Barriers and RAS: Trading immunity for growth?

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FRONTLINE DEFENSE

Mucous epithelium (slimy barriers)

Lives and learns

Dynamic

An early indictor

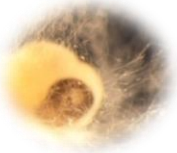
Barrier health is key!

A Living Pro-active System

1. Physical barrier*
2. Probiotic substrate
3. Immunologically active

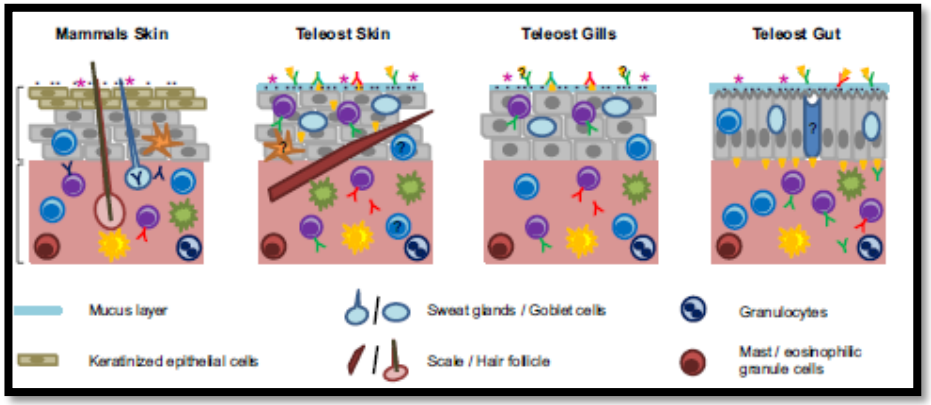
Antibakterial

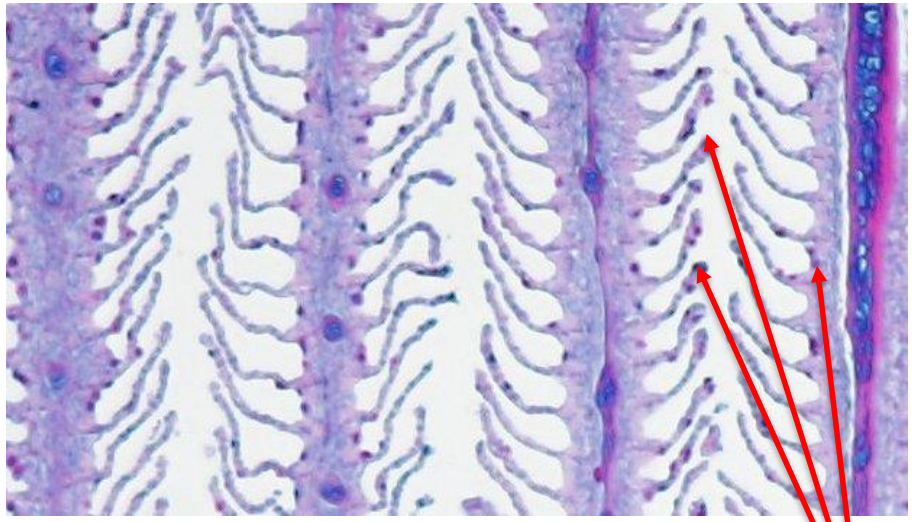
Antifungal



Antivirus

Antiparasitic





Mucous cell size and density is a measure of **barrier status**

*Gomez 2013**

Living barriers live and learn

Status quo:

- Focus on disease
- No early warning

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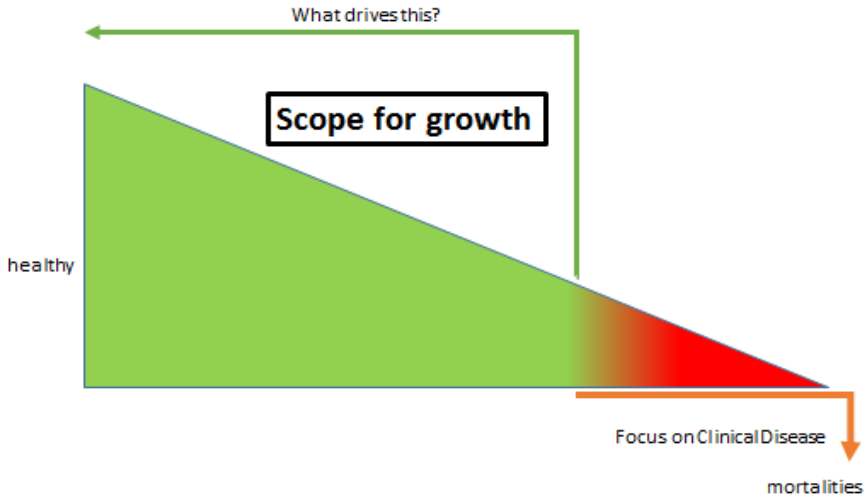
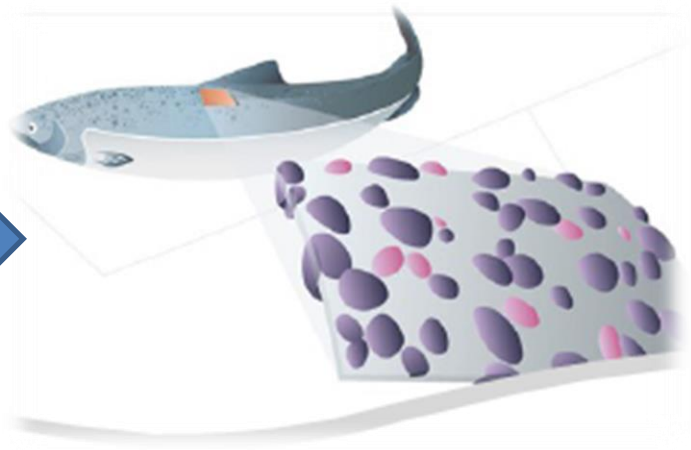
- Focus on barrier health
- Early warning

Protecting health vs detecting disease



Reactive: «Dead or alive»
Growth, mortality (%)

Pro-active: Barrier tissues (skin, gills, guts)
Summarize most effects



Reactive: gene level/ PCR
Detailed, difficult to interpret

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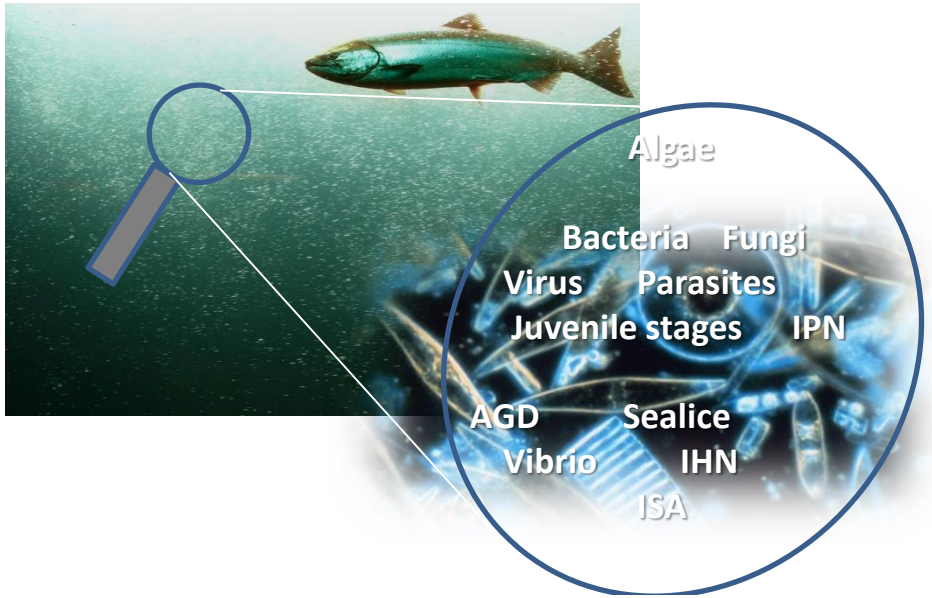
Frontline defense

Skin = Shield
- against environment

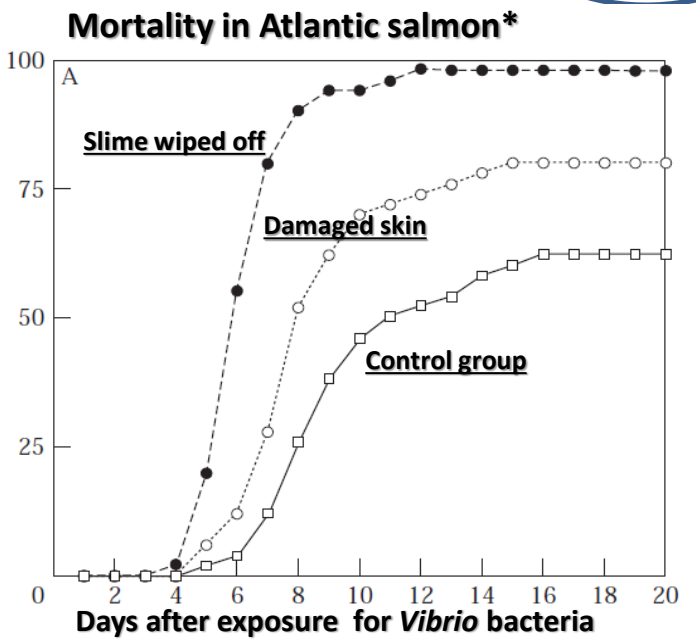
Gills = Sentinel
- *50% of total surface*
- *Respiration/excretion*

Gut = Foundation
- *Basis for immunity*
- *Influenced by diet*

Mucosal Barriers



Seawater is «pathogen soup»



Slime dried off
→100% mortality
(old news)

*Svendsen and Bøgwald 1997**

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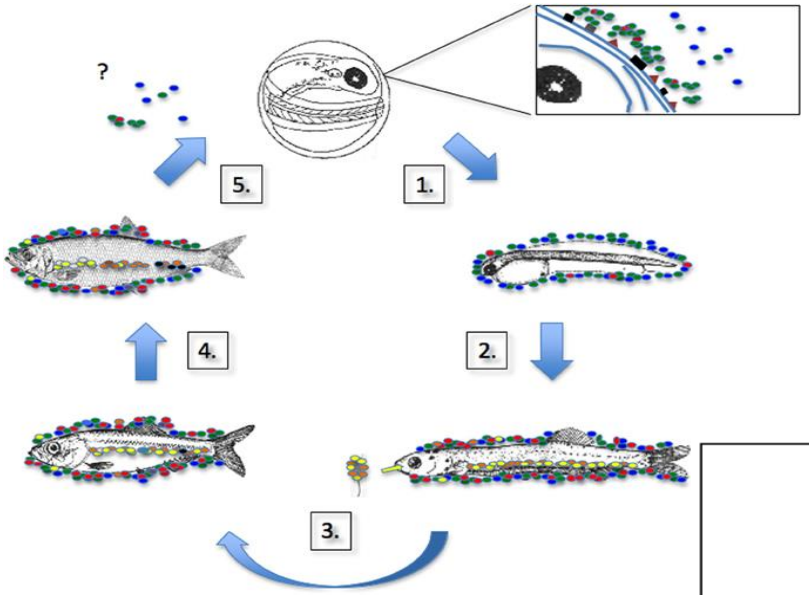
Basic principle:

Regardless of species…
Mucosal epithelium
is an ancient protection

Slimy barrier dynamics
= interaction with
microbiome and
environment

Living barriers are an innate immune system



Programming from Day 1

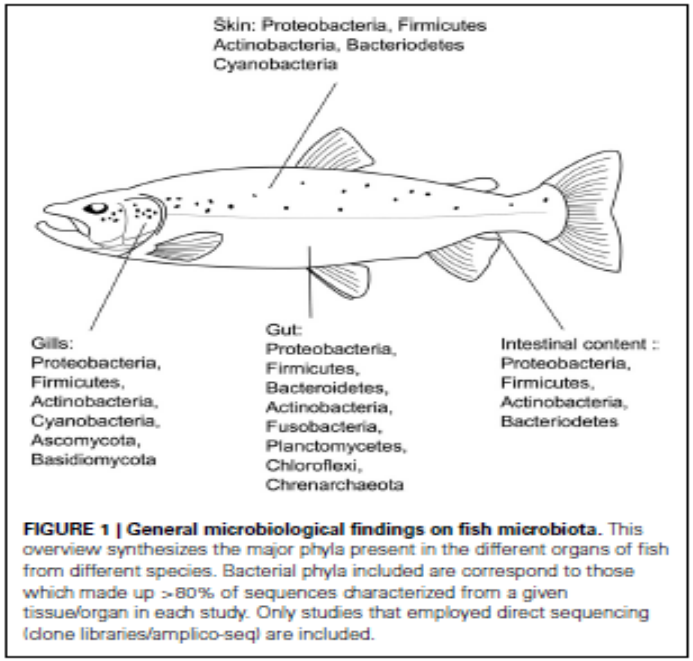


FIGURE 1 | General microbiological findings on fish microbiota. This overview synthesizes the major phyla present in the different organs of fish from different species. Bacterial phyla included are correspond to those which made up >80% of sequences characterized from a given tissue/organ in each study. Only studies that employed direct sequencing (clone libraries/amplico-seq) are included.

From Llewellyn et al 2014

Example microbiome for salmonids

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HYPOTHESIS Pathogenesis

- Biopsy based
- Response to immune-challenges is first in cell size then cell density
- Clinical conditions occur with both too much and too little
- Gills: The best early warning & indicator general health
- Foregut: Earliest response to diet

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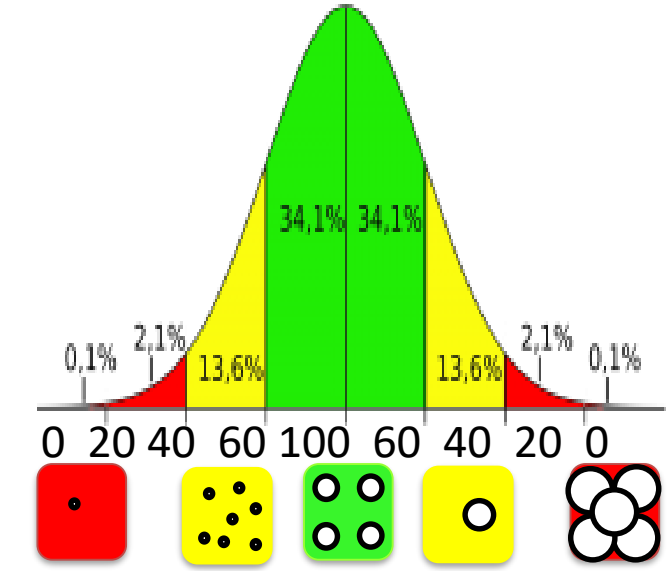
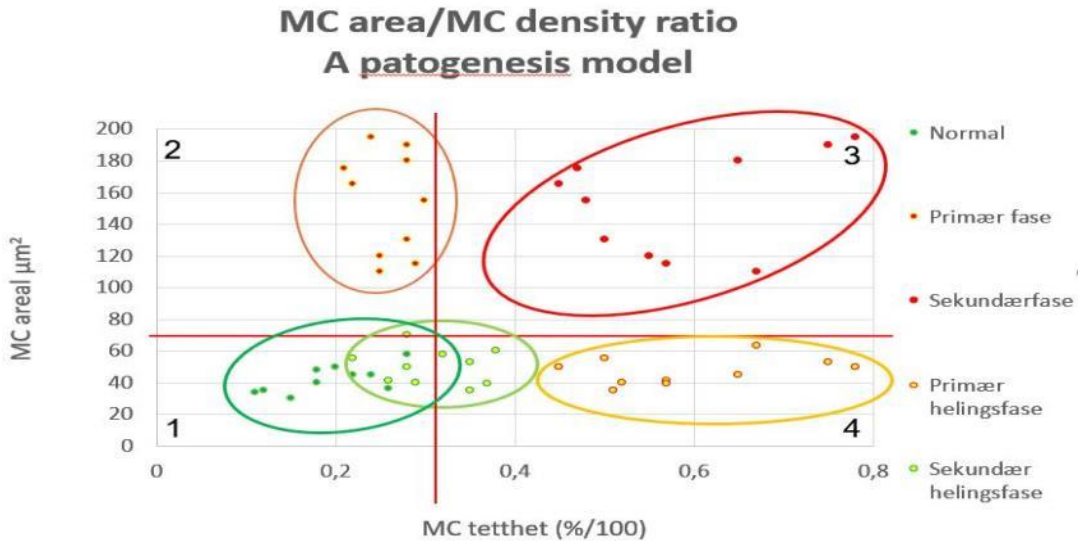
Quantify robustness of mucosal tissues

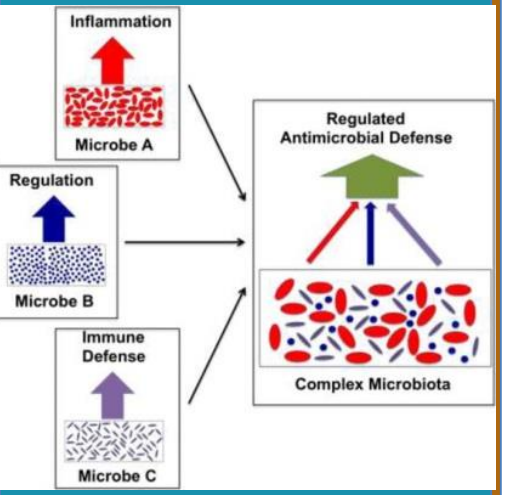


No mucus | Normal range of mucous cells | Only mucus



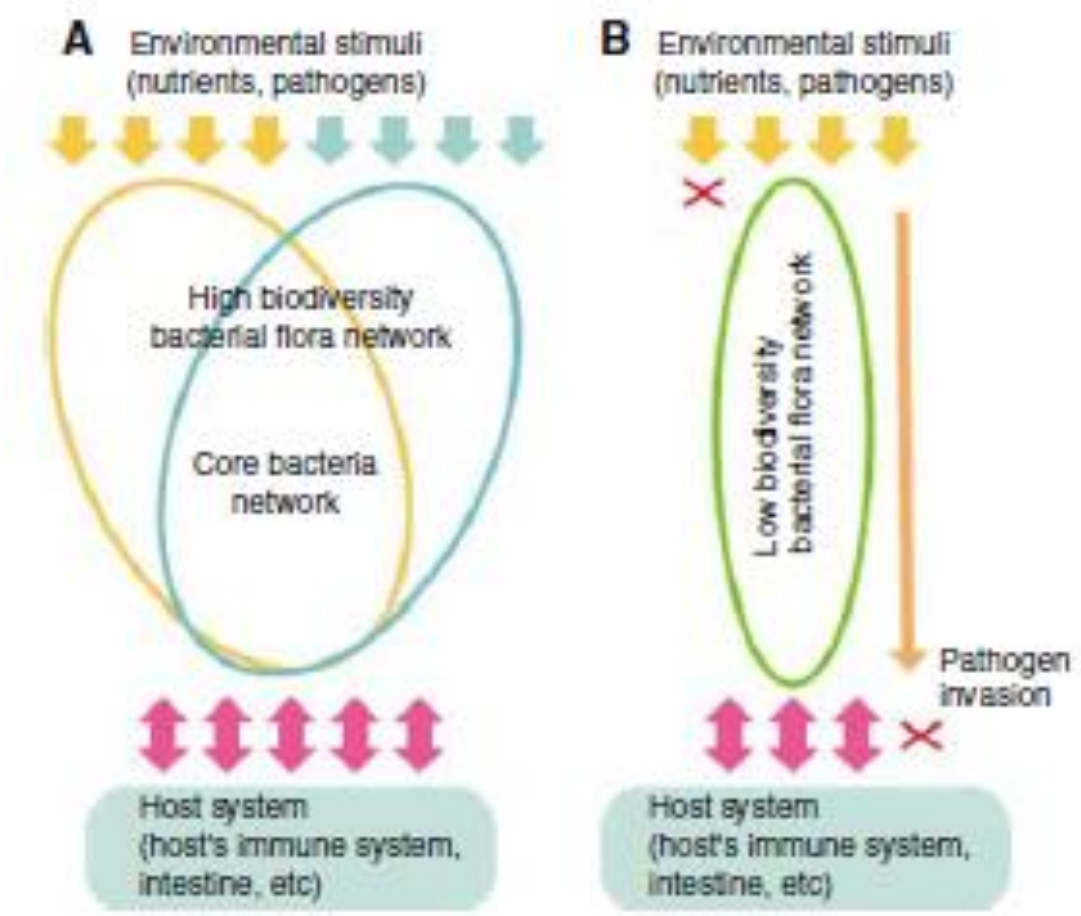
Basic Principle

Varied microbiome = better survival



From Pamer 2017

Wide microbial milieu **Narrow microbial milieu**



From Kitano & Oda 2006

"…the study of healthy individuals requires consideration of the microbiota at the community level" - Vadstein et al 2018

…"RAS gives stable microbiology, but ecology is a complex interaction between fish & microbes" - Bakke et al 2017

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Status 2018

SKIN:

Smolt origin important

Transfer weakened

Delousing weakens skin even further

GILLS:

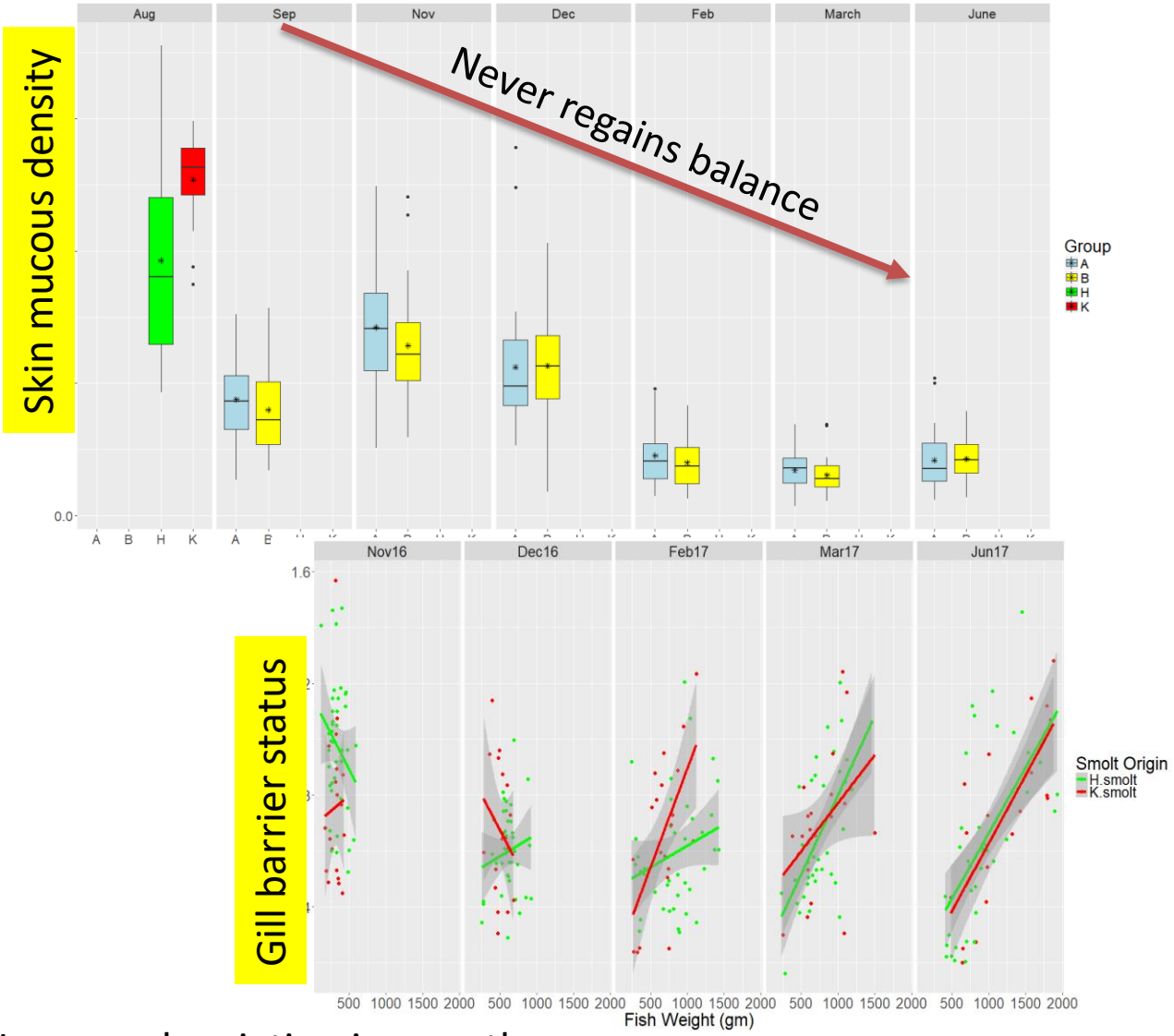
Correlated with growth

Increases with time

→ Earliest warning

«Industrial weakening» of salmon shield

From CAC Vindsvik: Marine Harvest, FHF, IMR, NIFES, Skretting, Quantidoc



Increased variation in growth

Found «winners» early,

«Losers» stay «losers»

NJORD Salmon AS

Smolt ongrowing farm at Tjeldbergodden:

Stable deep seawater

Stable temperature

Recieves both:

- **RAS smolt**
- **«Normal» smolt**

Fish held in 4 tanks (2 RAS and 2 Normal) Spring 2018

Measured barrier status:

- skin and gills

vs

- Growth and mortality

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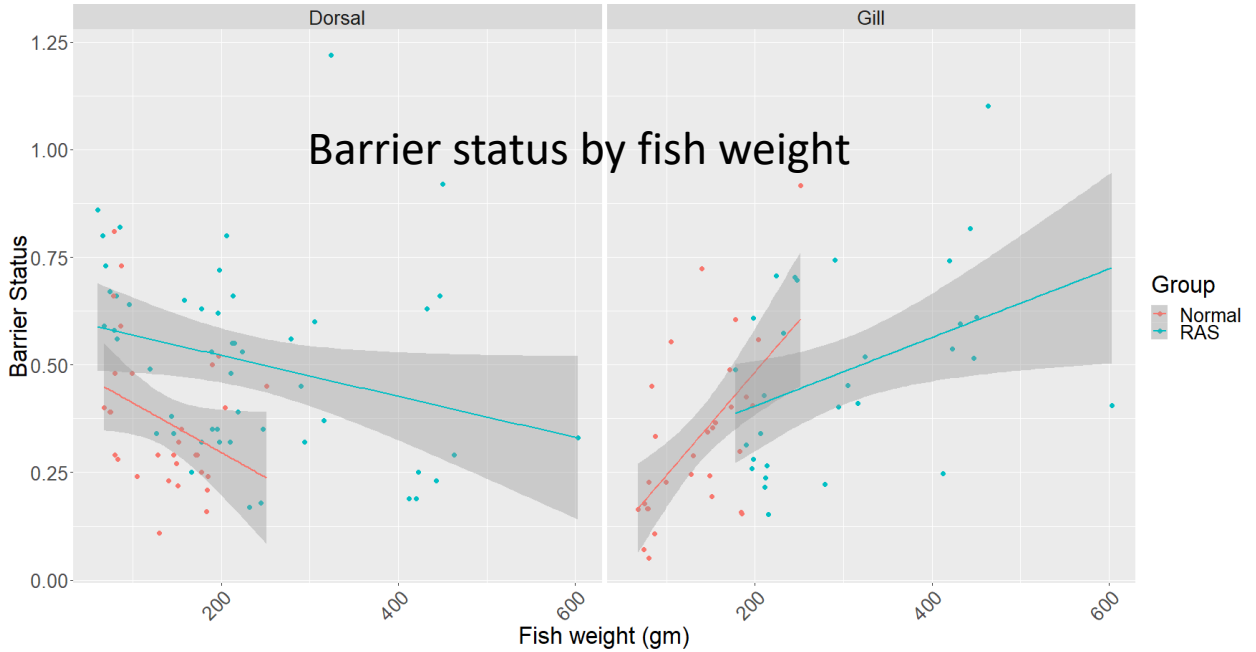
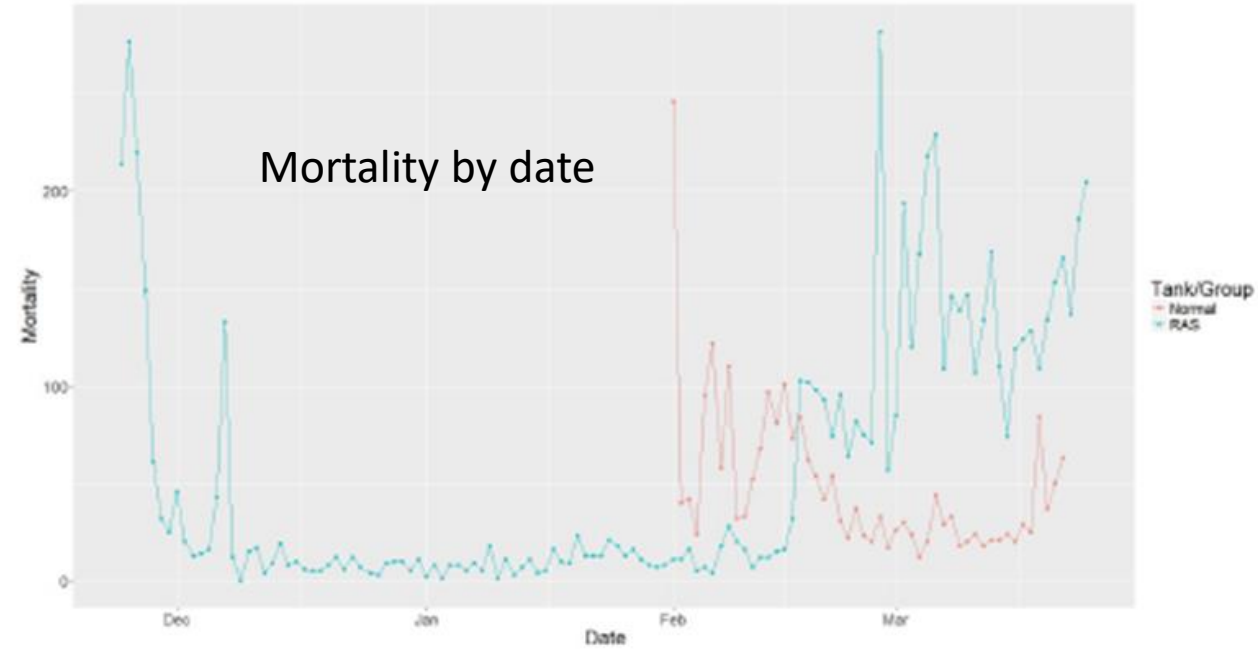
SKIN and GILLS

Gills: >50% of surface
- Oxygen uptake
- Metabolic excretion

The biggest RAS fish:
- Increased mortality
-Continued to die in sea
- Unspecific mortality

→ Adjusted for size:
RAS fish – weak gill barrier





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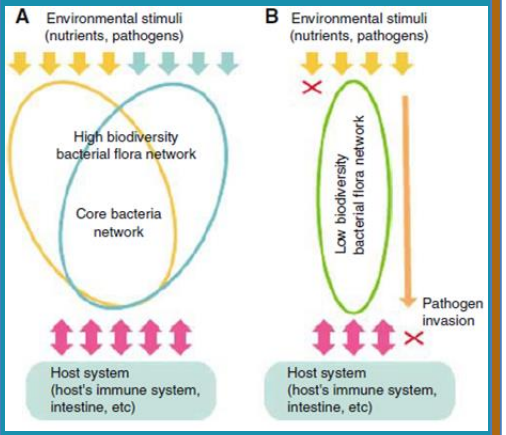
Njord Salmon as

Barrier status – trading growth for immunity?

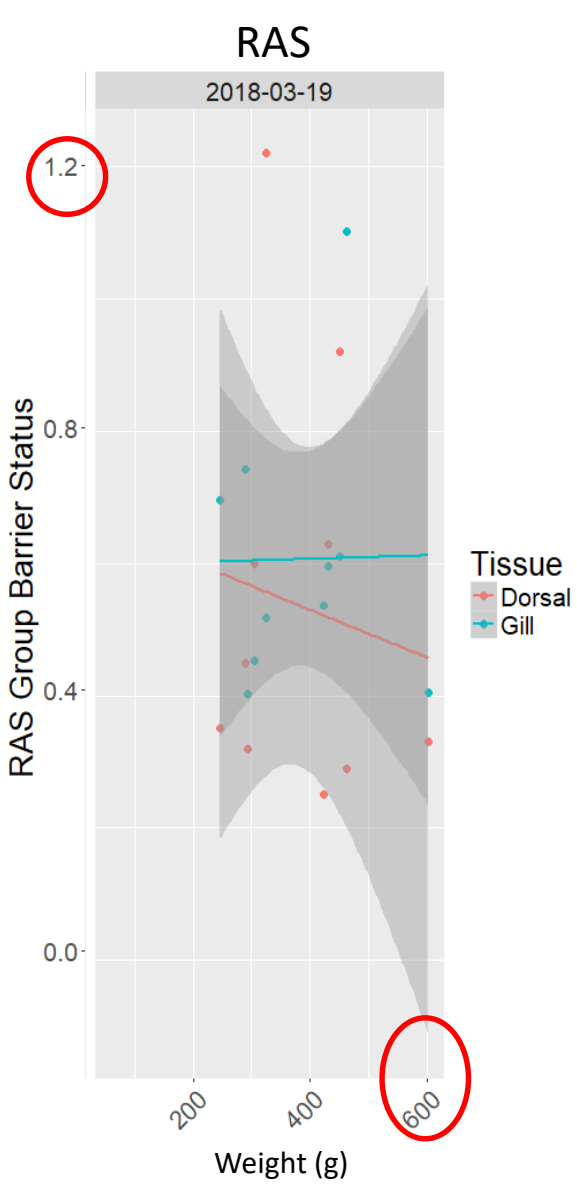
2 groups smolt in same fishfarm

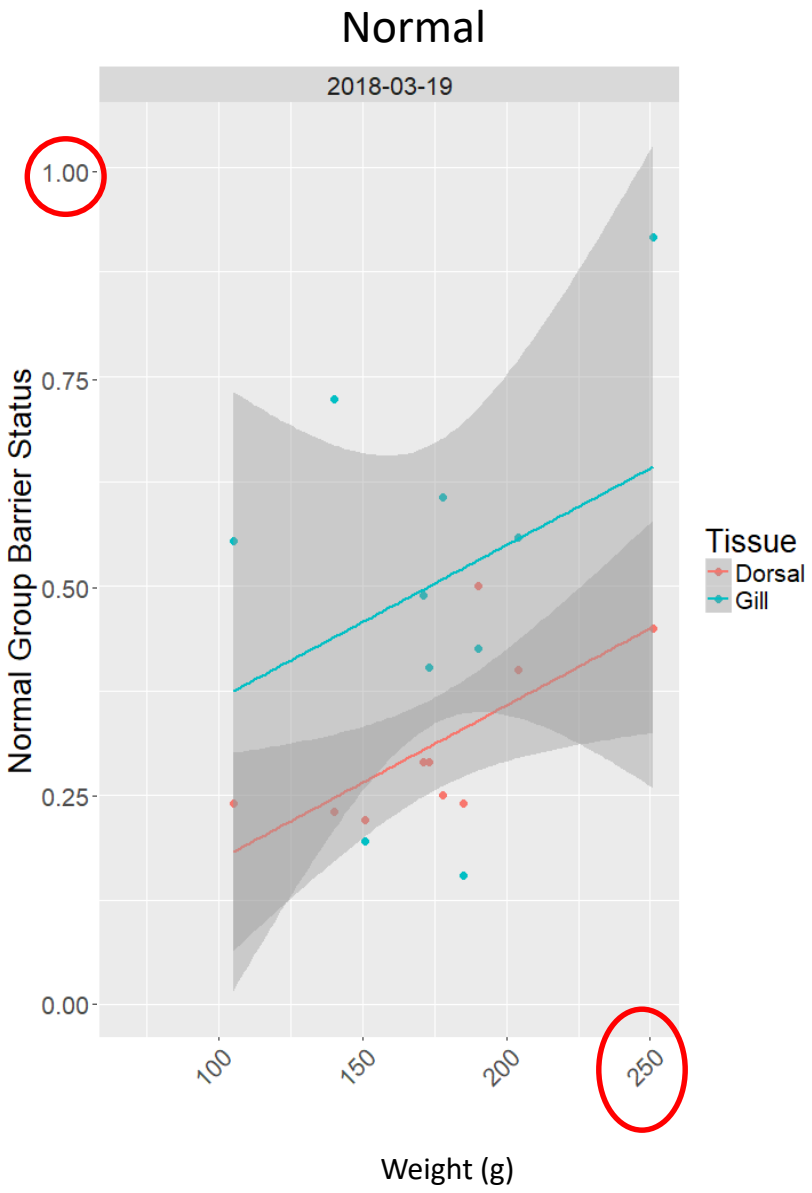
RAS weakens with size

Significant higher mortality in RAS group - 120 days in sea



Normal RAS





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Growth vs Immunity

Common garden - Salmobreed AS

Exposure:

3000 salmon smolt

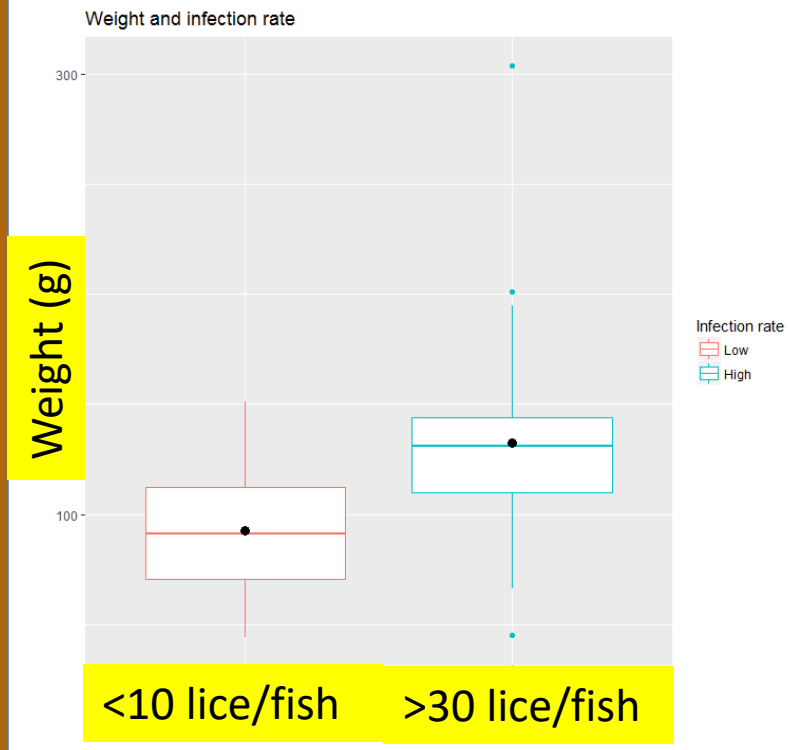
1 pulse of salmon lice

Reduced barrier status:

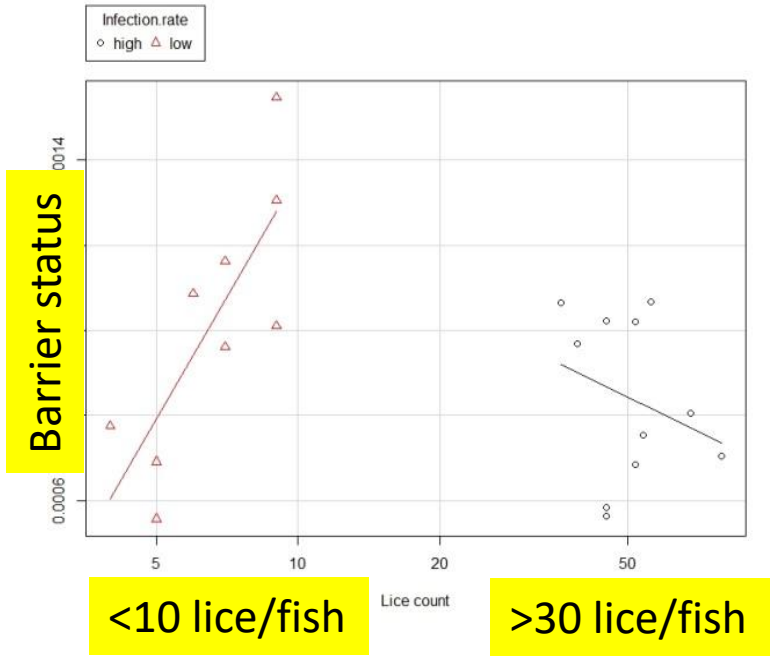
High growth?

OR

high lice count?



Early fast growth = more lice



Skin Barrier Status in smolts with low or high lice loads

From Hallberg (2018)

Peragill – project

Peracetic acid in RAS DTU Hirtshals

(Mild disinfectant)

Smolt, duplicate tanks:

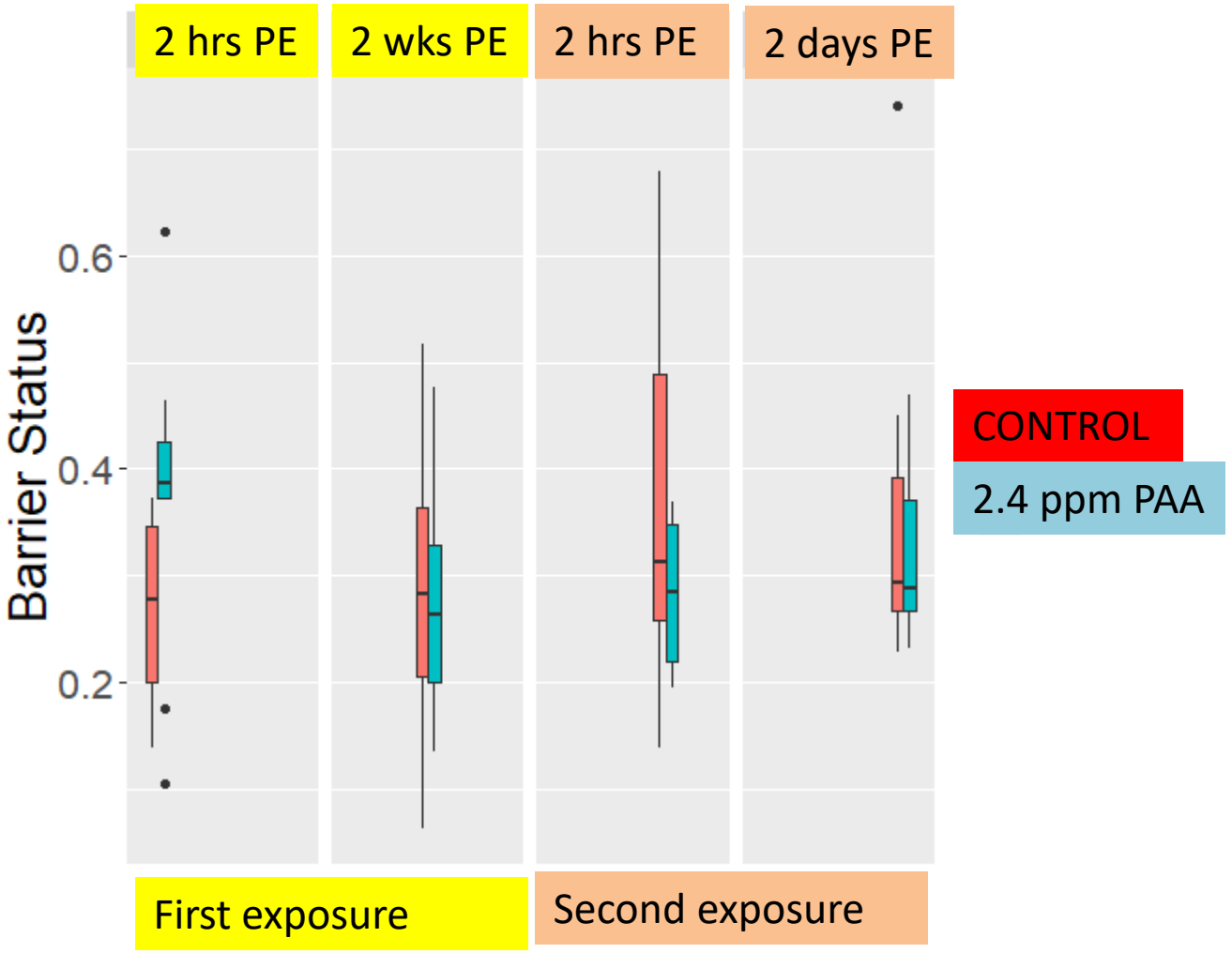
Peracetic acid in doses from 0 to 2.4 ppm

2.4 ppm increased barrier status <u>only at first exposure</u>

→ **Gills adapt to PAA with repeated exposure**

→ **LEARNING**

Gill Barrier Status vs Time Post Exposure



From: Haddeland et al (2019) in prep

Mucosal Barriers:

Reflects the costant interaction between fish and environment

Does the narrow microbiota in RAS systems make a immunological «naive» smolt?

CONCLUSIONS

1. Match vs mismatch in RAS microbial environment vs seawater challenges may underlie late mortality in large RAS fish

2. Evidence supports growth at the cost of general immunity (RAS vs Normal, lice loads, etc.)

3. Gill mucosal barriers learn and adapt

4. Can fish be trained to be more robust later within the current RAS environment?

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Verifying Barriers

Technology:
- Objective
- Reproducible
- Comparable
- Statistically robust

Application:
- Steering production
- Monitoring
- Verifying

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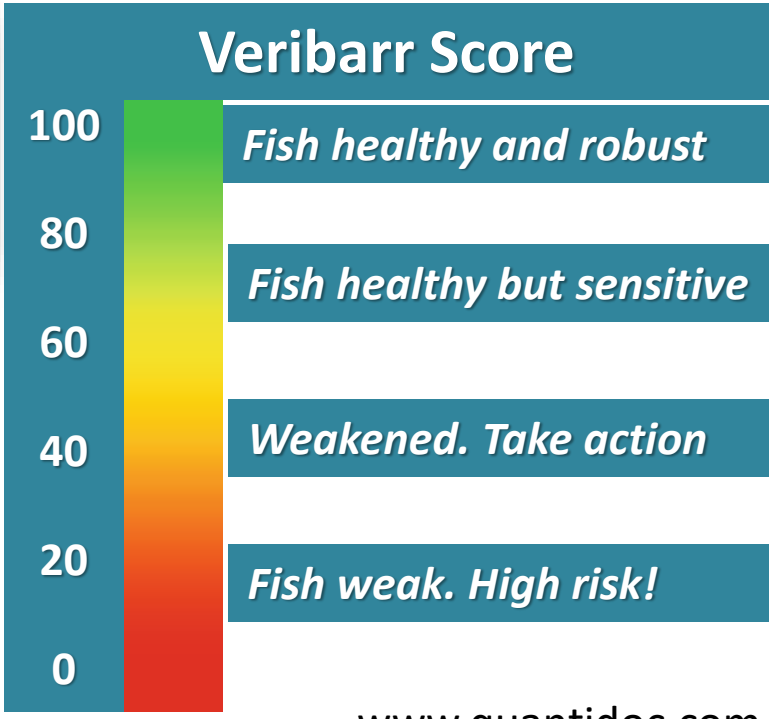
Veribarr™ an early warning tool for fish health

Quantifying tissue response
- Applied to skin, gills and guts
- Protocols for 7 species, applied in 7 countries, 60+ trials so far
- Diet, handling, technology, breeding, farm system, ecotoxicology

Quick method available in 2019

We measure – you improve



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