

November 5th 2013

Melanin in organs, abdominal wall (peritoneum) and fillet

- Increase knowledge
- Possible implications for industry
  - Demanding markets
  - Sorting

Nofima

Quality classes
- Superior

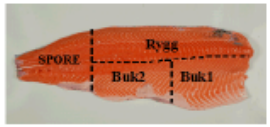
Produktet har følgende karakteristikker ved pakketidspunktet:

a) glansfullt skinn uten betydelig skjelltap,
b) ingen gjengrodde sår som reduserer helhetsinntrykket
c) ingen åpne sår
d) ingen bloduttredelser eller skader i buk eller muskulatur
e) ingen melaninflekker i muskulatur
f) hel, fast bukhinne
g) hele eller avhelte (gjengrodde) finner
h) naturlig strømlinjeformet fasong
i) ingen vesentlige blødninger i skjellommene ved gattfinnen eller under bukhinnen

National registration form

Melanin i filet (score)			
Ingen misfarging	0	Flekk 3 - 6cm	4
Grå skygge	1	Område større enn 6cm	8
Flekk mindre enn 3cm	2	*Gjennomsnitt*	*0.24*

registreringene sendes til turid.morkore@nofima.no



BESVAR SPØRSMÅL⇒

		Melanin i filet				Høyre=1	% blek	% gaping
Filet	Total score	Buk 1	Buk 2	Rygg		Venstre=2	Blek, 0-1	Gaping, 0-1
1	0	0	0	0				
2	0	0	0	0				
3	1	1	0	0				
4	0	0	0	0				
5	1	1	0	0				
6	1	1	0	0				
7	0	0	0	0				
8	0	0	0	0				
9	0	0	0	0				
10	0	0	0	0				
11	0	0	0	0				
12	0	0	0	0				
13	0	0	0	0				
14	0	0	0	0				
15	0	0	0	0				
16	0	0	0	0				
17	0	0	0	0				
18	0	0	0	0				
19	0	0	0	0				
20	0	0	0	0				
21	0	0	0	0				
22	0	0	0	0				
23	1	1	0	0				
24	1	1	0	0				
25	0	0	0	0				
26	0	0	0	0				
27	0	0	0	0				

SPØRSMÅL SOM BESVARES FOR HVER GRUPPE FISK SOM BEDØMMES

Navn på prosessanlegg					
Navn på bedømmer					
Lokalitetsnavn (nr)					
Dato ved slakt	07.10.2011	Sjøtemp			
Dato ved måling/klokker	11.10.11 15.40	Superior		Ordinær	
Vektklasse	4-5.	Merd nr		Lot nr.	

TILLEGGSINFORMASJON

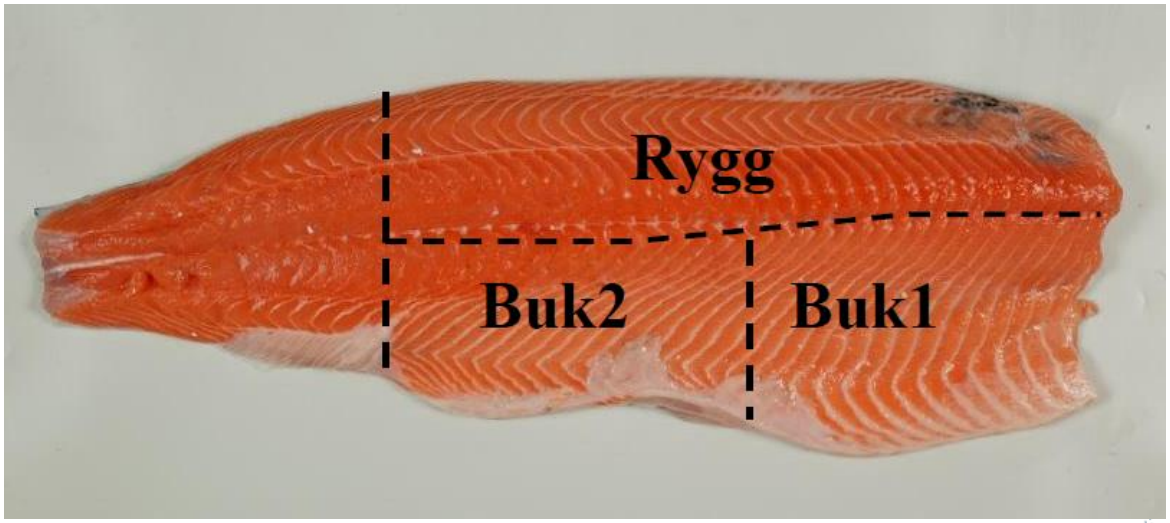
Smolt				
Leverandør:				
Utsett, mnd/år:		Stamme:		
	NEI	JA		
Maskinvaksinert			Vaksinetype:	

Slaktefisk

Sultetid, dager:		Fôrtype før slakt*:		
	NEI	JA	NEI	DAGER
Brønnbåt		X	Ventemerd	1
			NEI	JA
Avvik for partiet	NEI	JA	Brusk/deform	
Bløt filet			Annet:	
	Slag	CO_2	Strøm	Annet
Avliving			X	
	Vet ikke	Nei	Ja	Navn/tidspkt/dødelighet[2]

National registration form

Filet areas

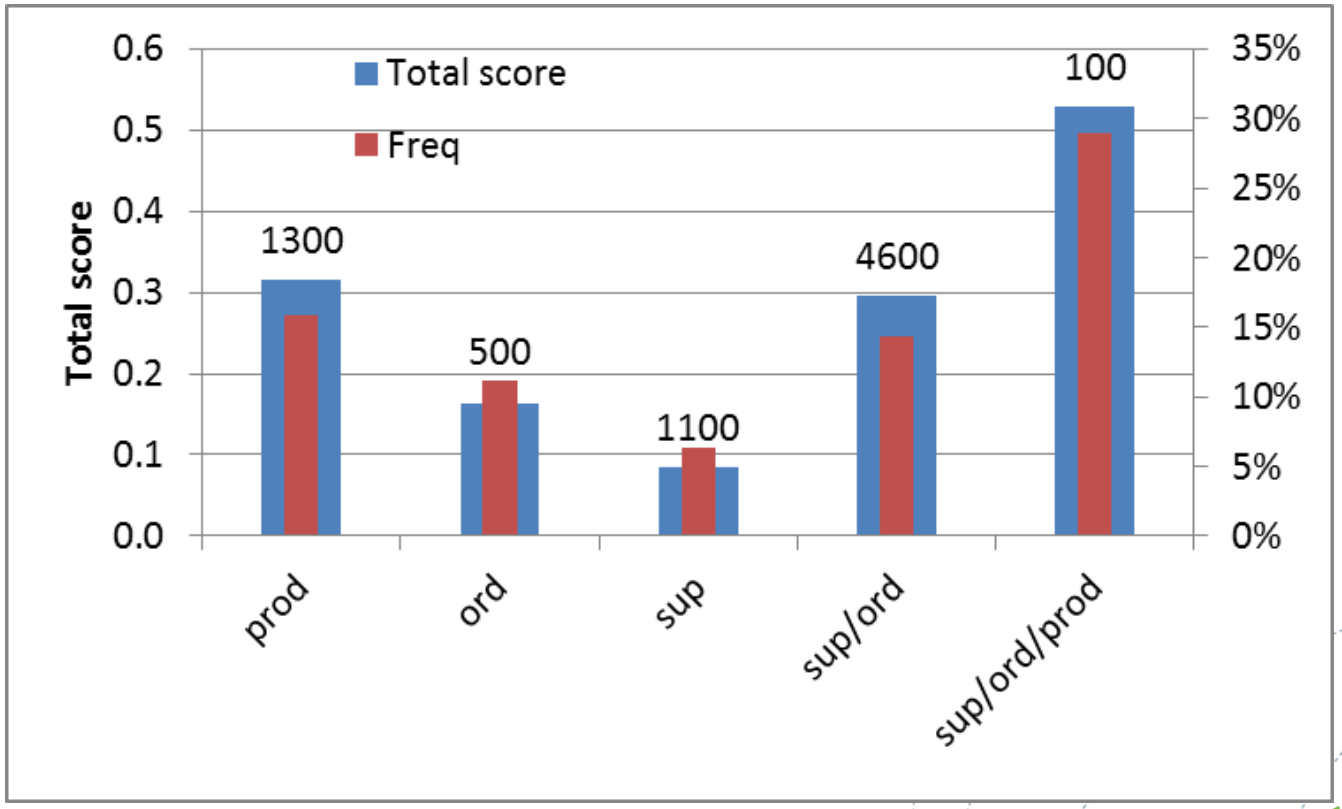


Scale

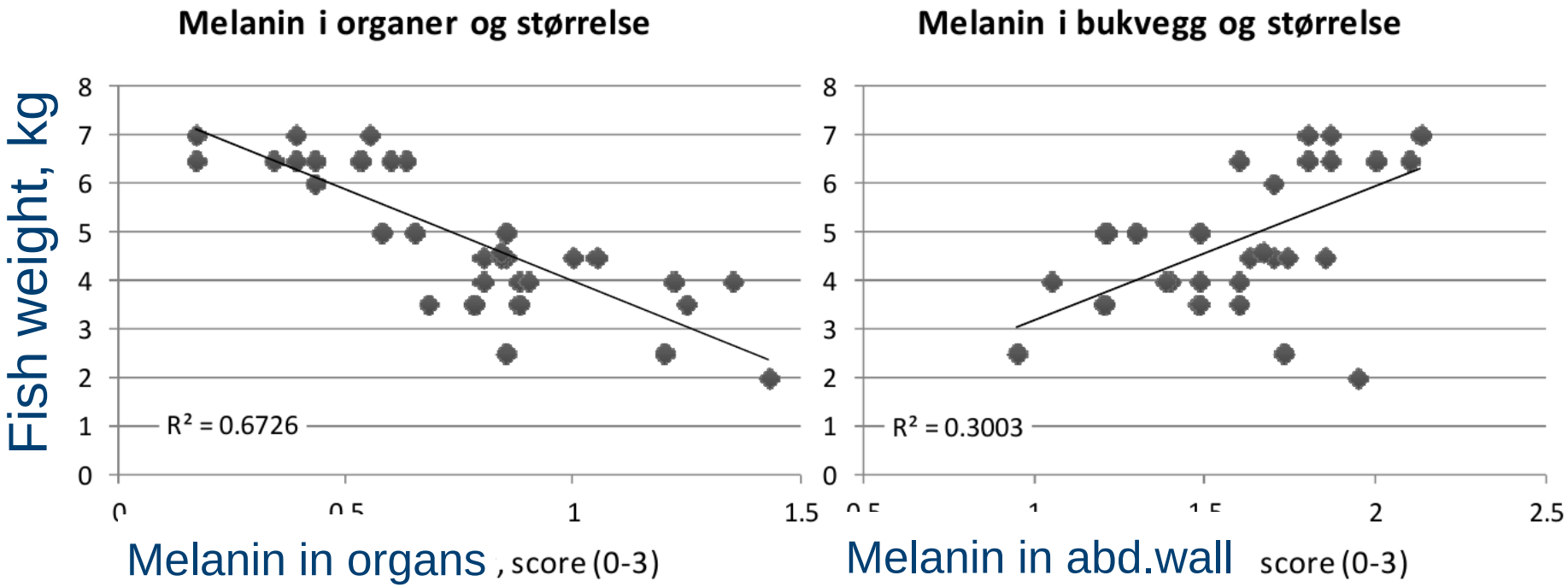
Melanin i filet (score)			
Ingen misfarging	0	Flekk 3 - 6cm	4
Grå skygge	1	Område større enn 6cm	8
Flekk mindre enn 3cm	2		

Quality classes – dark spots in fillet

- Tendency of higher frequency of dark spots in fillets of Production fish
- No clear differences between Superior / Ordinary



Organs and abdominal wall



Melanin in organs , score (0-3)

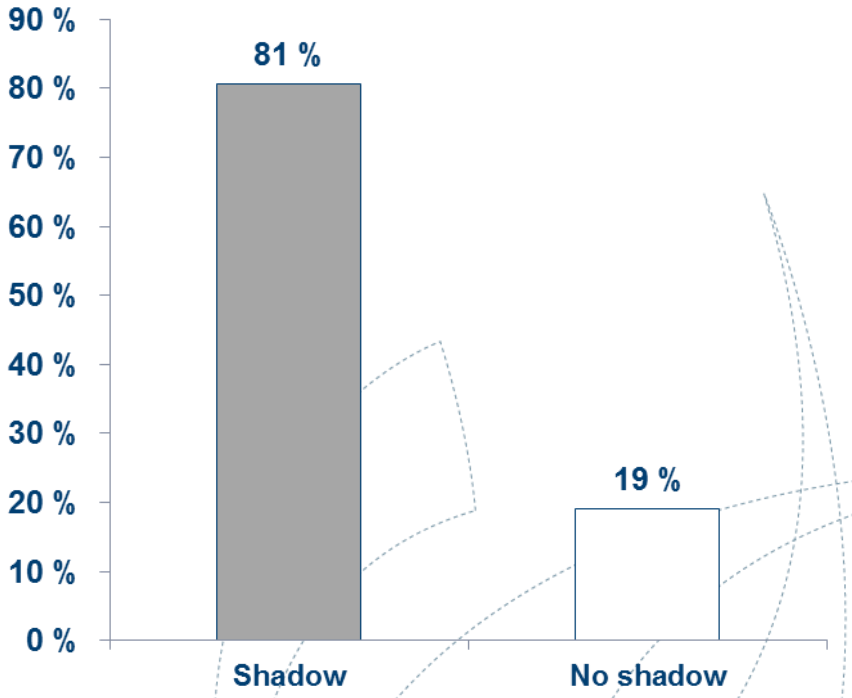
Melanin in abd.wall score (0-3)

- On individual basis (n = 915), the correlation between melanin in organs and abdominal wall = 0.24

Relationship between dark spots in abdominal wall and fillet
- preliminary results

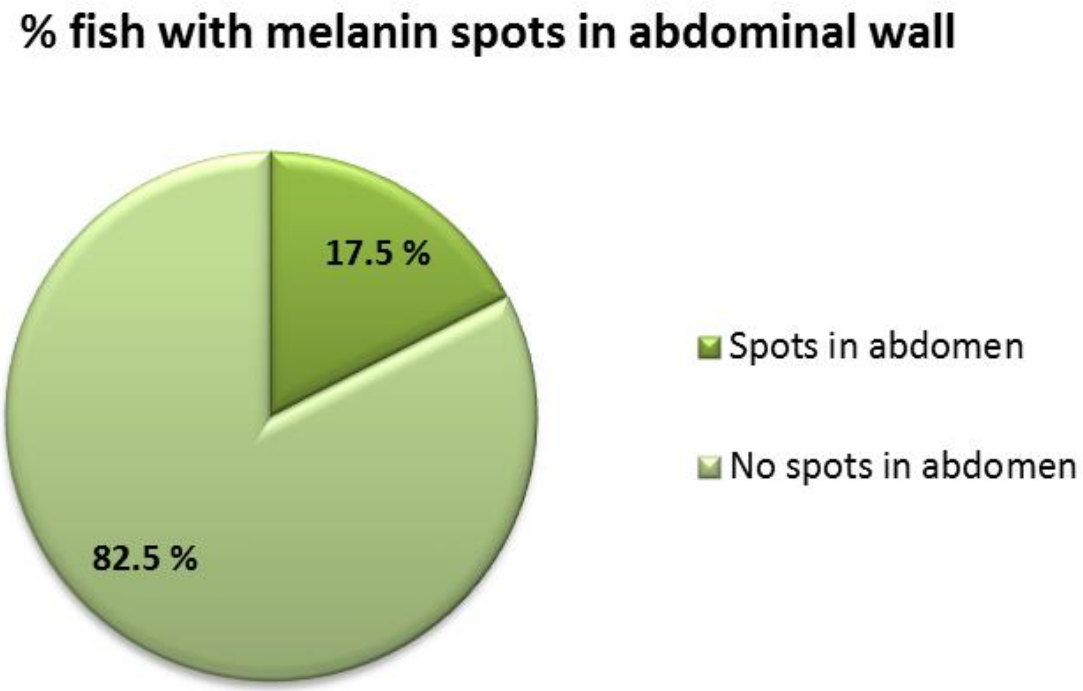
Relationship between dark spots in abdominal wall and fillet - Test 1

- Class: Superior (3.7 – 5 kg)
- 400 fish, visually evaluated before and after trimming
- «Shadow» in the abdominal wall and clear spots in the abdominal wall and fillet were recorded

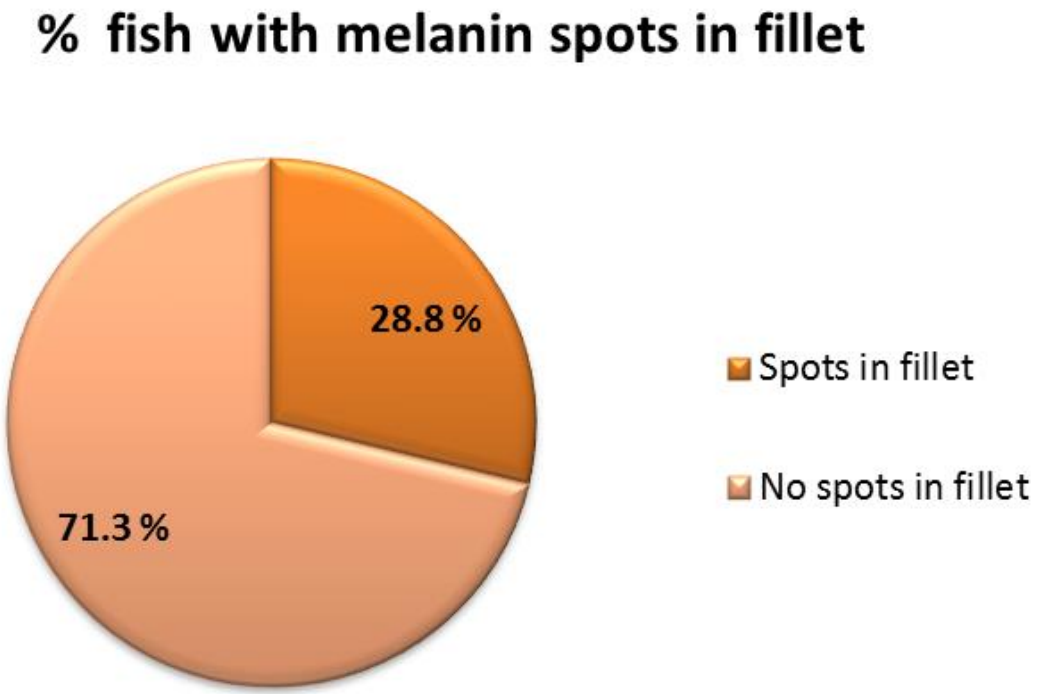


Nofima

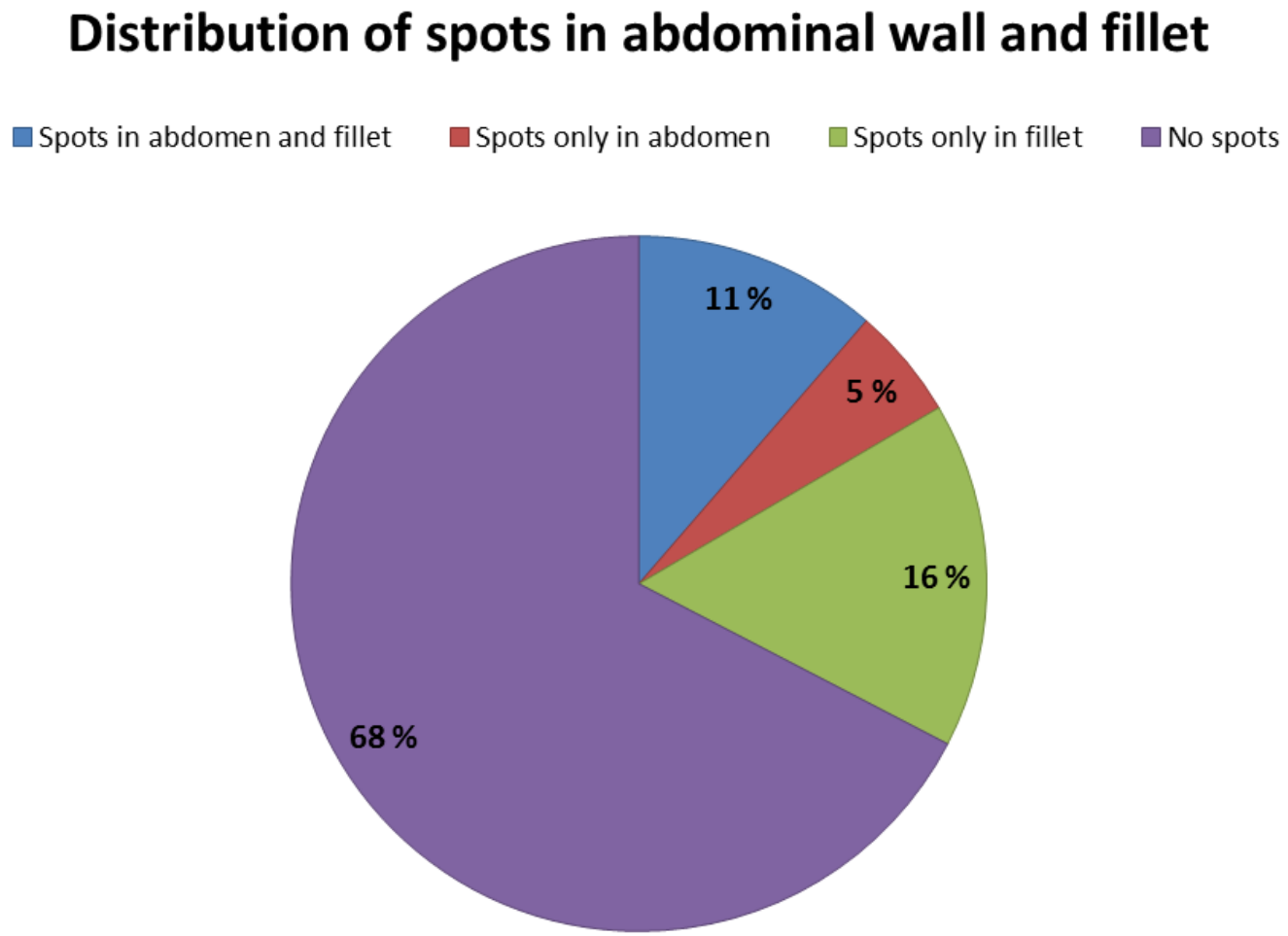
Relationship between dark spots in abdominal wall and fillet - Test 1



Relationship between dark spots in abdominal wall and fillet - Test 1

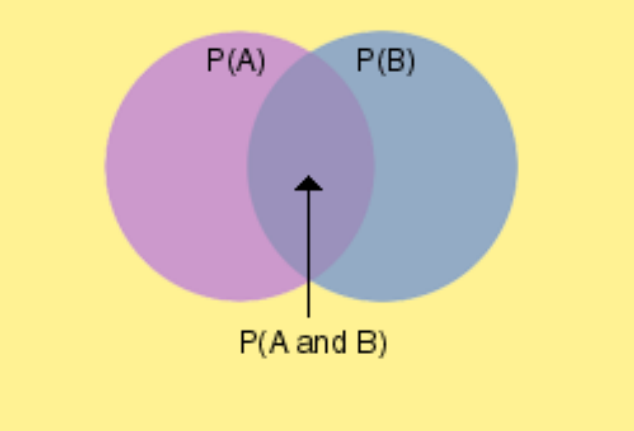


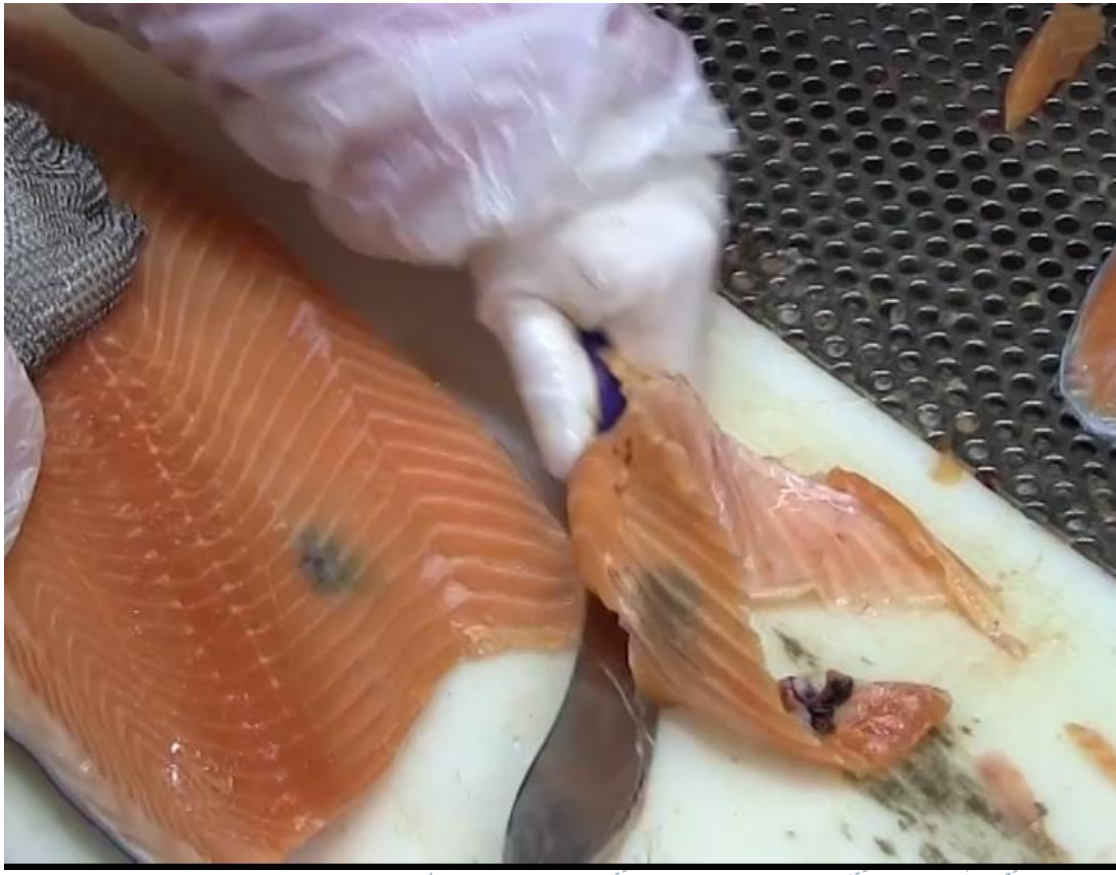
Relationship between dark spots in abdominal wall and fillet - Test 1



Probability – test 1

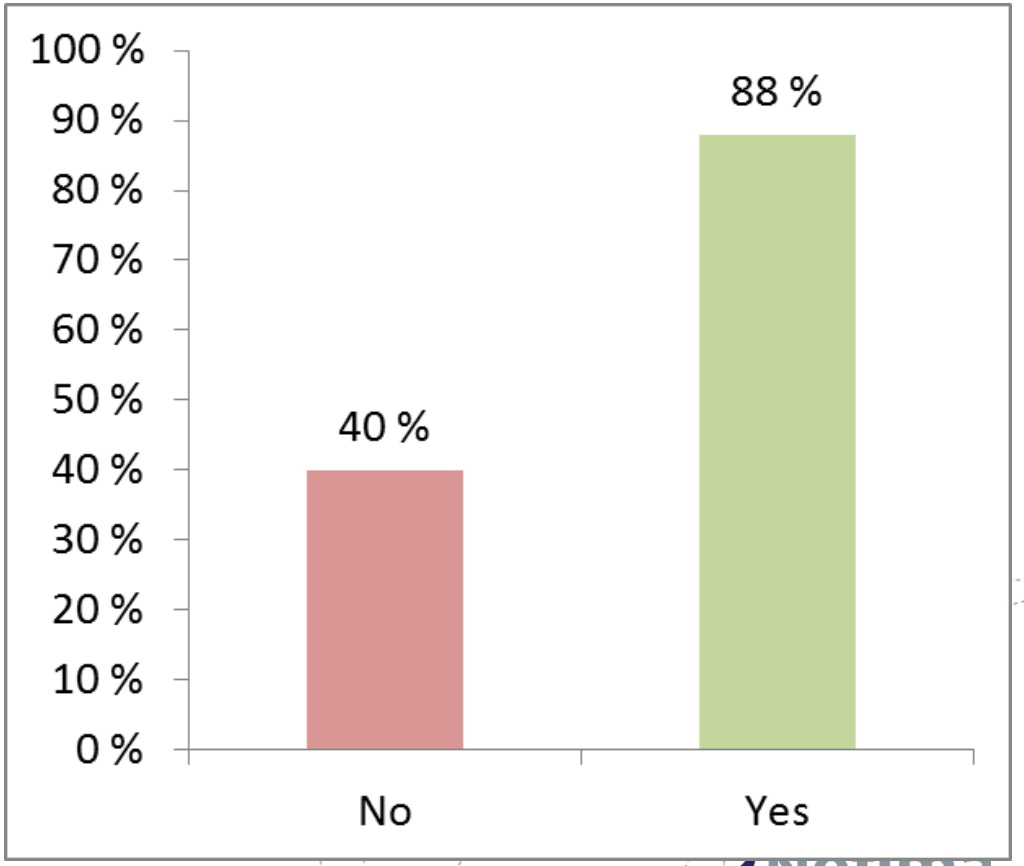
- Probability of having a spot in the fillet when there is a spot detected in the abdominal wall = 0.69
- Probability of having a spot in the abdominal wall when there is a spot in the fillet = 0.41





Relationship between dark spots in abdominal wall and fillet - Test 2

- Class: Superior/Ordinary (4-5 kg)
- 50 fish with (= YES) and 50 without (= NO) spots in the abdominal wall
- Visually evaluated before and after trimming
- Highly significant difference



Preliminary conclusions

- No clear relationship between melanin in organs and abdominal wall
  - But appear to have different causes
- Higher frequency of dark spots (and perhaps also larger spots) in muscle than in abdominal wall
- Sorting based on dark spots in abdominal wall may be important for high quality products


Thank you!!