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New objective method for mucosal epithelia measures applied to gills

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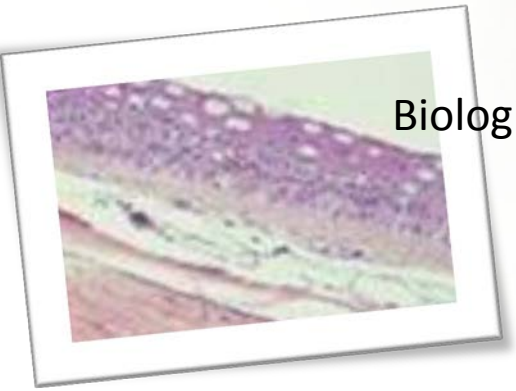
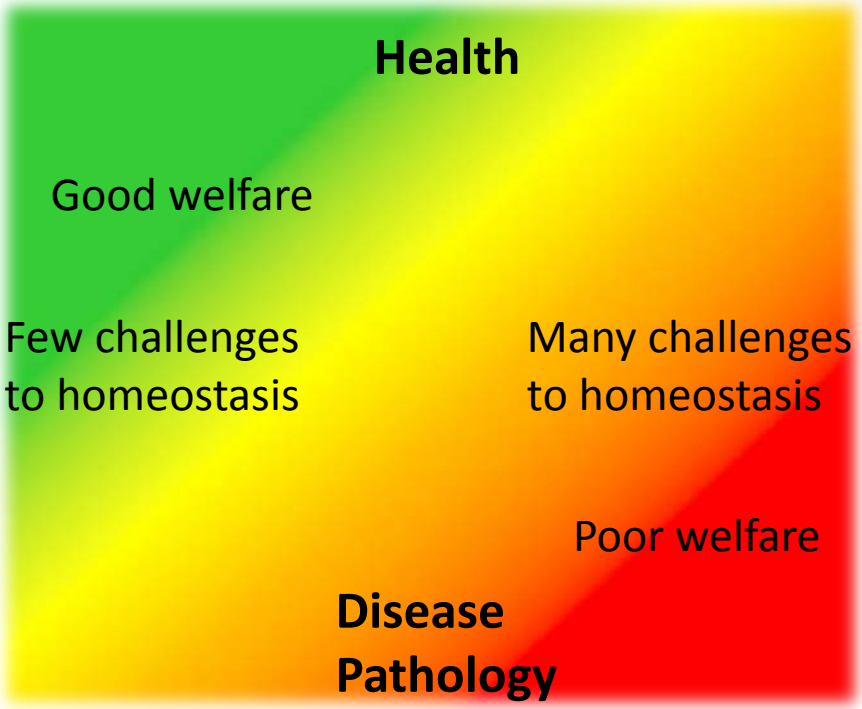
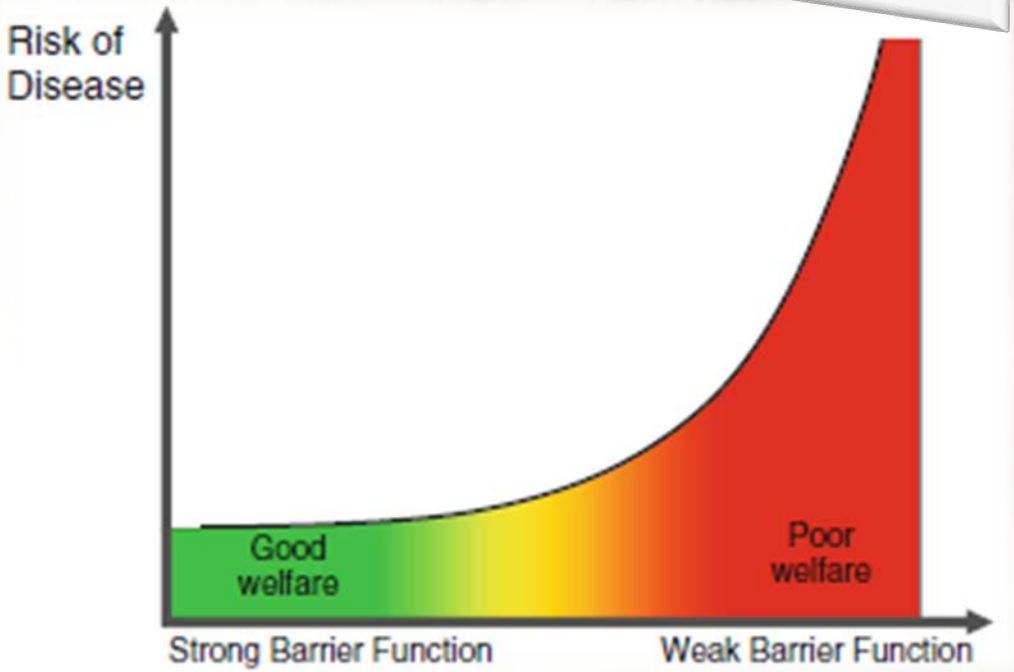
MUCOSAL EPITHELIUM = SKIN, GILLS, GUTS = BARRIER FUNCTIONS



Biological function is a cornerstone of fish welfare
permeability, immunity





Redrawn from Segner et al 2012 Fish Physiol Biochem (2012) 38:85–105

Numerical density gives an indication but comparisons are difficult

Table 1 Site selection of parasites in the total fish population [*Gyrodactylus derjavini* week 1 and week 6 post-infection, surface area of different body parts (mean of ten fish), mucous cell density in (uninfected) rainbow trout skin and fins (ten fish examined; mean of all zones measured or mean of the zones with the lowest mucous cell density, standard deviation in parentheses)]

Microhabitat	Total number of parasites		Parasites per cm^2		Surface area per fin (cm^2)	Mucous cell density (mean number/ mm^2, all zones)	Mucous cell density (mean number/mm^2, zones with lowest count)
	Week 1	Week 6	Week 1	Week 6			
Caudal fin	106	852	0.58	6.98	1.14	116.7 (38.3)	86.1 (37.8)
Dorsal fin	23	66	0.66	2.80	0.22	309.8 (81.1)	300.5 (90.3)
Pectoral fin	240	241	1.71	2.56	0.88	285.1 (66.6)	249.8 (33.9)
Pelvic fin	182	208	2.03	3.47	0.56	269.3 (77.8)	259.5 (56.5)
Anal fin	84	131	1.66	3.82	0.32	217.7 (38.6)	211.5 (58.9)
Adipose fin	21	26	1.31	2.43	0.10	236.6 (58.2)	217.3 (56.9)
Corpus	64	368	0.06	0.48	7.24	273.5 (87.6)	265.5 (104.9)
Cornea	36	439	0.75	13.67	0.30	49.9 (72.7)	0 (0)

Buchmann & Bresciani (1998)

- Mucus cell density varies with location on body
- Strong negative correlation between mucus cell density and parasite density?

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Mucosal mapping

Q: how do you take one (1) slice of the sky and find out how big the balloons are and how tightly packed they are?

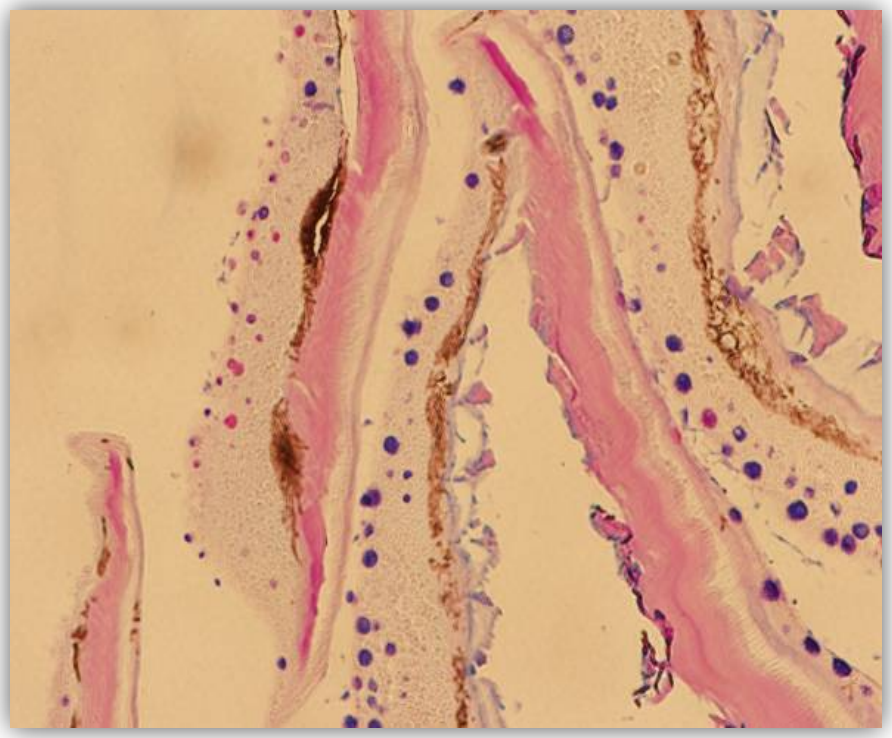
A: Design-based stereology

Design-based stereology (Pittman Protocol)

Examples of salmon skin sections giving statistically robust objective measures of:

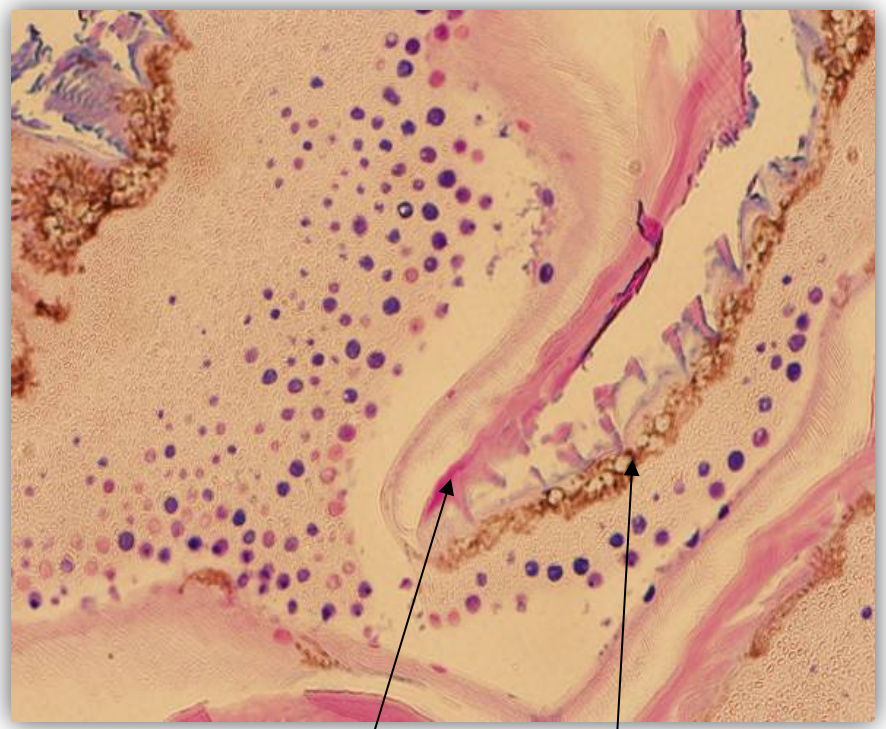
i) mucous cell area ii) mucous cell density iii) area/density (tissue dynamics)

Control

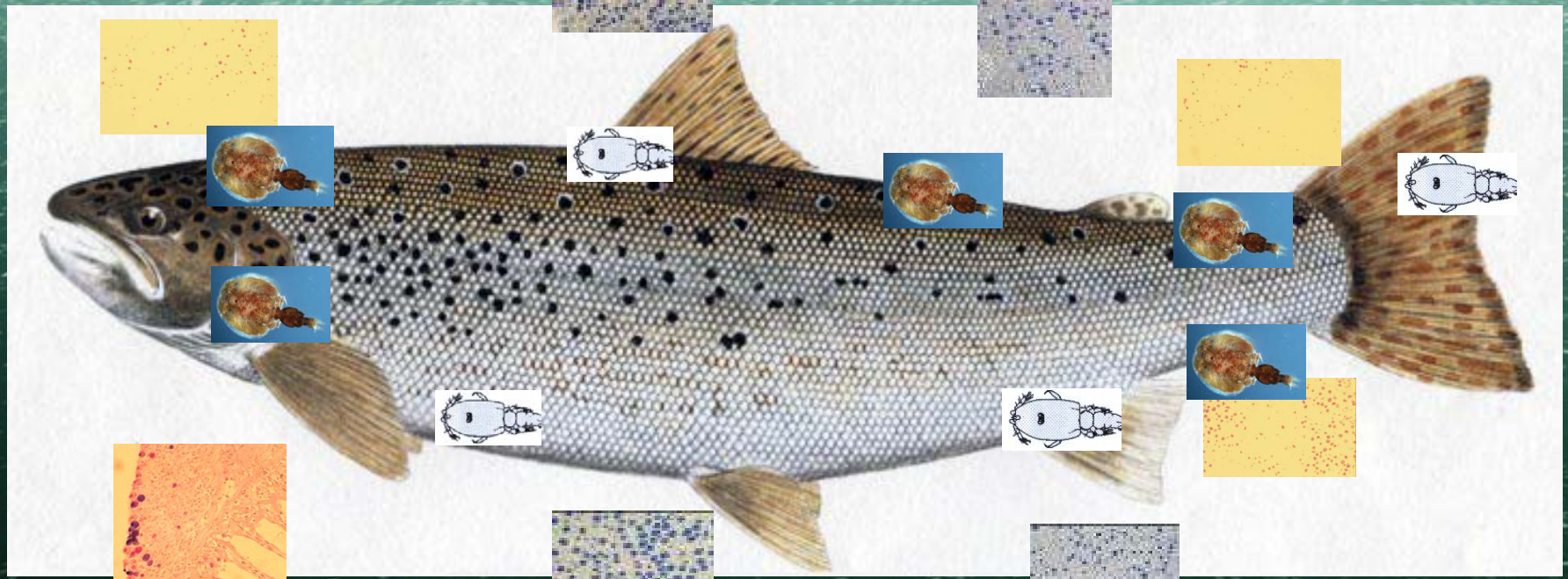


mucus cells
pink – neutral mucins
blue – acid mucins

Aquate SPMP



MUCOSAL MAPPING IN SALMONIDS



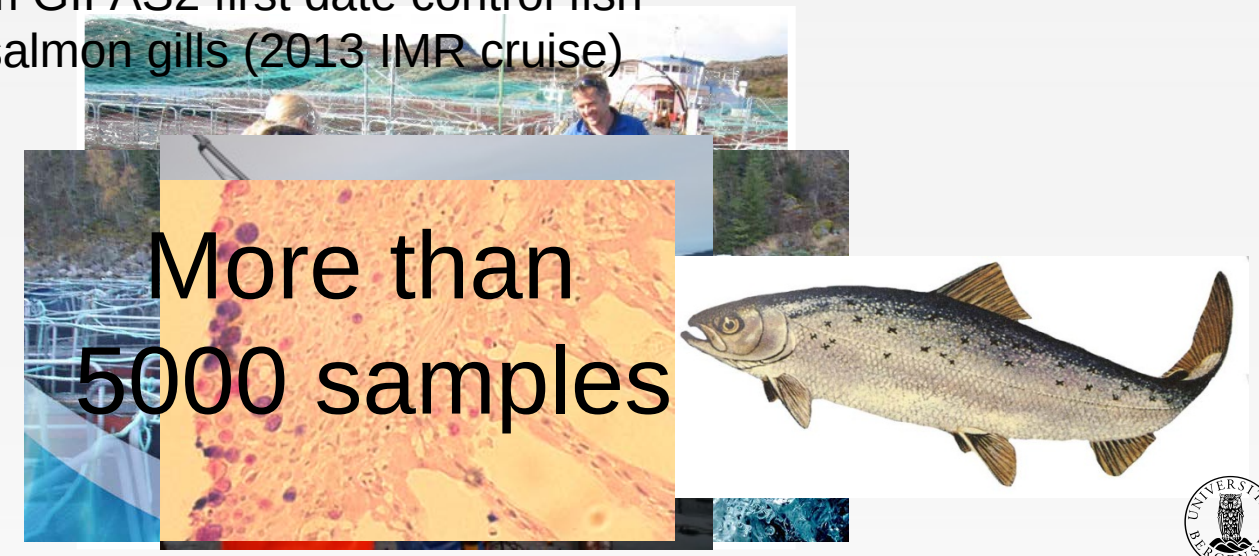
Industrial scale application of the protocol

1. Sulefisk - field trial on commercial farm, 4 cages with 2 control 2 Aquate-fed groups
   - 280 000 salmon (about 50 m tons), 4 sampling dates, 7 months duration

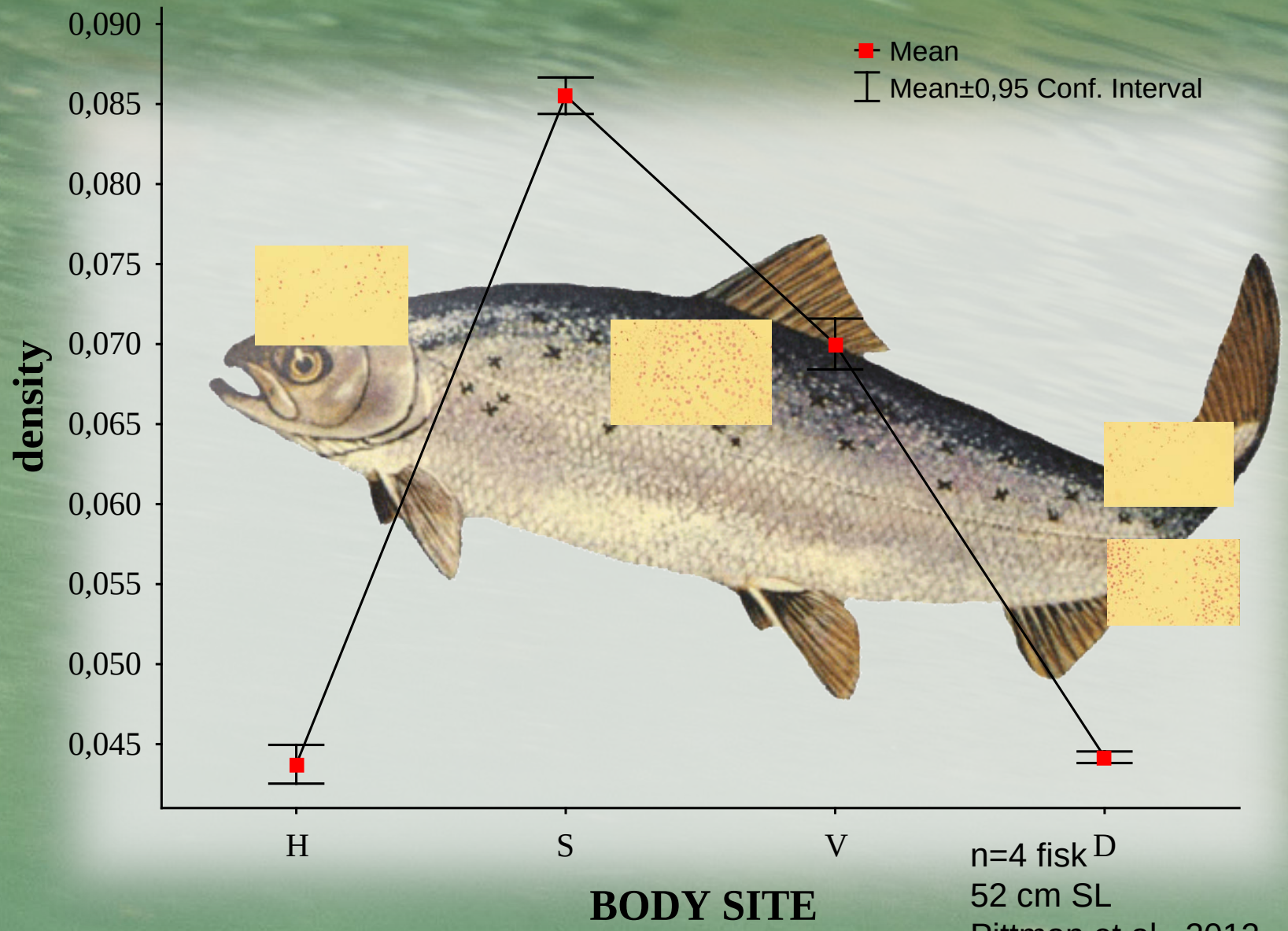
2. Gifas1 - field trial on research station, 60 000 salmon, 12 cages with 3 cages for each of 4 diets (control, plus three other diets), 5 sampling dates over 4 months
3. Gifas2 - field trial on research station, 60 000 salmon, 12 cages dose-response to 2 levels of trial diet plus control diet, 3 sampling dates over 2 months

4. Averøy - controlled trial at Salmobreed, two specially bred families
   - 1 sampling date

5. Gill test - gills from GIFAS2 first date control fish plus WILD salmon gills (2013 IMR cruise)

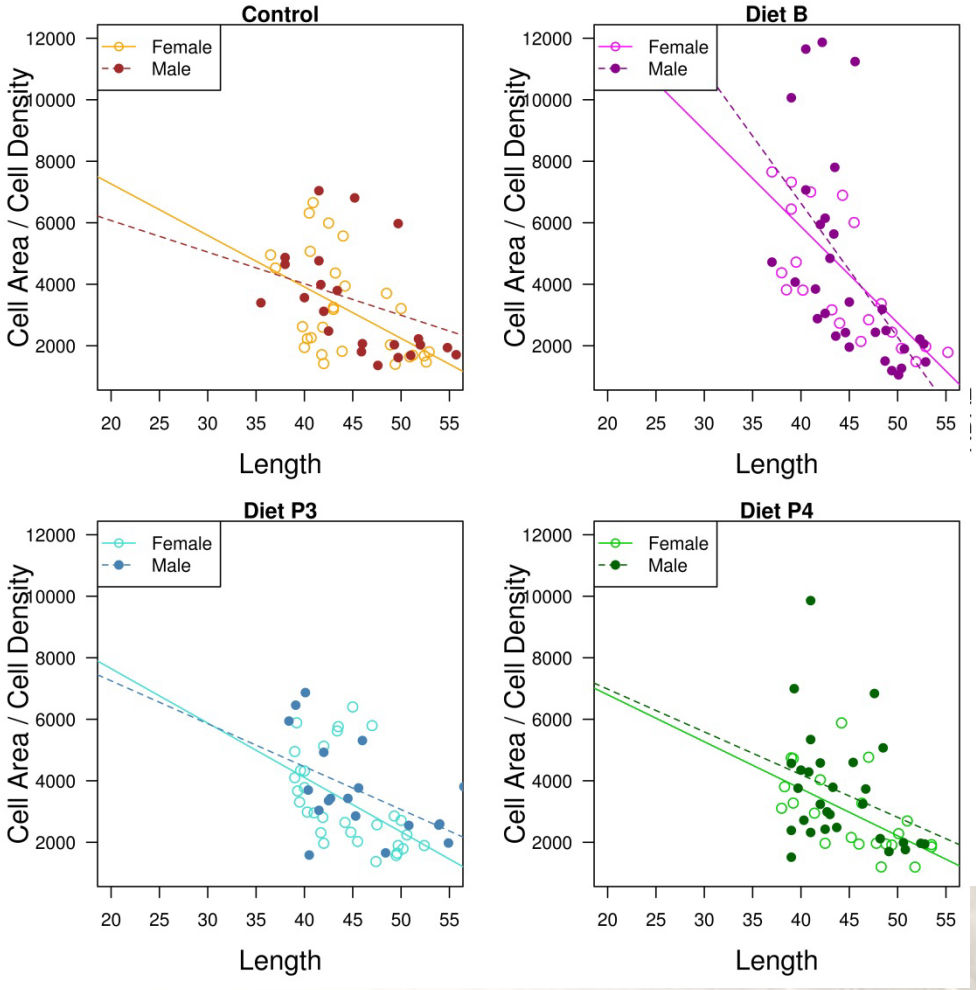


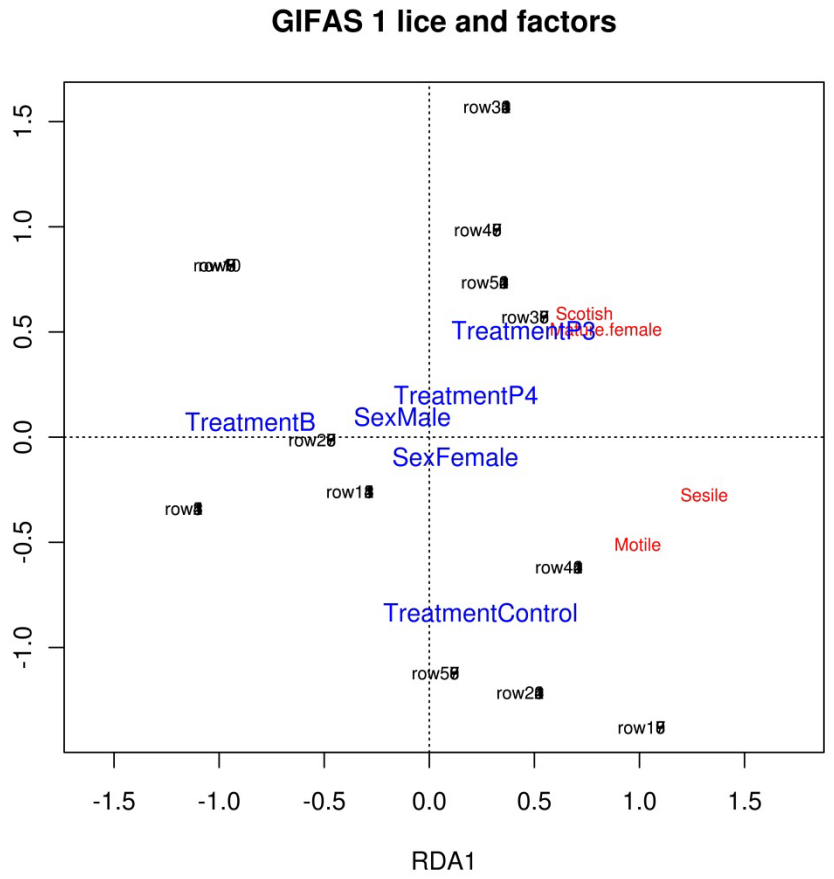
Significantly denser mucous cells on the dorsal ($p<0.05$) ie. how much of the epithelia is mucus



n=4 fisk
52 cm SL
Pittman et al., 2012

GIFAS1: Some diets promote rapid change in area/density of mucous cells- these diets repel lice at all stages (multivariate analysis of lice abundance and treatment; F-test p=0.05)

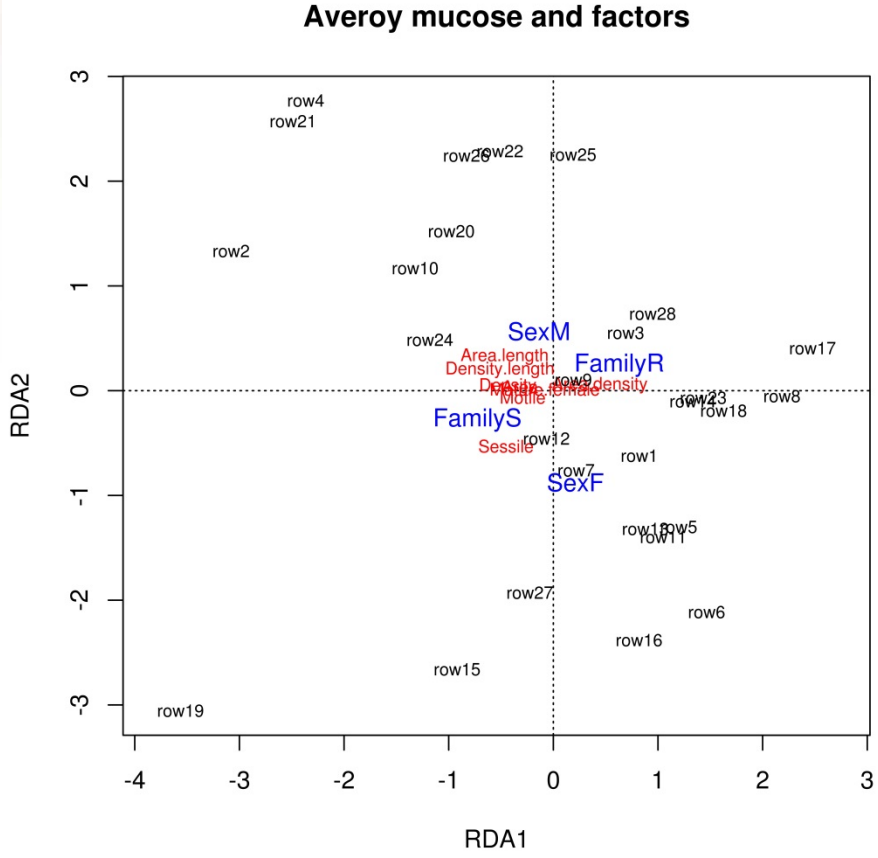


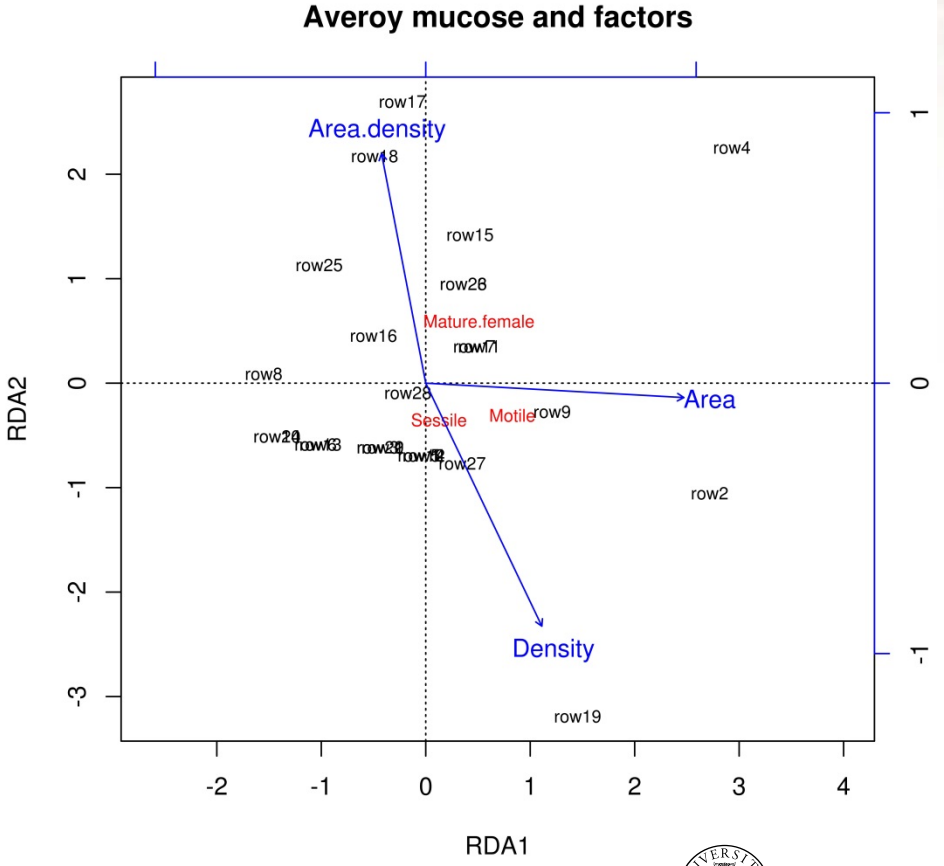


RDA on last date of trial

Family does not explain lice counts, but mucosal measures do

-PCA analysis shows mucosal values are the factors most related to resistance of lice at all stages (family is, so far, irrelevant)





N=29 salmon

Pittman et al., in prep

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MUCOSAL MAPPING

- Resolves significant differences of as little as 7 square microns (area of *E. coli*)
- Shows how the mucosal tissue fills itself with mucous cells
- Focusses on mucous and epithelial cells
- Comparable across species, tissues, time

- Gills and guts…

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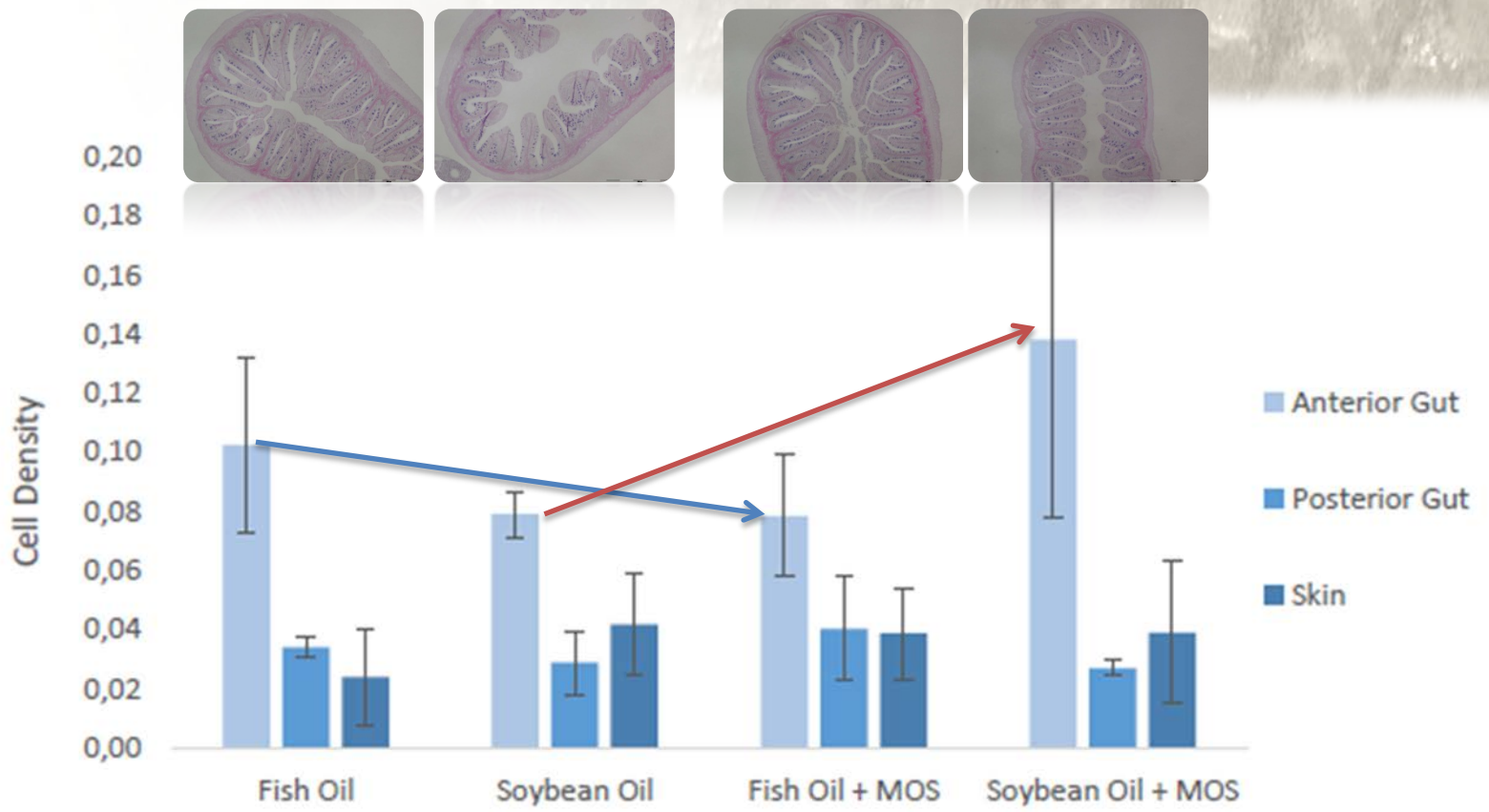
Mucosal Mapping in Seabass guts and skin



From: Custodio 2014; Custodio et al. in prep; n=36
Photos: S. Torrecillas

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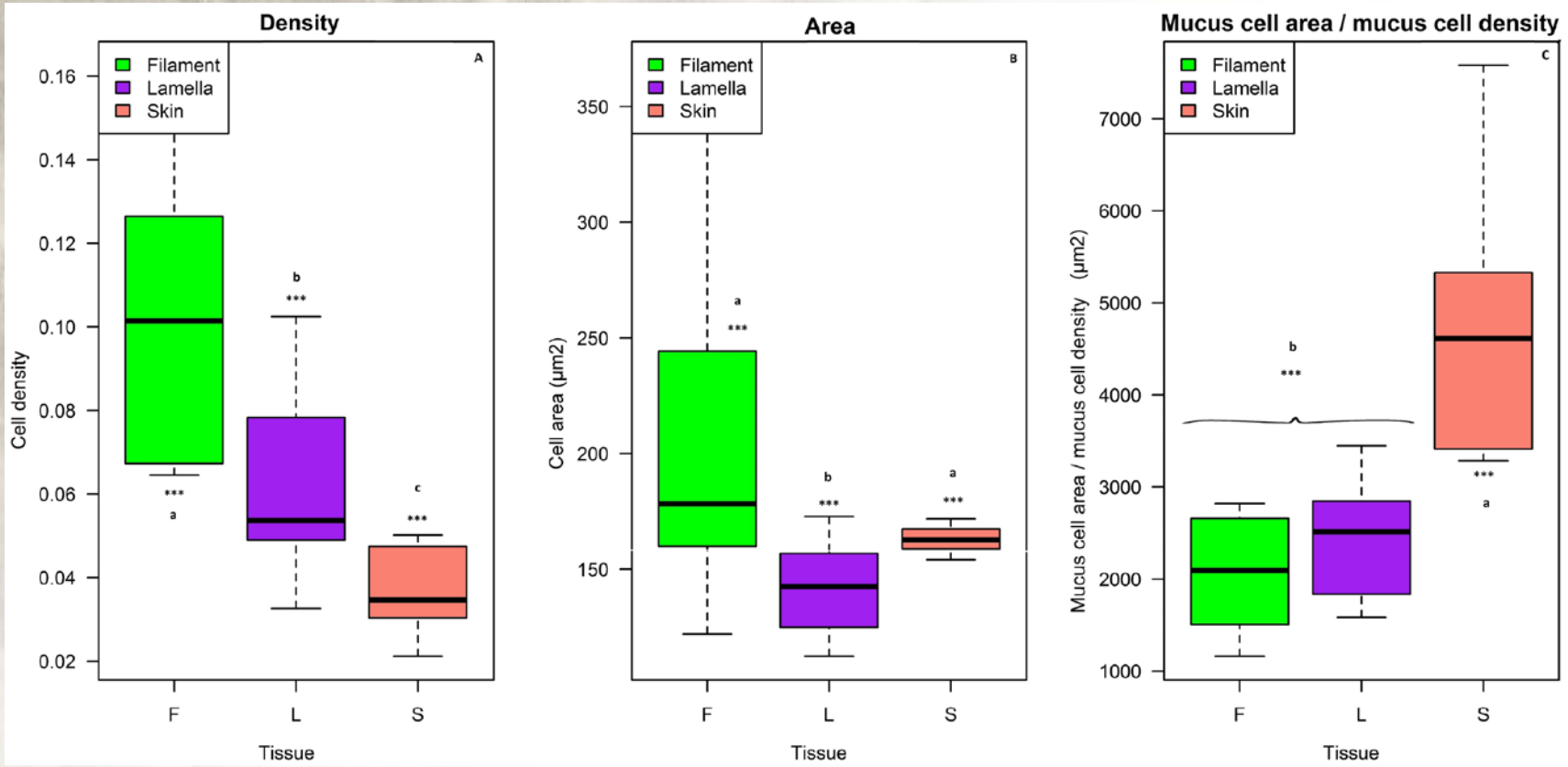
Mucosal Mapping on salmon gills and skin

- highly significantly different mucous cell populations
- different control mechanisms



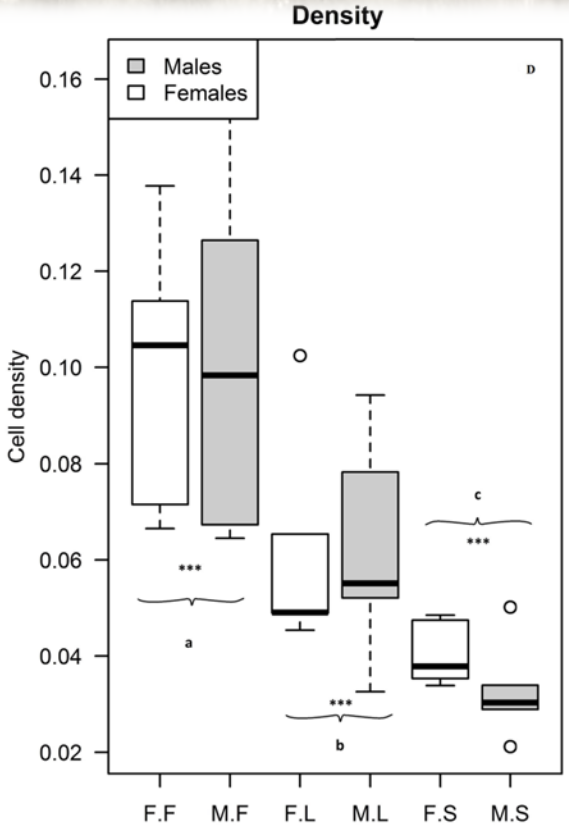
N= 10 salmon, 2nd gill arch

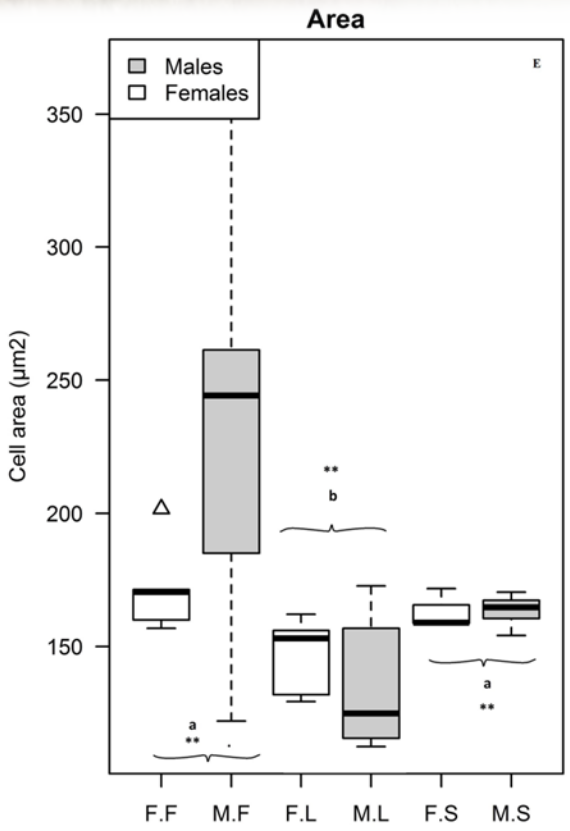
*** p<0.0001

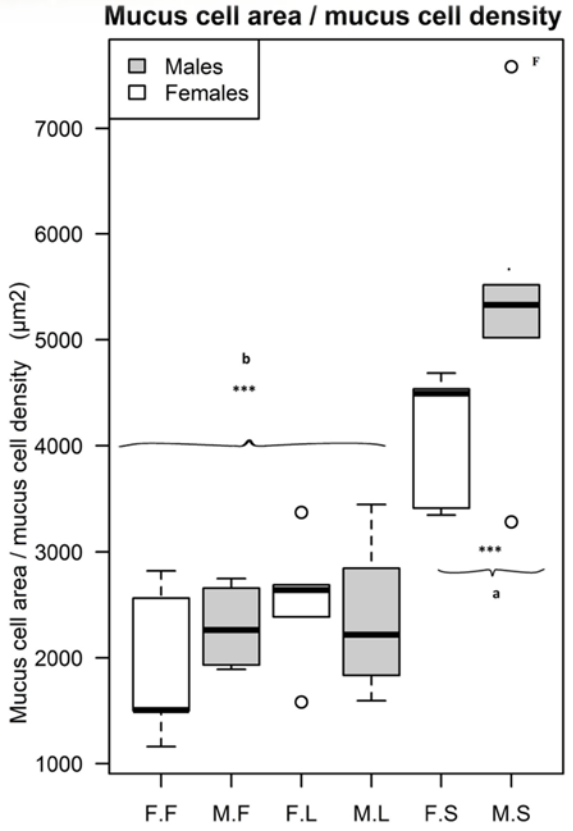
* p<0.05

From Campo et al., submitted

Salmon gills and skin: trend to significant sex differences in mucosal variables







BATEMAN'S PRINCIPLE: FEMALES INVEST MORE IN IMMUNITY

From Campo et al., submitted

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Mucosal Mapping of two tissues in salmon: 3 populations of mucous cells

Gills have two distinct populations,
one in filament (largest size and highest density)
and one in lamellae (smallest cells, medium density)
Skin has medium sized mucous cells and lowest density

Table 3: General overview of the significant results and trends obtained, based in F-test.

Measure variable	Significant factor	*p*-value
Density	Tissue	3.56E-05***
Area	Tissue	0.00207**
Area	Tissue:Sex	0.05604 ·
Area/dens	Tissue	9.78E-07***
Area/dens	Sex	0.0887·

From Campo et al., submitted

One section per fish for Mucosal Mapping: how the numbers behave

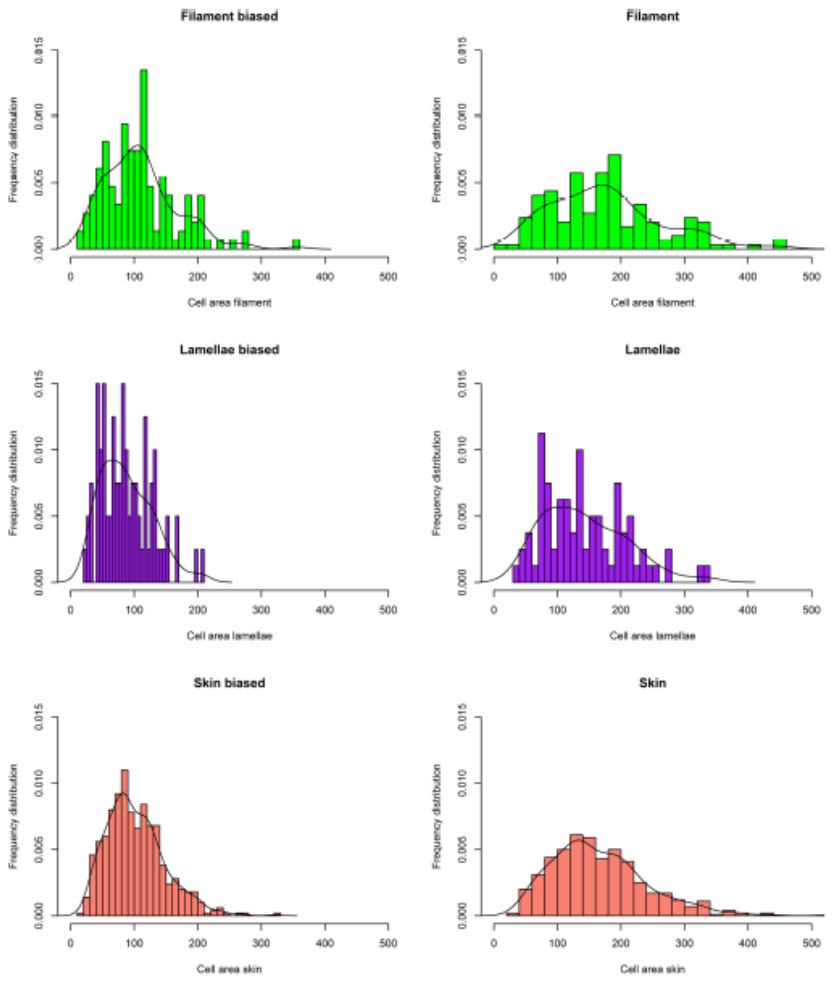


Figure 2: Mucous cell area frequency distribution in filaments, lamellae and dorsolateral skin mucosal tissue. The conversion from biased to unbiased changes completely the distribution pattern from a Poisson distribution to an approximately normal distribution. $N = 728$ mucous cells from 10 Atlantic salmon individuals.

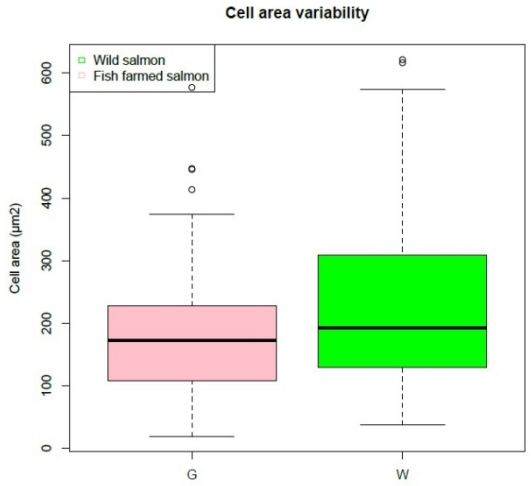
Table 4: Study of the error

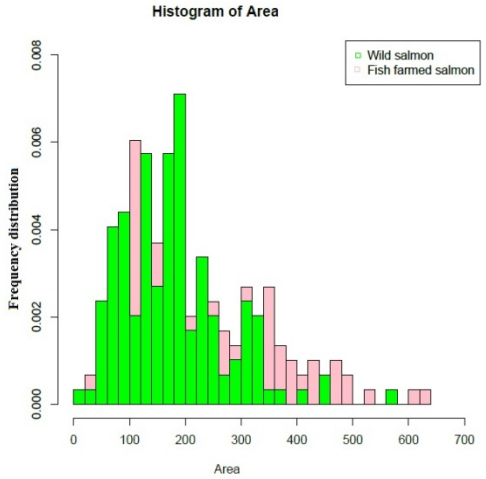
	Epithelium area	Mucous number	Area of mucous cells
FILAMENT			
Average	174098.007	62	204.07
Standard deviation	31735.89	47.70	70.87
Standard error est(SE)	10035.77	15.08	22.41
Coefficient error est(CE, %)	0.06	0.24	0.1098266620
LAMELLA			
Average	95622.687	37.9	141.47
Standard deviation	27730.666	11.76104	21.14
Standard error est(SE)	8769.206	3.719169	6.68
Coefficient error est(CE, %)	0.091706	0.098131	0.04725
SKIN			
Average	908002.055	193.6	163.09
Standard deviation	168279.5	47.91473	5.75
Standard error est(SE)	53214.66	15.15197	1.8191296
Coefficient error est(CE, %)	0.058606	0.078264	0.011154

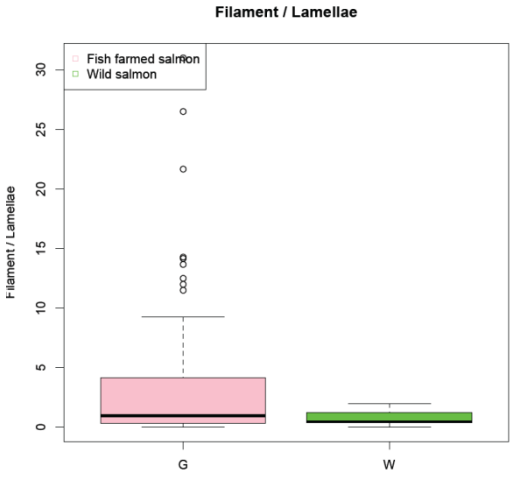
Correction for not sectioning at equator of mucous cells = unbiased!

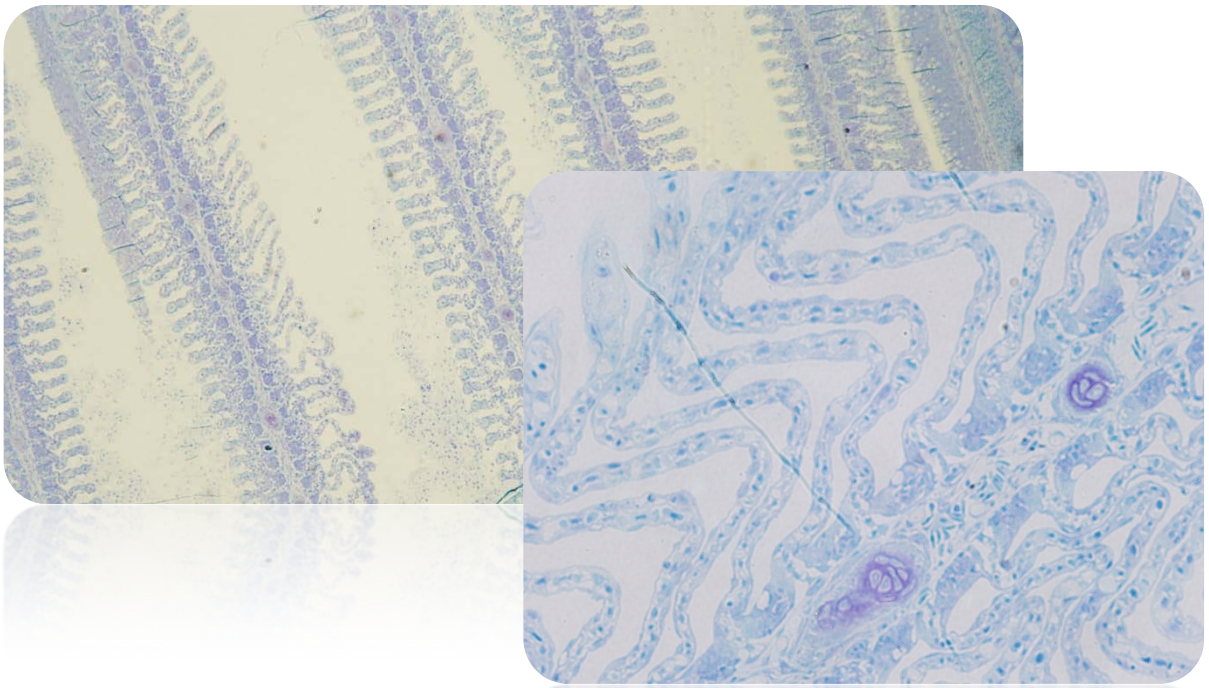
From Campo et al., submitted

Mucosal Mapping of wild salmon gills:









Wild had shorter lamellae
No mucous cells in lamellae

Work continues…!

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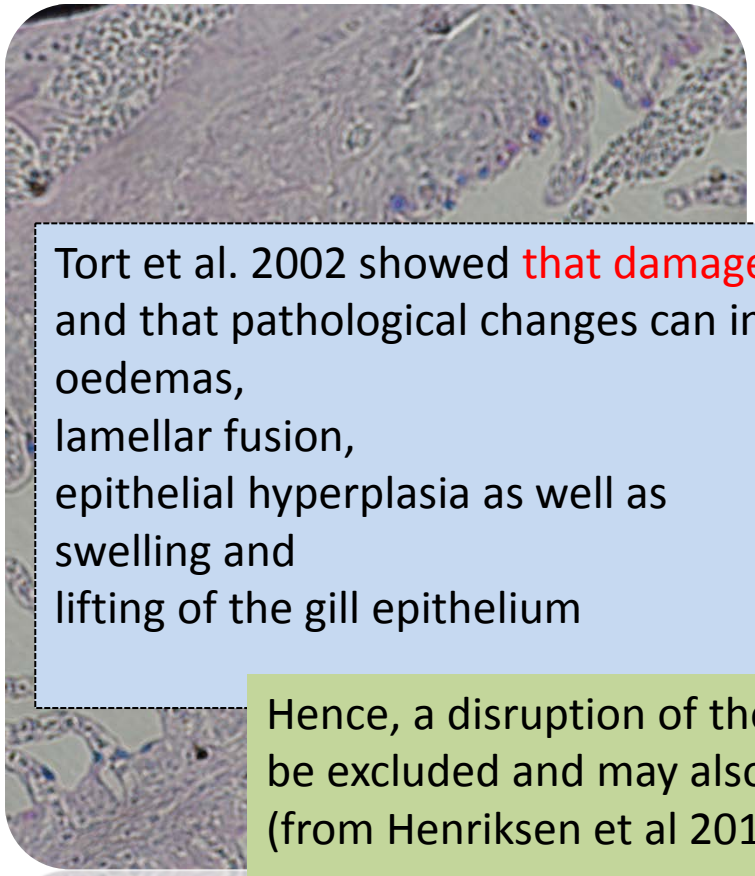
Mucosal Mapping of salmon gills:



Biophysical model
-cell size from pressure by neighbouring cells
= tensile strength of barrier

Tort et al. 2002 showed that damage due to H_2O_2 primarily occurred in the gills and that pathological changes can include:
oedemas,
lamellar fusion,
epithelial hyperplasia as well as
swelling and
lifting of the gill epithelium

ation?

ilable?

Hence, a disruption of the epidermal integrity cannot be excluded and may also lead to an increased vulnerability to pathogens. (from Henriksen et al 2013)

Immune and inflammatory responses in AGD affected salmon gills and Mucosal Mapping

question	Mucosal Mapping answer
Epithelial hyperplasia	Ratio of filament:lamellar epithelia Differential diagnosis wrt mucous cell proliferation?
Early immune responses	Changes in area/density ratio of mucous cells in epithelia
Upregulation of immune cells, immune signalling (f.eks. IgT, IgM, pIgR)	Number and area/density of mucous cells or other marked cells
Therapeutic interventions	General response capacity of mucosal epithelia of gills

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Conclusions:

The integrity of barrier functions of the skin, gills and guts may be indicated by mucous cell size and density in the epithelia (biophysical model)

Mucosal Mapping gives a snapshot of health which may have clinical significance

In Salmon, there are two distinct populations of gill mucous cells in the filaments and the lamellae.

There are trends to sex differences in the mucous cell populations of gills and of skin ♀

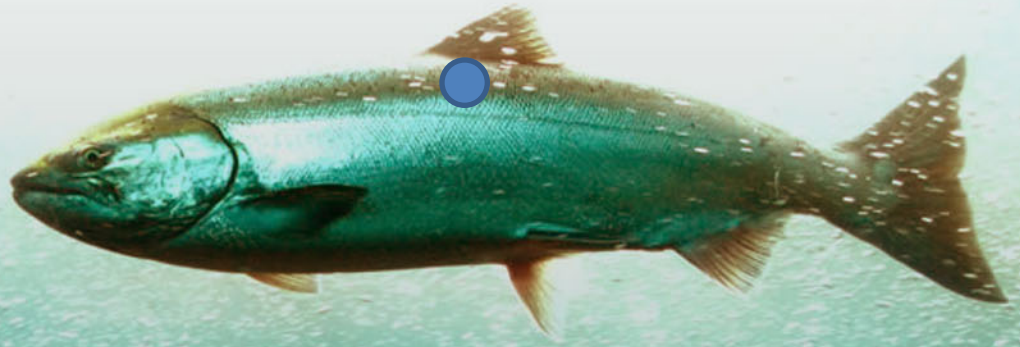
Gill mucous cell density is higher than skin, and cell size is smaller (higher tensile strength, faster motility)

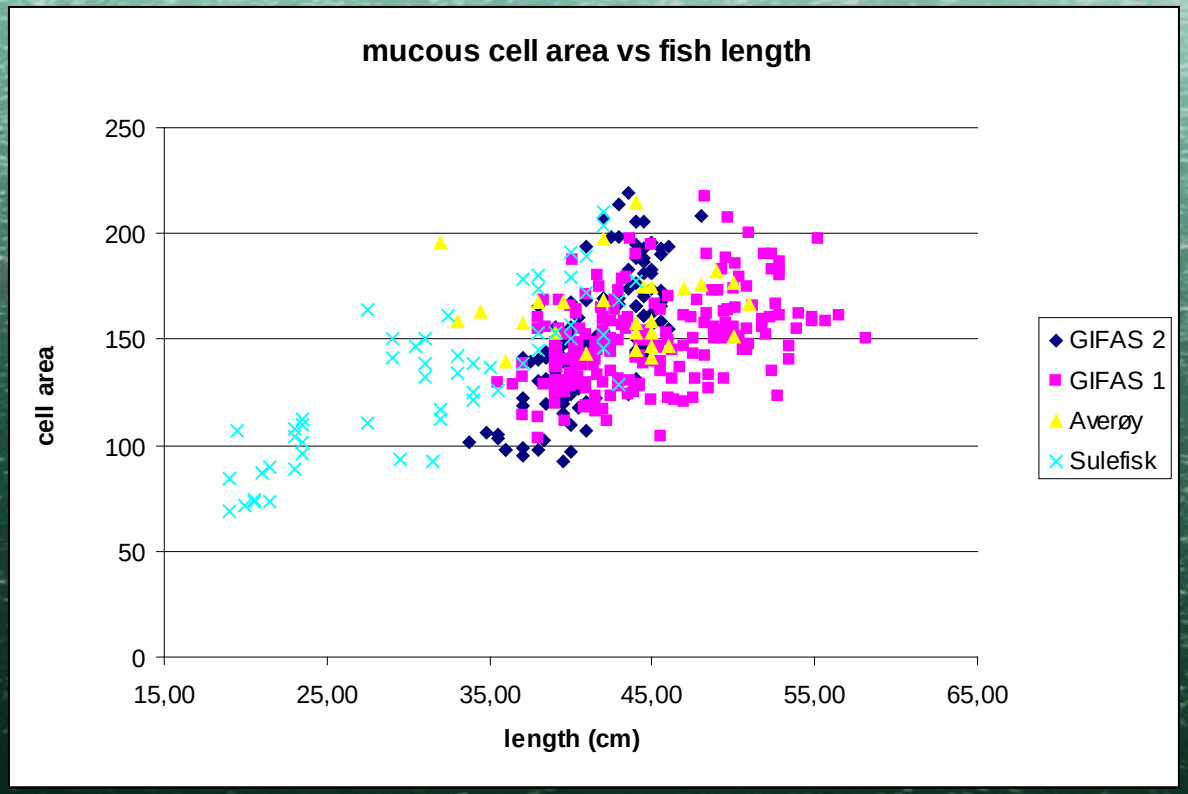
The mucosal epithelia of gills and skin are regulated differently

Small differences in all three mucosal epithelia reflect differences in the ability of the fish to maintain good health.

Site signatures

The data clusters: what more can we learn?





Mucosal Mapping in Salmonids

Any questions?

Call Quantidoc

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Previous studies on mucous cells in salmon skin (that's it folks!)

Source	Species	Section location	Stain	Thickness	Units of report
Buchmann & Bresciani (1998)	*Oncorhynchus mykiss* (Freshwater stage)	Varied	Alcian Blue	?	Number per mm2
Fast et al. (2002a)	*Oncorhynchus mykiss* *Salmo salar* *Oncorhynchus kisutch*	Mid body	Hematoxylin & Eosin	5 µm	Number per 100 µm length Width of largest
Harris & Hunt (1975a&b)	*Salmo salar* *Salmo trutta*	Varied	Lead citrate Uranyl acetate Toluidine blue	500-600 Å 1-2 µ	Size (µ) Did not report density.
O'Byrne-Ring et al. (2003)	*Salmo salar*	Shoulder flank	Periodic acid-Schiff/alcian blue	5 µm	Number per mm2
Fast et al. (2002b)	*Oncorhynchus mykiss* *Salmo salar* *Oncorhynchus kisutch*	Mid body	Hematoxylin & Eosin	5 µm	Number per 100 µm length
Roberts & Powell (2003)	*Salmo salar*	Gills	Periodic acid-Schiff/alcian blue	5 µm	Number per inter-lamellar unit
Roberts et al. (1970)	*Salmo salar* *Salmo trutta*	Varied in head region	Multiple stains (LM) Reynolds' citrate stain (EM)	8 µm 600 Å	Observations
Van der Marel et al. (2010)	*Cyprinus carpio*	Varied	Periodic acid-Schiff/alcian blue	4 µm	Number of goblet cells per sample

Healthy mucus = Healthy skin= Healthy fish

- The slimy skin of fish is the first line of defence against pathogens and damage
- Mucosal Mapping is the only statistically robust and objective quantification of mucous cell sizes, their density and their dynamics,
- 3 years of development and testing, 3 peer-reviewed publications
- Applied to 5 field trials of farmed Atlantic salmon, 1 of seabass
- Mucosal dynamics are significantly affected by diet, by body site, by fish size and sometimes by fish family or sex
- Useful for enhancing fish health, for interpreting molecular results, for breeding and for product development. Easy to compare between trials, species, tissues etc.
- Open for further development with new potential partners