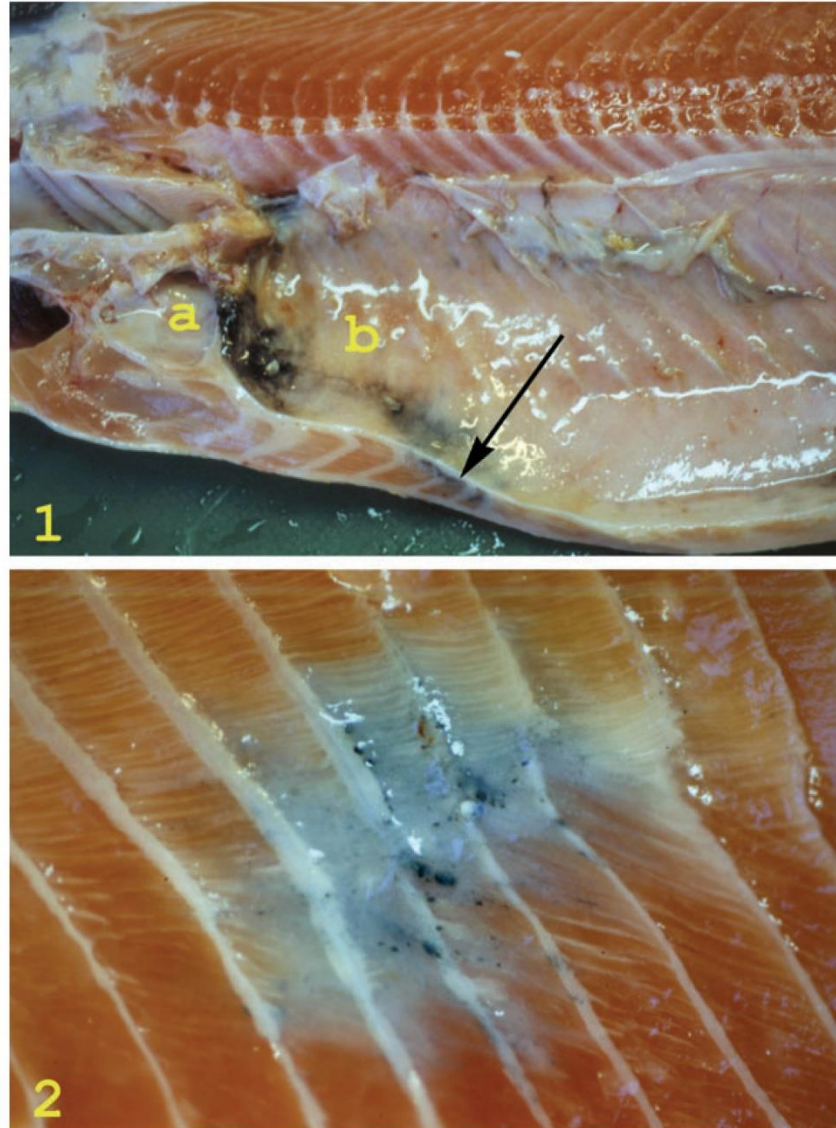


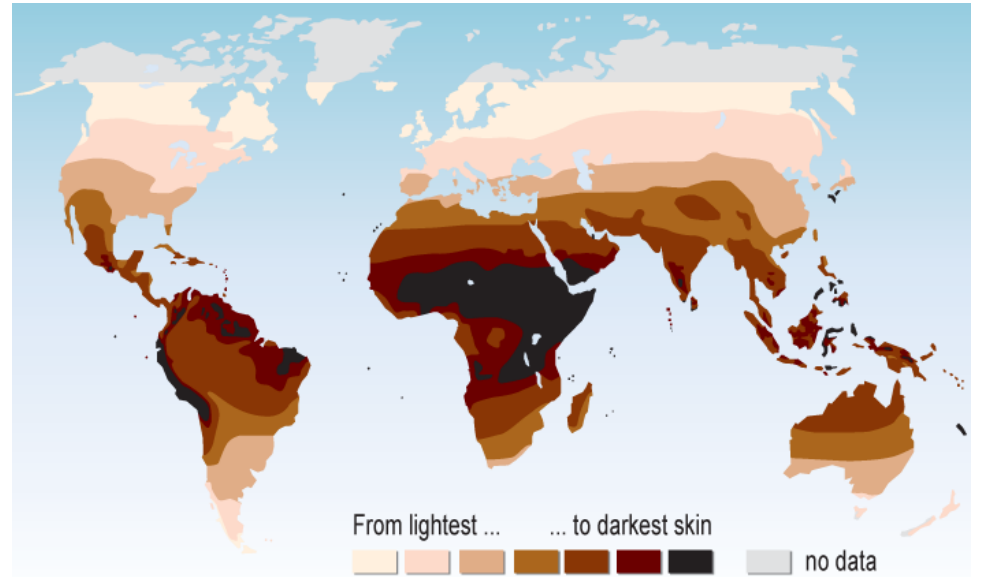
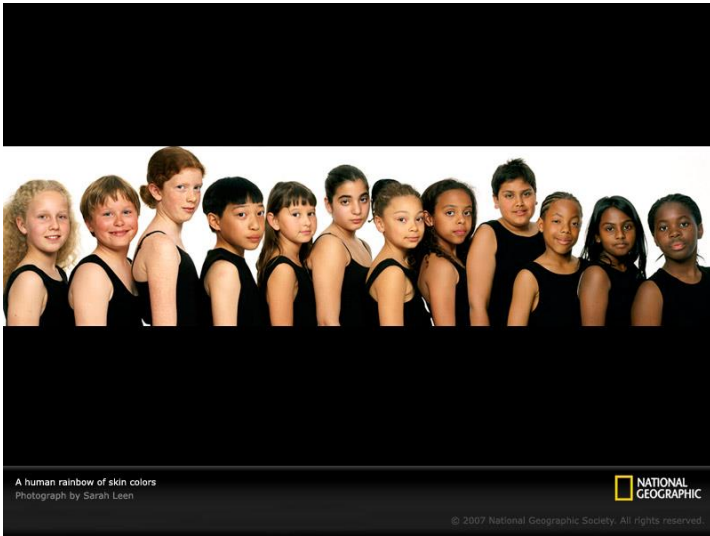
# Melanin

Erling Olaf Koppang  
Veterinærhøgskolen NMBU  
Oslo



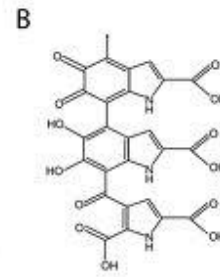
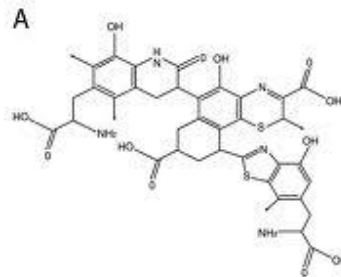
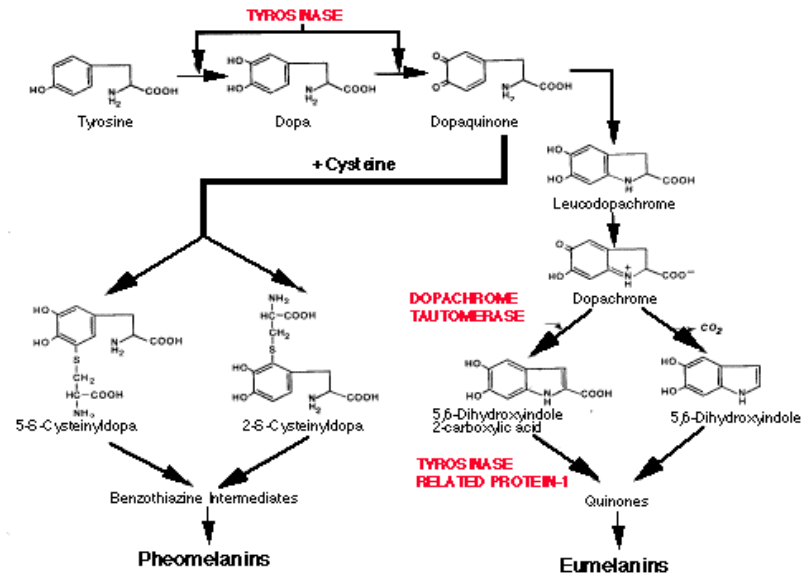
**Figure 1** Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).

**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.

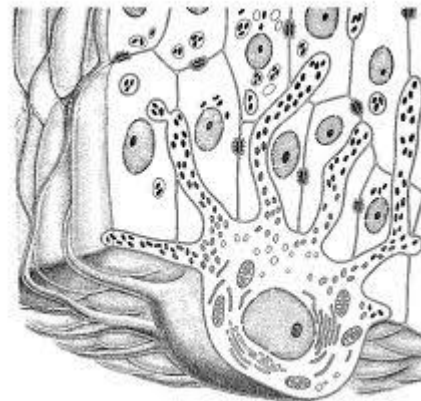
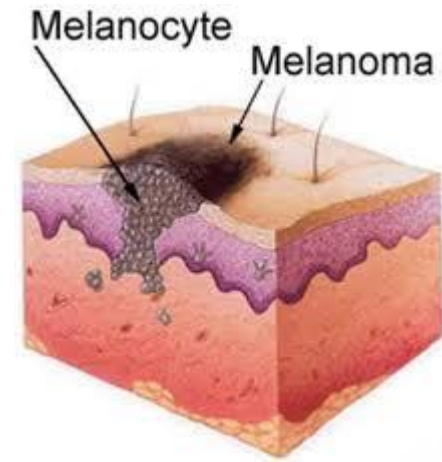
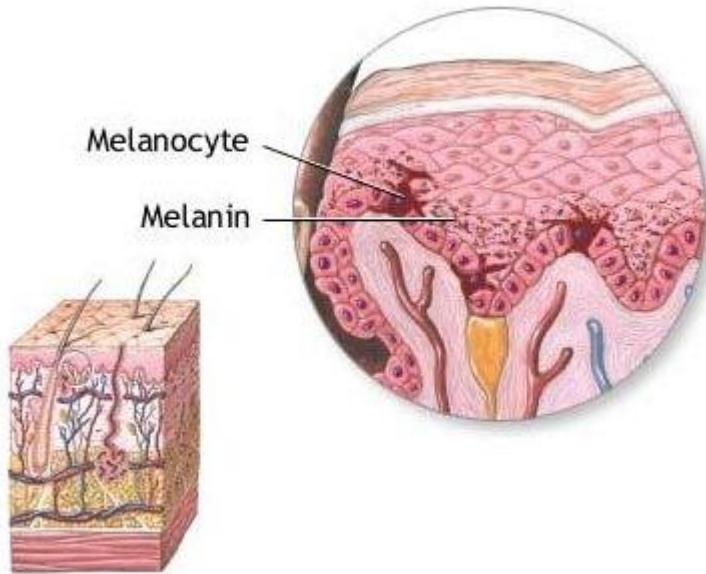


# 1. Hva er melanin?

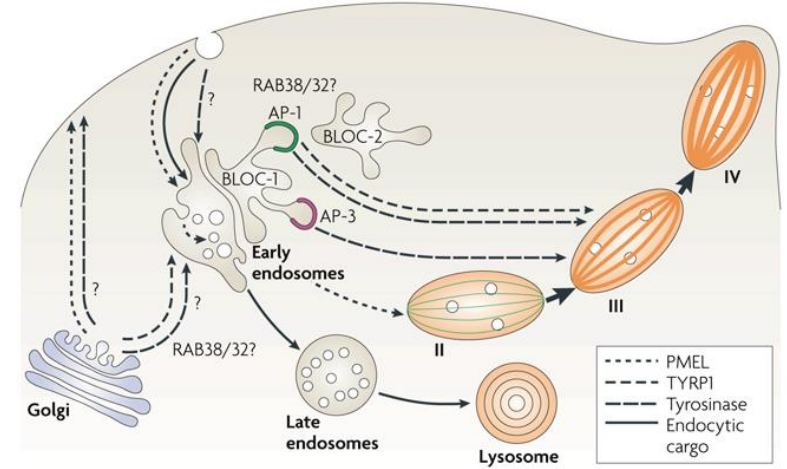
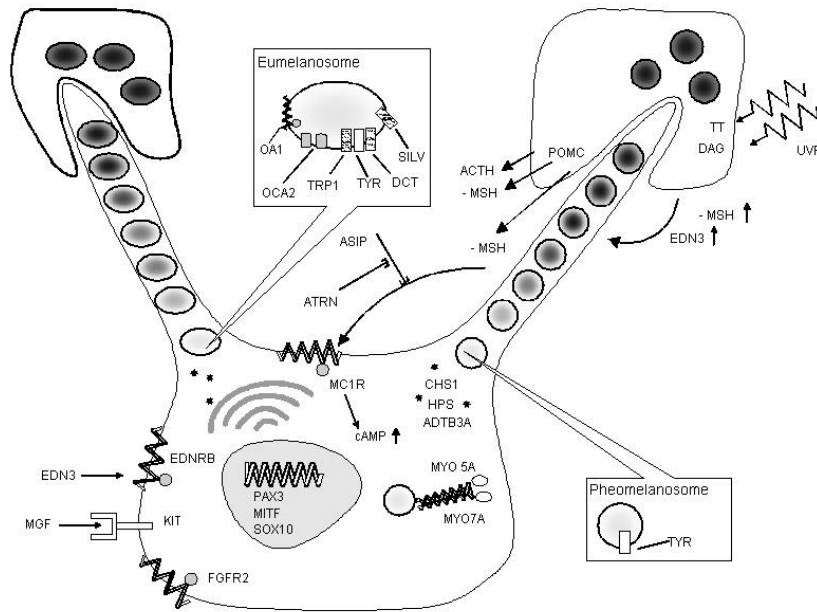
## The Melanin Chemical Pathway



# Hvor dannes melanin?

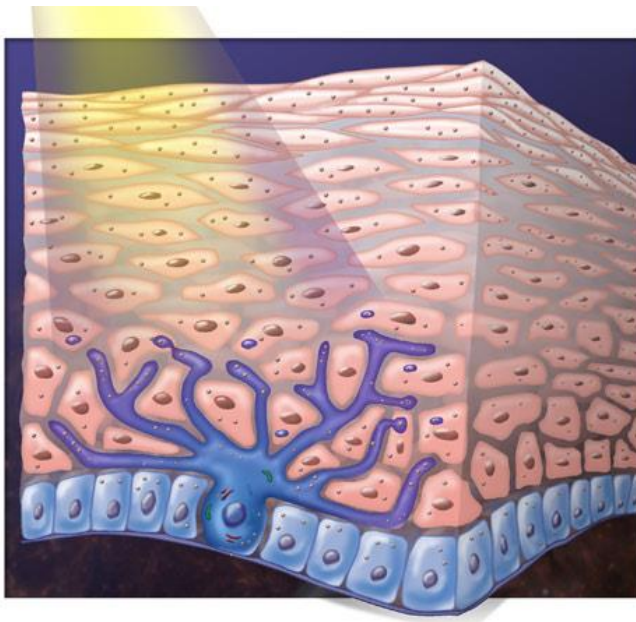
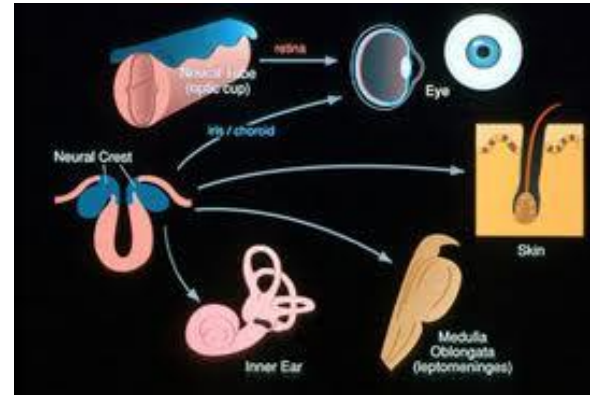
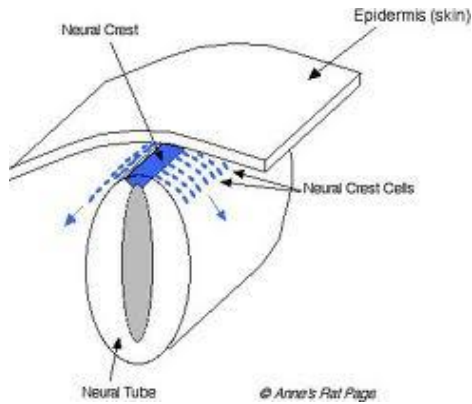


# Melanosom: den intracelluære melaninfabrikken



Nature Reviews | Molecular Cell Biology

# Hvor finner vi melanocytter?



- Hud: Beskytte mot sol.
- Øye: Beskytte mot sol og regulering av lys.
- Hjerne: ??
- Øre: ??

## 2. Hvilke funksjoner har melanin?

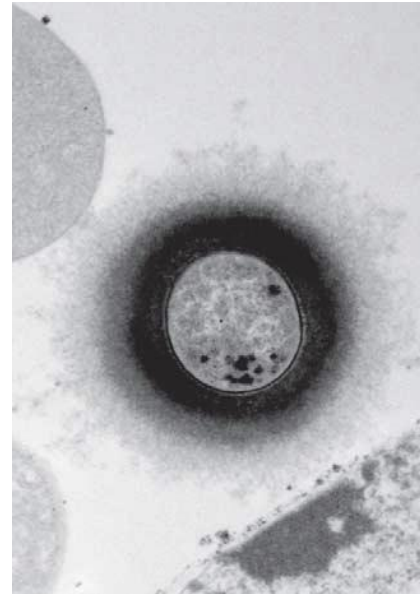




## 2.b. Betennelse og melanin



Christensen et al.  
Trends Parasitol. 2005;21;192



# Hva er funksjonen?

- Infeksjoner ikke studert
- Så langt: antioksidanter – anoxi!
- Binder kobber og jern
- Binder en rekke toksiske komponenter

# Foreløpig oppsummering

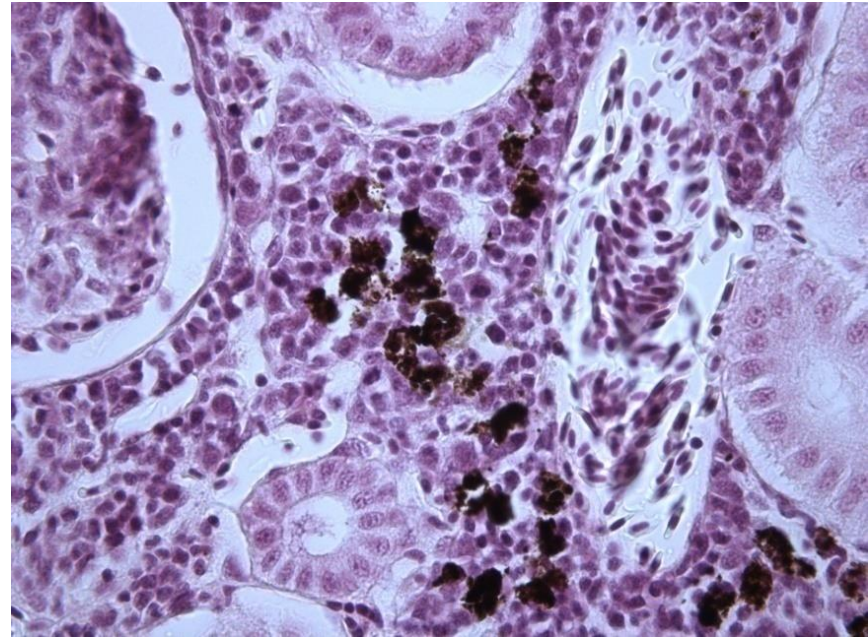
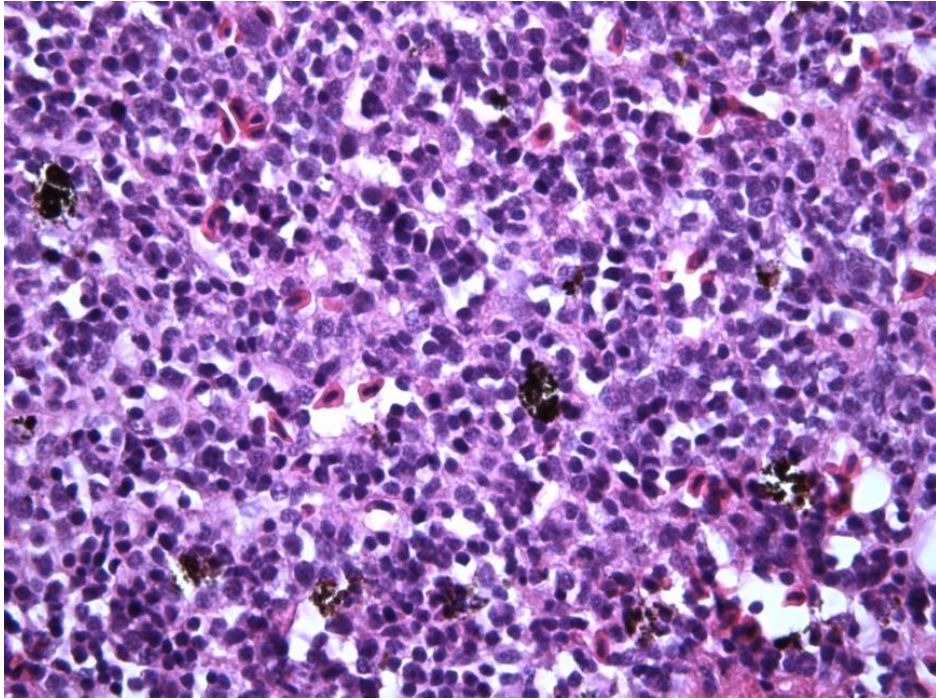
- Melanin er en et makromolekyl som har antioksydativ effekt
- Melanin kan binde og inaktivere toksiner, jern og kobber
- Hos varmblodige virveldyr dannes melanin i celler av ectodermal opprinnelse
- Hos amfibier kan melanin også dannes i cellepopulasjoner av mesenchymal opprinnelse

# Fisk og melanin

## Tilbake til hovedsaken: Melaninflekker hos laks

- Men først: Hva er de normale forholdene hos laks?







# Mørke flekker - utvikling

	Frekvens	Buk	Rygg	Antall reg.
<b>2011</b>	<b>13.4%</b>	<b>12.6%</b>	<b>0.8%</b>	<b>35.000</b>
<b>2012</b>	<b>16.1%</b>	<b>15.3%</b>	<b>0.8%</b>	<b>25.000</b>
<b>2013</b>	<b>17%</b>	<b>16.1%</b>	<b>1.8%</b>	<b>40.000</b>

Midt- og Sør:

17-20%

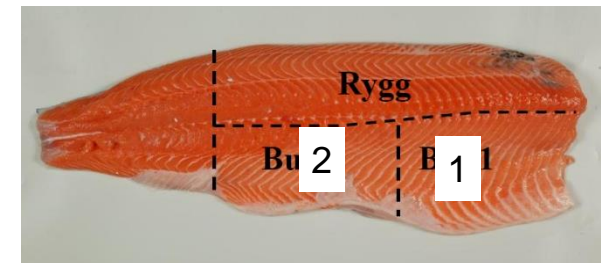
Hovedsakelig Buk 1 + 2

Nord

13%

Hovedsakelig Buk 1

- men frekvensen av mørke områder økt i ryggmuskel  
i 2013



## Melanogenesis and evidence for melanosome transport to the plasma membrane in a CD83<sup>+</sup> teleost leukocyte cell line

Erlend Haugarvoll<sup>1\*</sup>, Jim Thorsen<sup>1</sup>, Morten Laane<sup>2</sup>, Qirong Huang<sup>1</sup> and Erling Olaf Koppang<sup>1</sup>

<sup>1</sup>Institute of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Ullevålsveien 72, PO Box 8146 Dep., 0033 Oslo, Norway

<sup>2</sup>Institute of Molecular Bioscience, University of Oslo, 0316 Oslo, Norway

\*Address correspondence to Erlend Haugarvoll, e-mail: erlend.haugarvoll@veths.no

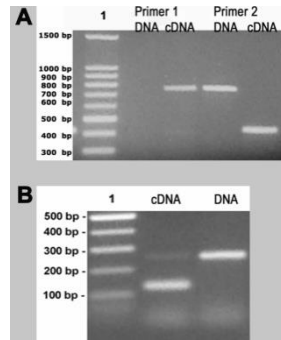
### Summary

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

Received 1 April 2005, revised and accepted for publication 14 December 2005

### 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; Orlov, 1995; Raposo et al., 2002). Common for

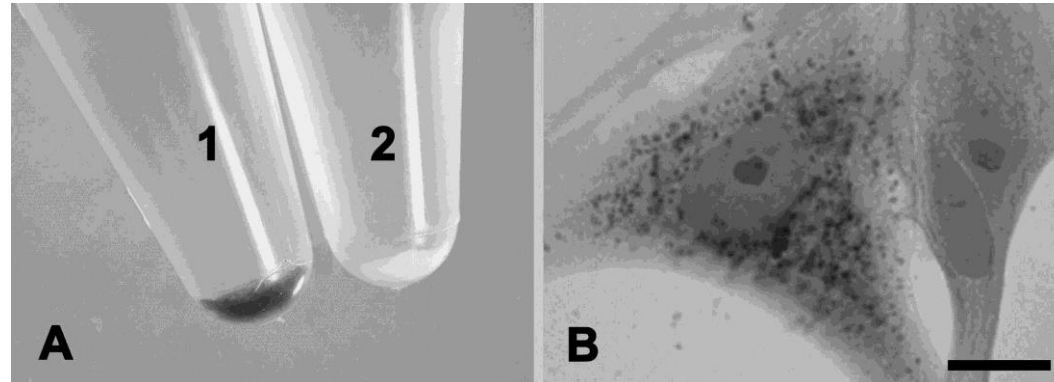


- Primer pairs recognising a fish CD83 homolog

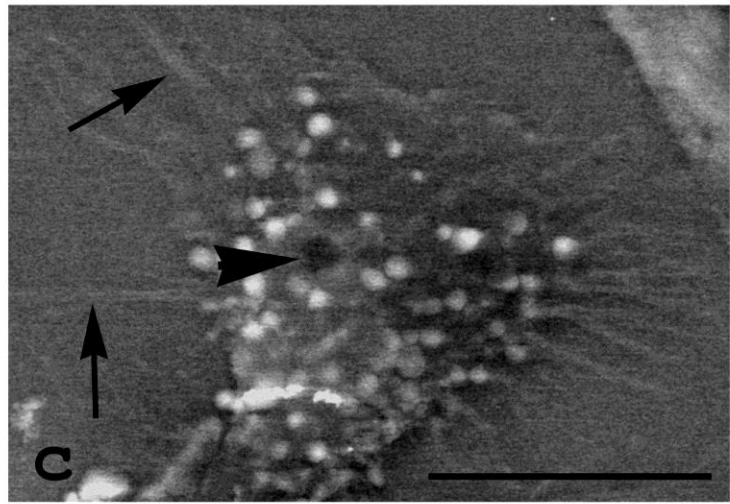
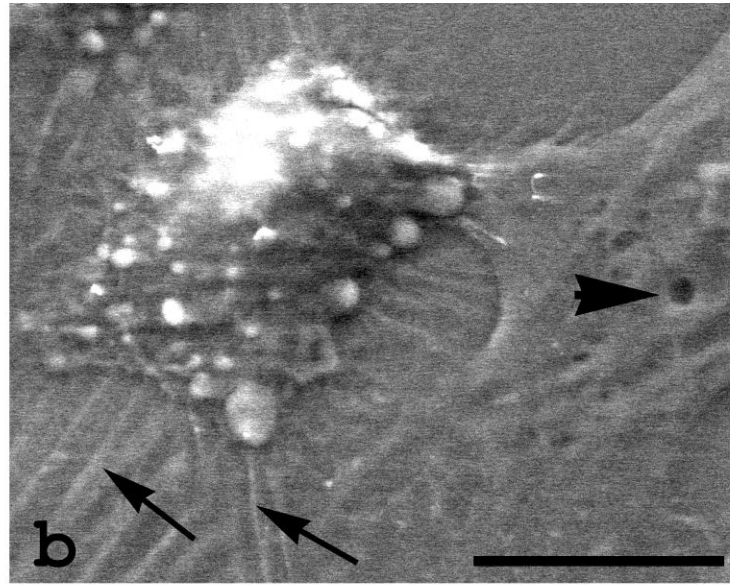
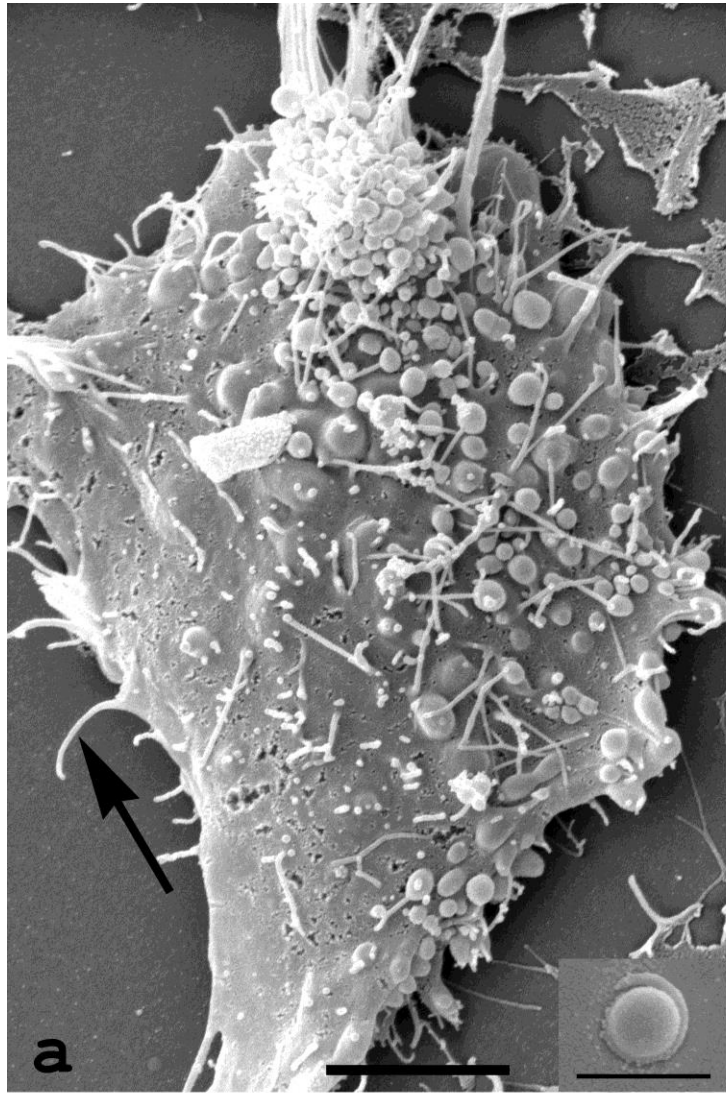
- Primer pair detecting Dct/TRP-2

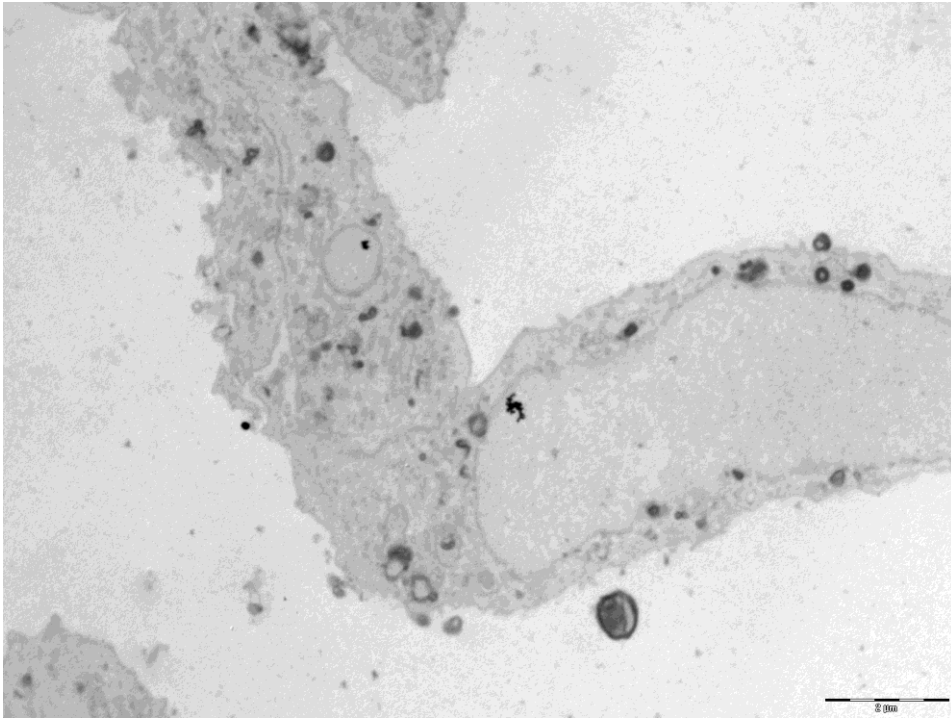
- A; 0,1 mM PTU inhibit tyrosinase dopachrome production from L-DOPA

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

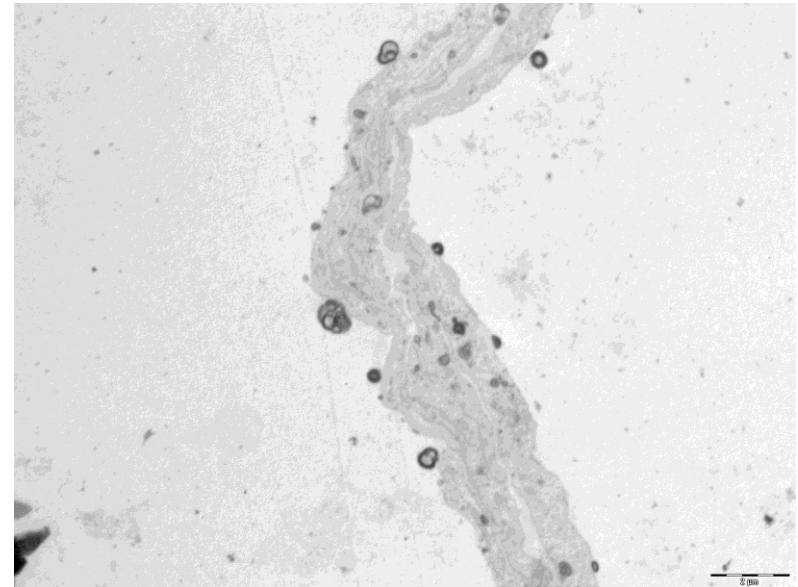
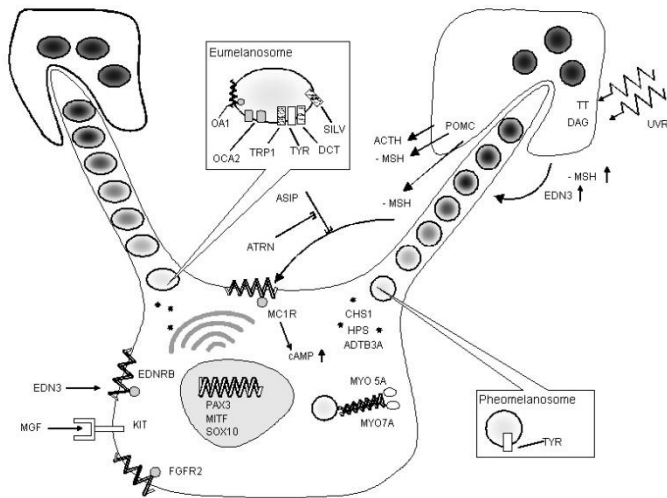








Haugarvoll et al. Pigment Cell Res  
2006;19:214-225



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

Jim Thorsen\*, Bjørn Høyheim and Erling O. Koppang

Institute of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway  
\*Address correspondence to Jim Thorsen, e-mail: jim@jim.no

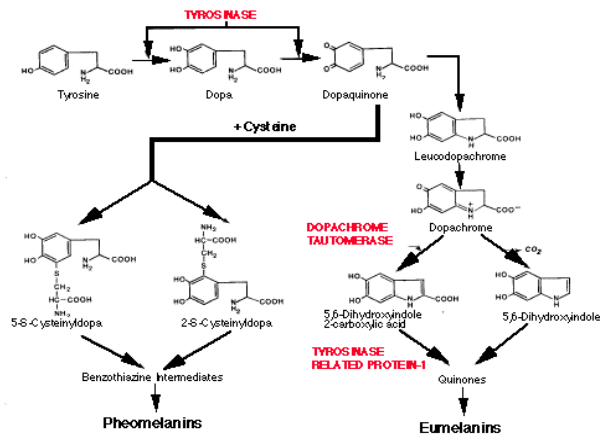
## Summary

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-

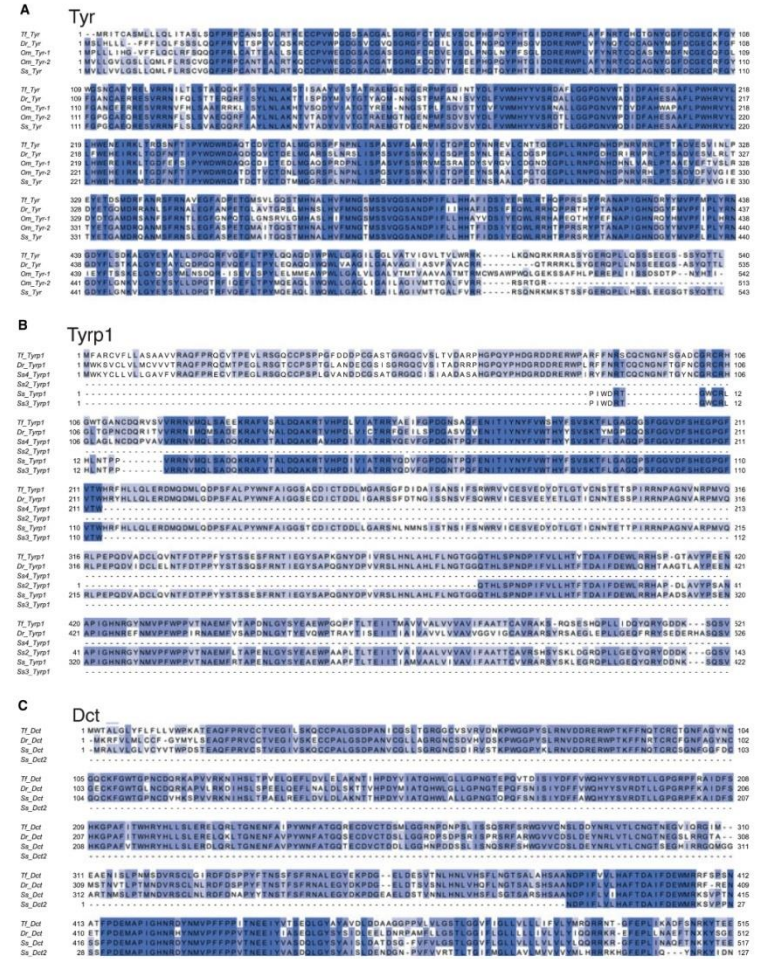
## Introduction

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).

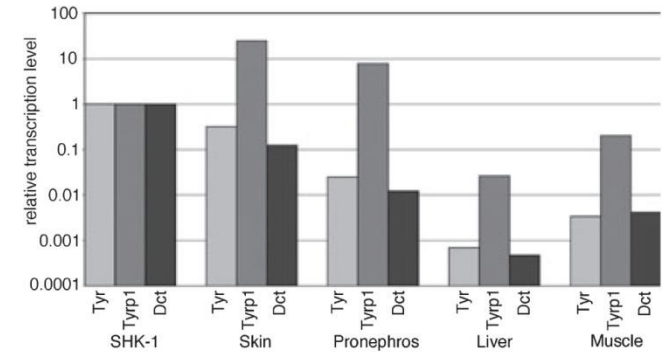
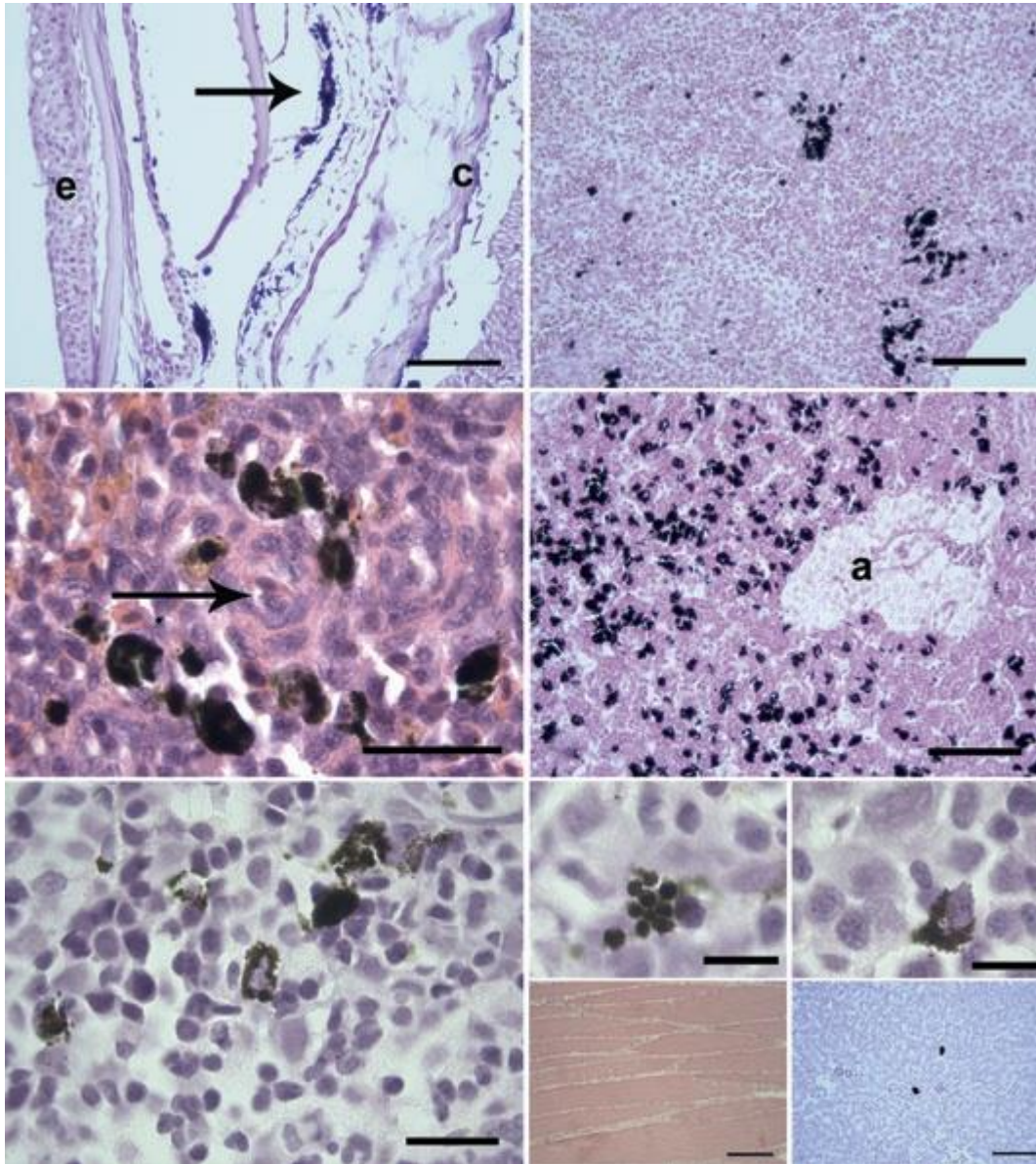
## The Melanin Chemical Pathway



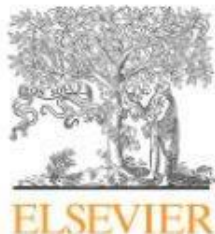
Thorsen et al.



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**Figure 3.** Histograms showing real-time quantitative polymerase chain reaction data of the tyrosinase (Tyr), tyrosinase-like protein-1 (Typr1) and dopachrome tautomerase (Dct). Tissues investigated are indicated. The data are presented exponentially and relative to the SHK-1 values (set as 1). The values presented are the average of triplicate runs for each gene and tissue.



## 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<sup>a</sup>, Lars Austbø<sup>b</sup>, Melanie König<sup>b</sup>, Henning Sørum<sup>c</sup>, Espen Rimstad<sup>c</sup>, Erling O. Koppang<sup>a,\*</sup>

<sup>a</sup>Section of Anatomy and Pathology, Department of Basic Science and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway

<sup>b</sup>Section of Genetics, Department of Basic Science and Aquatic Medicine, Norwegian School of Veterinary Science, Oslo, Norway

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Melanin

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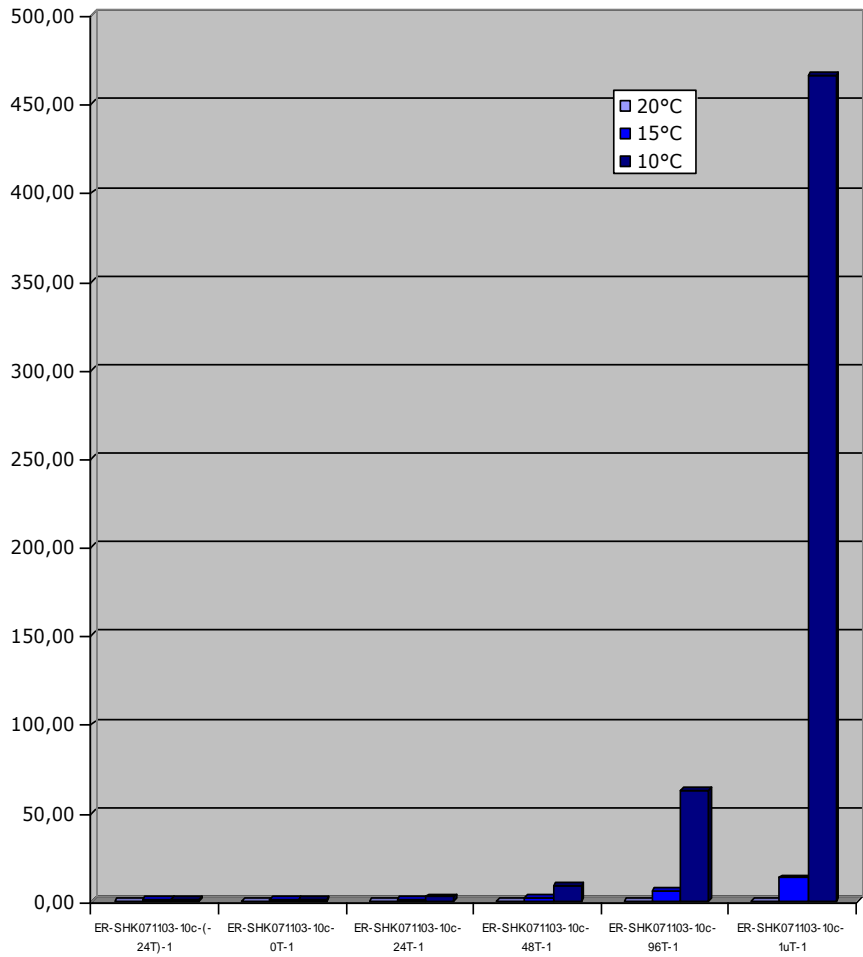
Melanomacrophage

SHK-1 cells

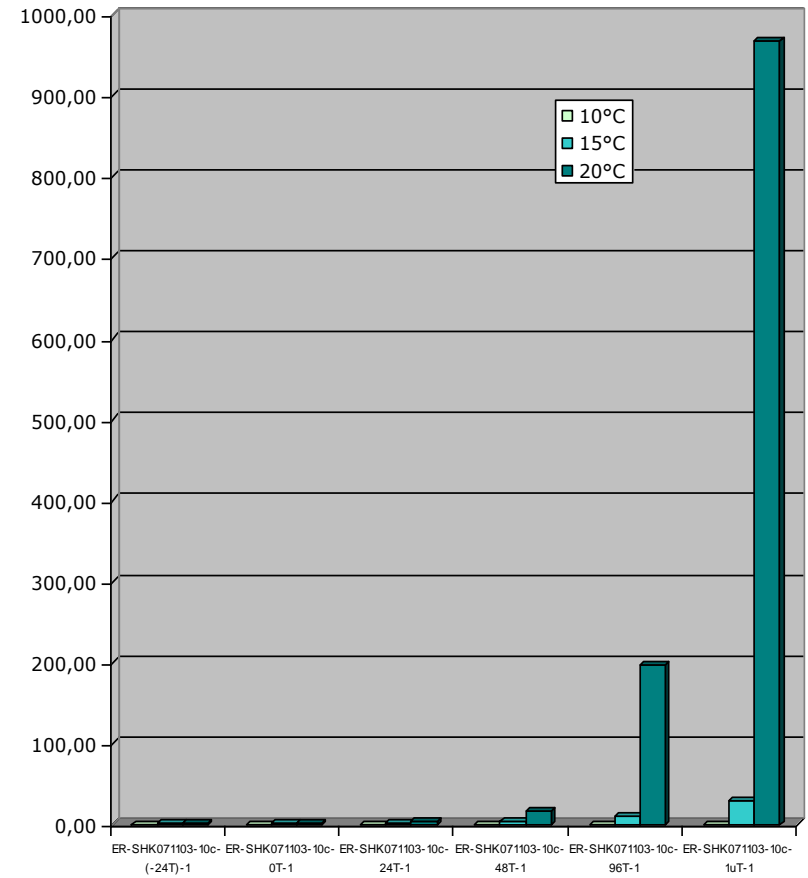
### ABSTRACT

The present study was performed to address putative links between the immune and pigmentary systems. A pigment-producing leukocyte-like cell-line (SHK-1 cells) of Atlantic salmon (*Salmo salar* L.) was exposed to different temperatures, poly I:C, bacterin or infected with virus (infectious pancreatic necrosis virus or infectious salmon anaemia virus). The effect of this stimulation regarding the transcription-pattern of the tyrosinase gene family (melanin genes) and the immune-related genes MHC class II and IFN-1 was analysed using real-time RT-qPCR. At 10 °C cultivation, tyrosinase and dopachrome tautomerase remained unregulated. At 15 °C, a moderate up-regulation was induced, while at 20 °C, these genes were up-regulated in an exponential manner over time. Temperature did not affect the transcription of the immune-related genes. Virus infections, poly I:C or bacterin had no influence on the transcription of the melanogenesis-related genes, but triggered the immune-related genes. Our findings revealed no connections between the pigmentary and immune systems, but demonstrated a hereto undiscovered temperature-effect on the tyrosinase gene family.

### Tyrosinase gene in SHKcells 070910 under 10c, 15c og 20c



### Dct gene in SHKcells 070910 under 10c, 15c og 20c



## 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<sup>1</sup>, L Austbo<sup>2</sup>, A Nodtvedt<sup>3</sup>, T W K Fraser<sup>3</sup>, E Rimstad<sup>4</sup>, P G Fjellidal<sup>5</sup>, T Hansen<sup>5</sup> and E O Koppang<sup>1</sup>

<sup>1</sup> Department of Basic Science and Aquatic Medicine, Section of Anatomy and Pathology, Norwegian School of Veterinary Science, Oslo, Norway

<sup>2</sup> Department of Basic Science and Aquatic Medicine, Section of Genetics, Norwegian School of Veterinary Science, Oslo, Norway

<sup>3</sup> Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway

<sup>4</sup> Department of Food Safety and Infection Biology, Section of Microbiology, Immunology and Parasitology, Norwegian School of Veterinary Science, Oslo, Norway

<sup>5</sup> Institute of Marine Research, Marre Research Station, Matroedal, Norway

### Abstract

The presence of melanin in muscle fillets of farmed salmon represents a considerable quality problem for the salmon industry with major economic concerns. In this study, we have examined the presence of abnormal pigmentation in vaccinated versus unvaccinated Atlantic salmon, *Salmo salar* L., and evaluated possible differences between diploid and triploid fish. Furthermore, the impact of the smolt production regime at ambient (4.5 °C) versus elevated temperature (16 °C) was investigated. Pigmented muscle spots were analysed for the expression of genes involved in melanization (tyrosinase gene family) and immune-related response in addition to morphological investigations. The proportion of fish with intramuscular melanin deposits was not significantly different between vaccinated and unvaccinated fish, regardless of ploidy. However, an interaction between vaccination and smolt regime was shown, where smoltification at elevated temperature after vaccination increased the number of affected individuals compared with

vaccination followed by simulated natural smoltification. Furthermore, there were overall more fish with melanin spots amongst the triploids compared with their diploid counterparts. Transcription of the tyrosinase gene family confirmed an onsite melanogenesis in all pigment spots. The histological examination and the expression of the immune-related genes revealed a chronic polyphasic myopathy that was not affected by vaccination, ploidy or smolt production regime.

**Keywords:** diploid, melanin, melanogenesis, melanomacrophage, triploid, vaccination.

### Introduction

Pigmented muscle changes, often called 'black spots', represent a quality problem of considerable size for the Atlantic salmon, *Salmo salar* L., industry (Mørkøre & Heia 2012), and the aetiology and pathogenesis of this condition have so far remained an enigma (Berg *et al.* 2006; Larsen *et al.* 2012; Mørkøre 2012). Histological investigations of pigmented muscle lesions show that they are dominated by inflammation and pigmented cells (Koppang *et al.* 2005; Larsen *et al.* 2012), frequently termed 'melanomacrophages' in piscine morphological characterization (Agius & Roberts 2003).

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The proportion of fish with intramuscular melanin deposits was not significantly different between vaccinated and unvaccinated fish, regardless of ploidy. However, an interaction between vaccination and smolt regime was shown, where smoltification at elevated temperature after vaccination increased the number of affected individuals compared with vaccination followed by simulated natural smoltification. Furthermore, there were overall more fish with melanin spots amongst the triploids compared with their diploid counterparts.

FHF-finansiert prosjekt

**Mørke flekker i laksefilet**

**-årsaker til forekomst og forebyggende tiltak**

Leder: Turid Mørkøre, NOFIMA

**Ellers i prosjektet:**

Veterinærhøgskolen NMBU, Oslo

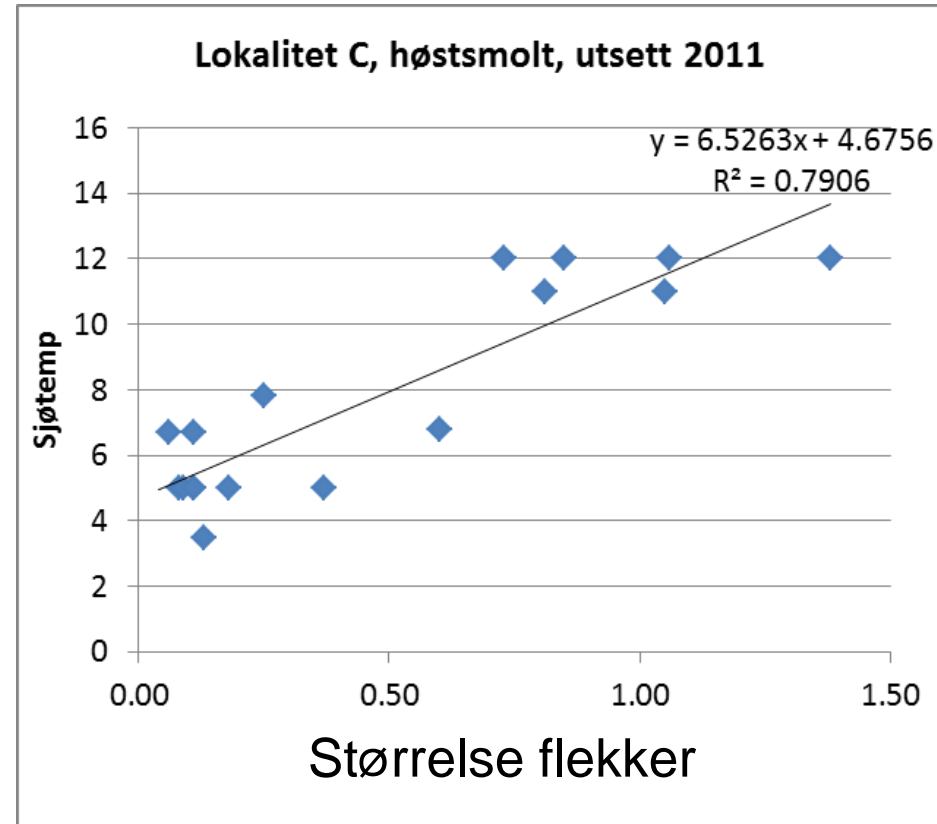
Veterinærinstituttet, Oslo

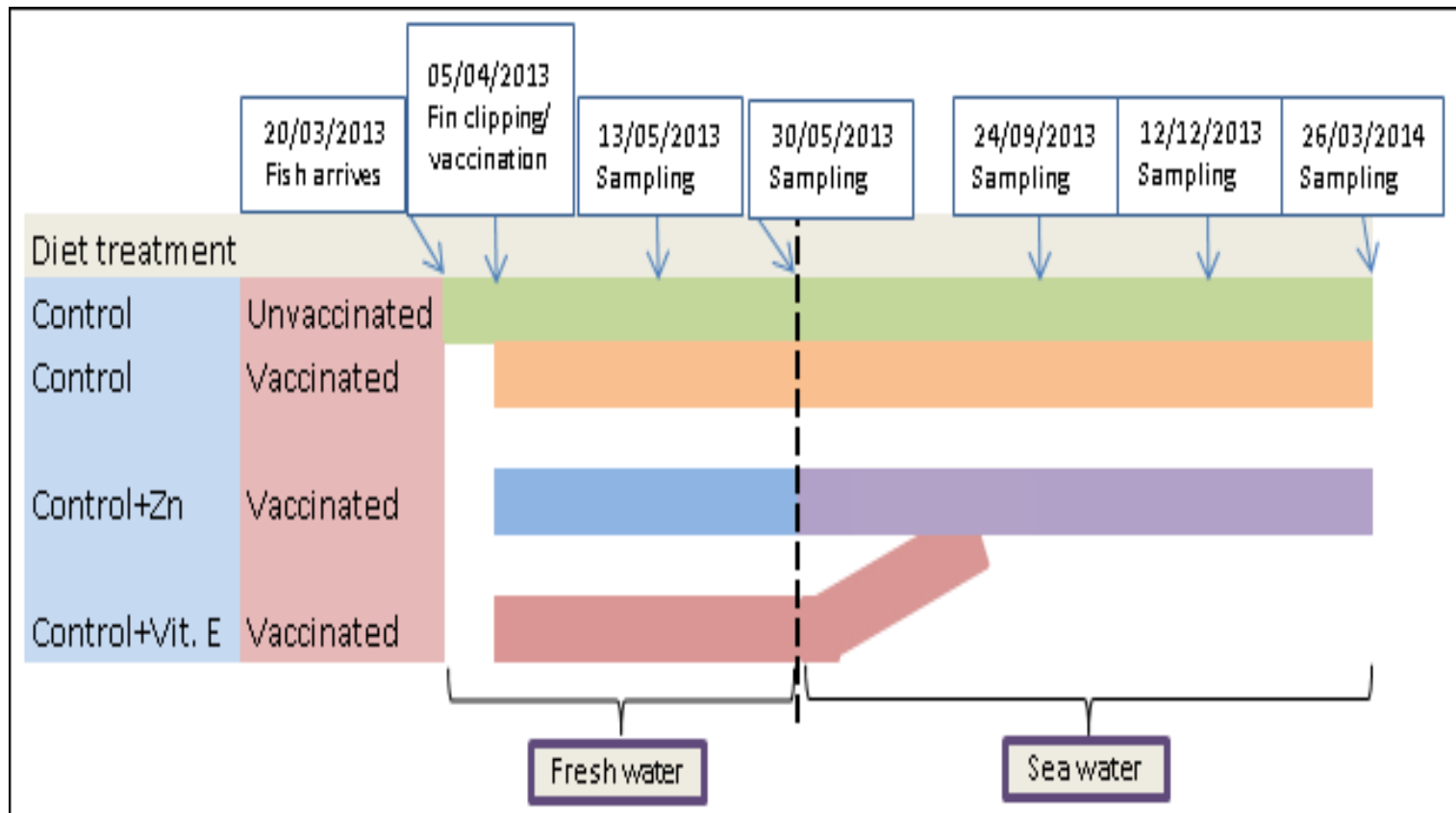
ICTAN (Institutt for matvitenskap,  
teknologi og ernæring, Madrid)



# Sjøtemperatur

- Ingen tydelig sammenheng, men noen unntak
  - Temperaturinduser t stress?
  - Økning utgjøres i hovedsak av flekker i rygg

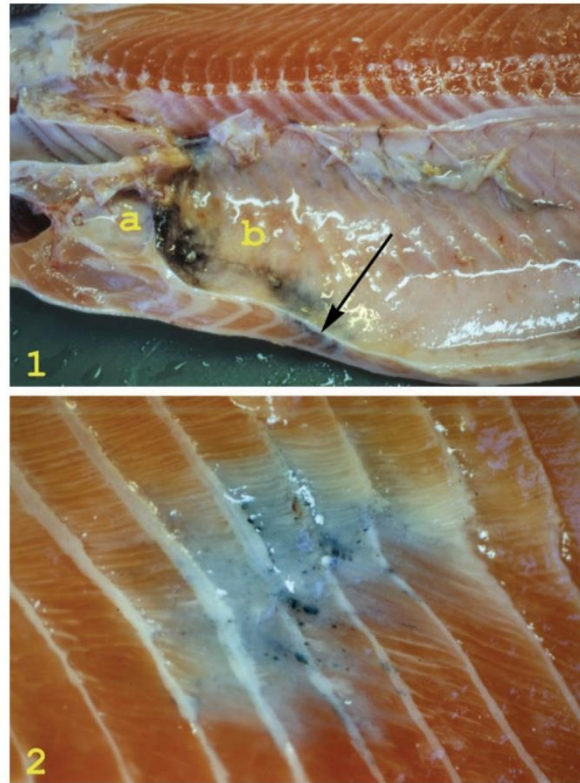




# 6. Misfarging av muskel – er det melanin?

Journal of Fish Diseases 2005, 28, 13–22

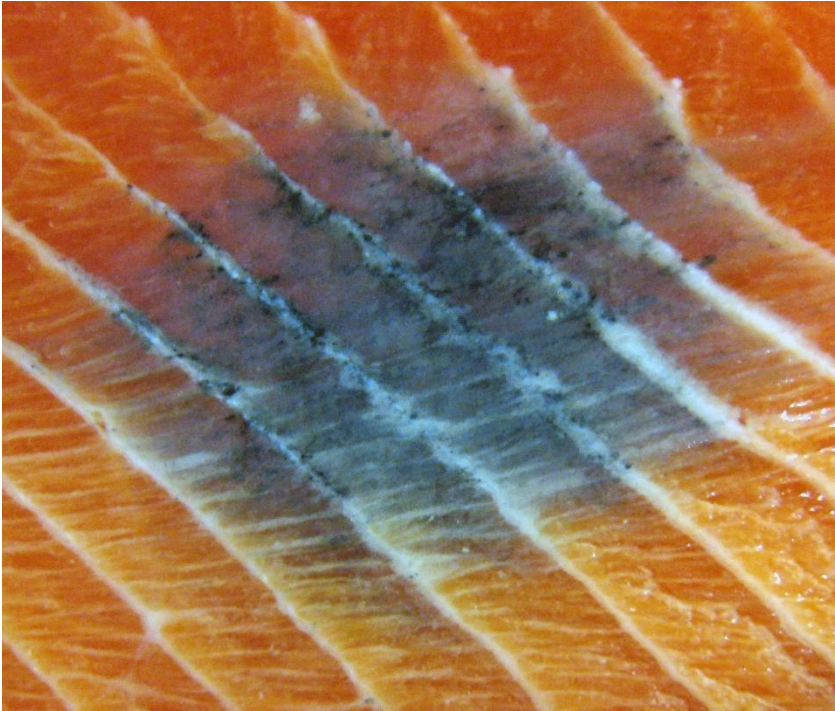
E O Koppang et al. Vaccine-associated pathology in salmon muscle

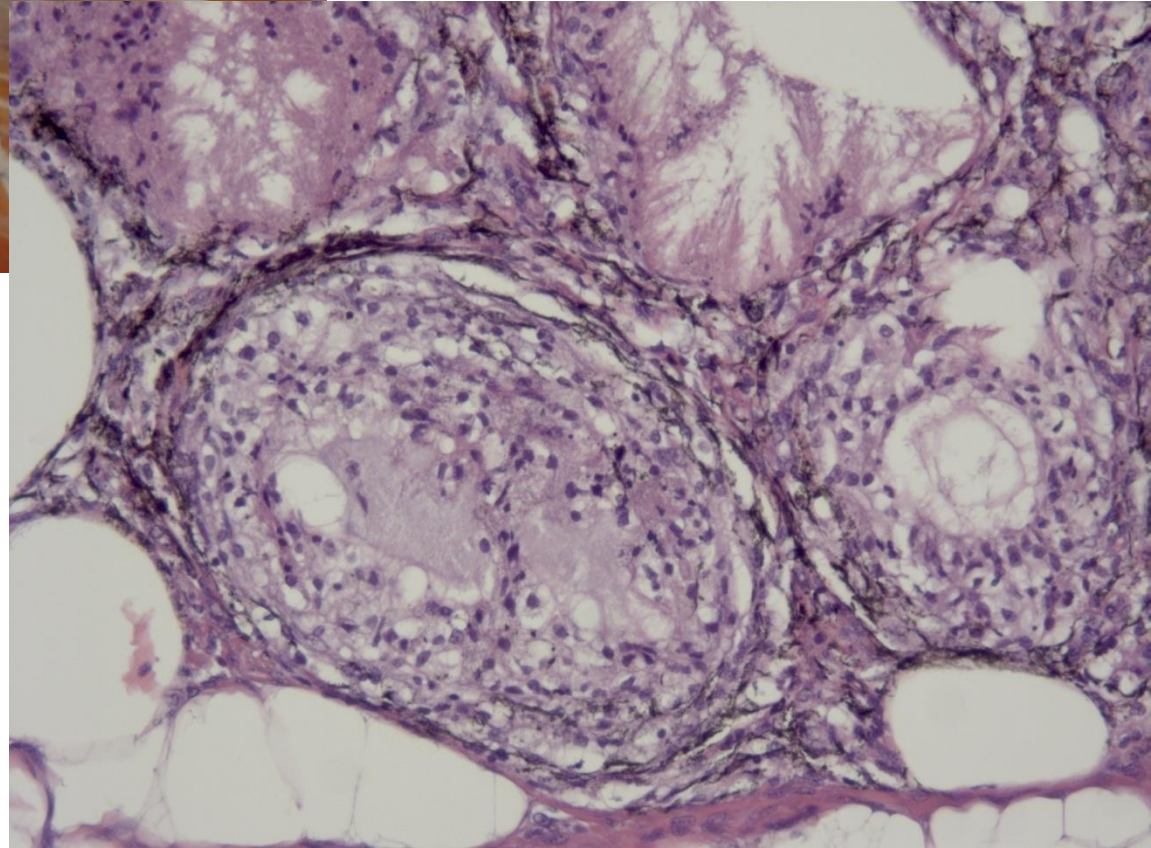
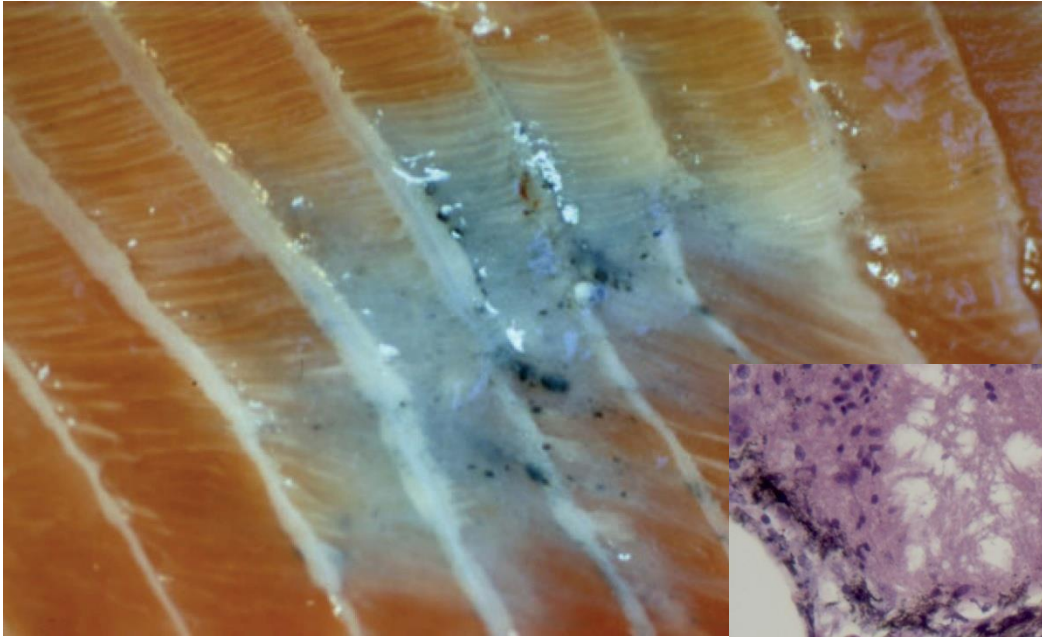


**Figure 1** Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).

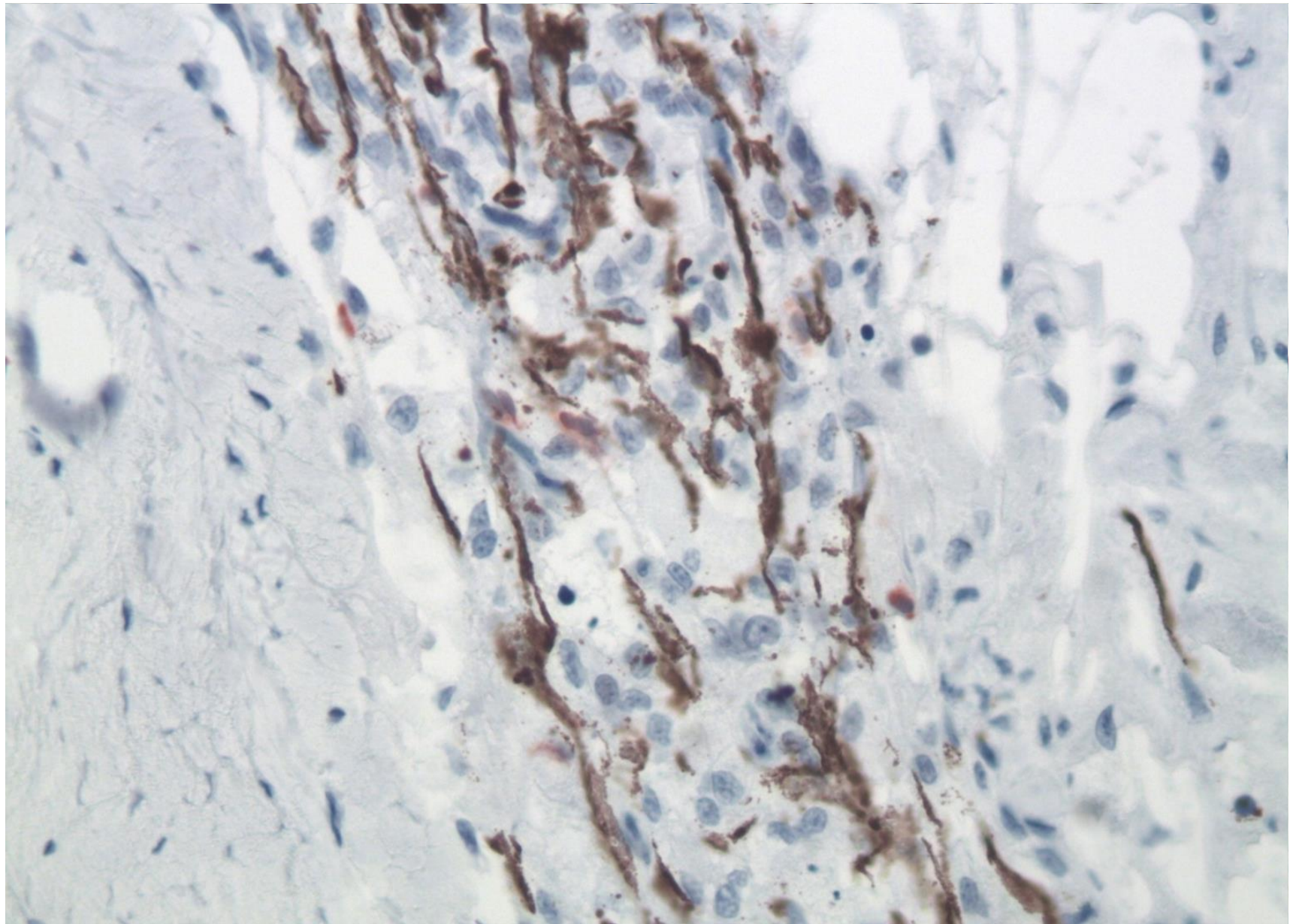
**Figure 2** A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular septa.

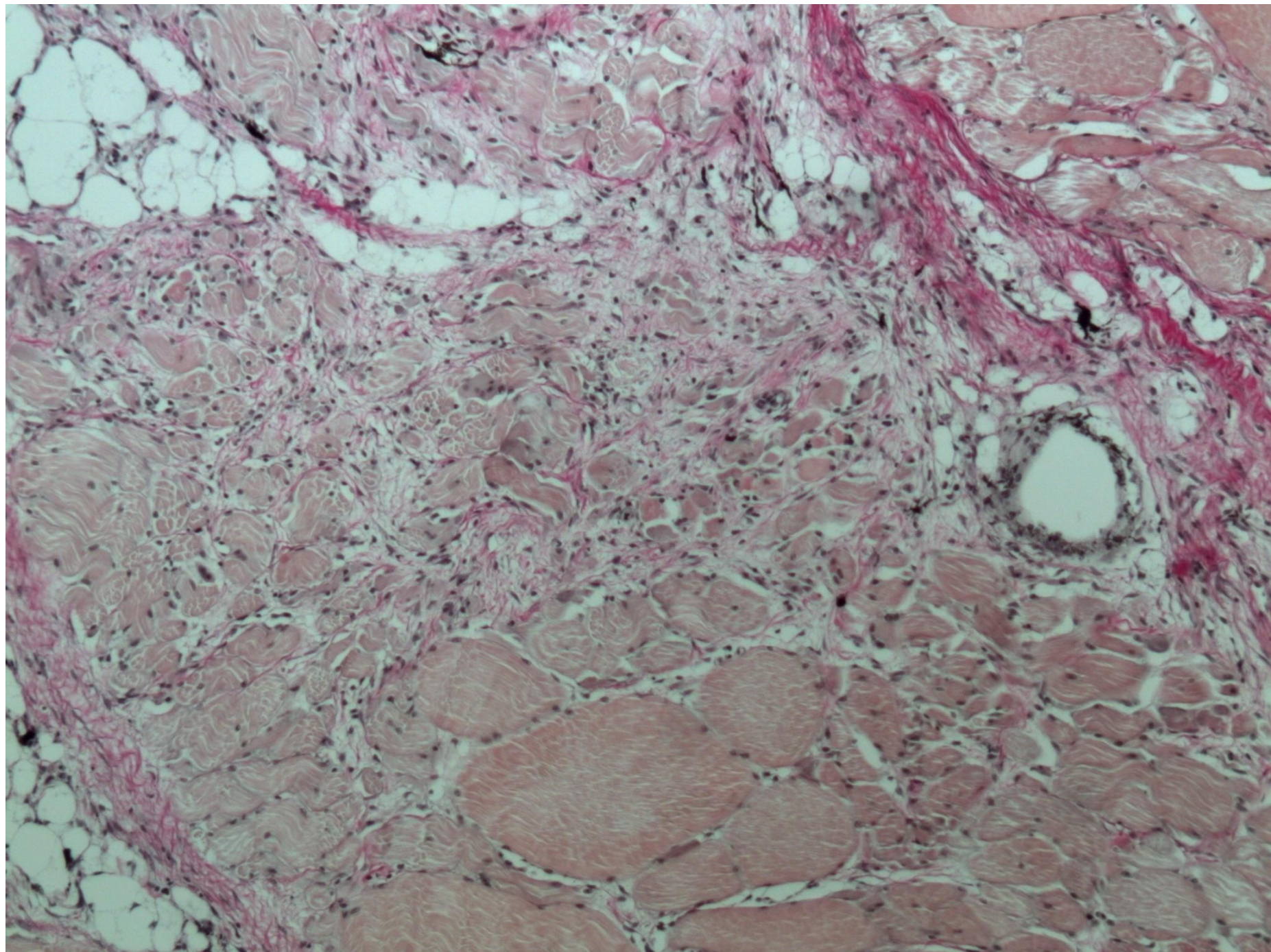
# Er melanin alltid melanin?

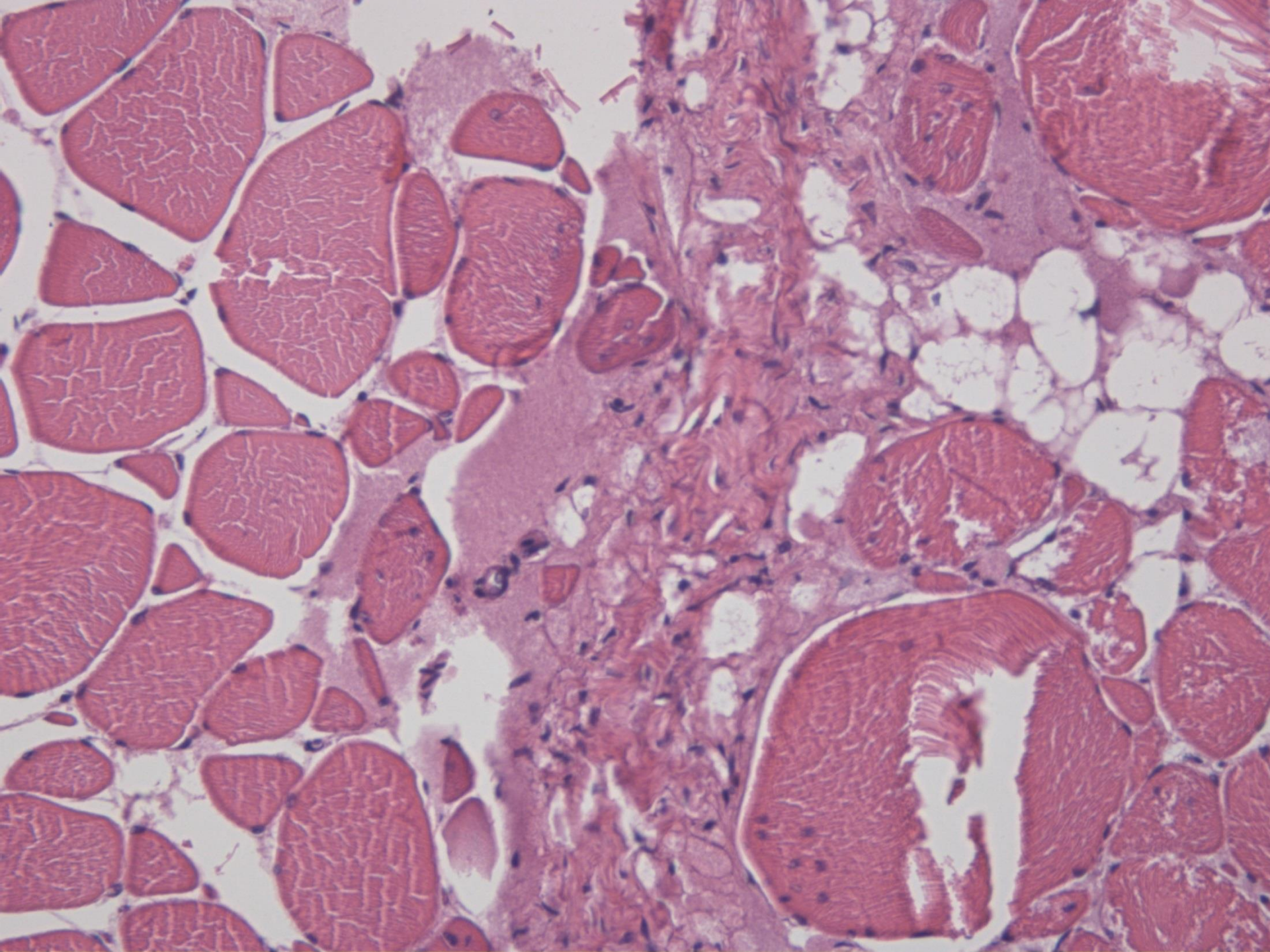




**Granuloma with melanisation in salmon fillet- probably**











8. Kan melanisering  
unngås?  
Ja, tydeligvis!



# Oppsummering

- Melaninproduserende celler finnes normalt i flere av fiskens vev utenom hud
- Slike celler er makrofag-lignende celler
- I forbindelse med kronisk betennelse kan det skje en akkumulering av slike celler i muskulatur
- Melanogenesen ser ut til å være temperaturregulert
- Det kan være at vaksinerings har noe å si i forbindelse med melanisering i muskel