QuantiDoc
Quantifying Protection of Health

VERIBARR™
Specifying Robustness

GILL HEALTH FROM ANALOG DESCRIPTIONS TO DIGITAL QUANTIFICATION: TOWARDS STANDARDS

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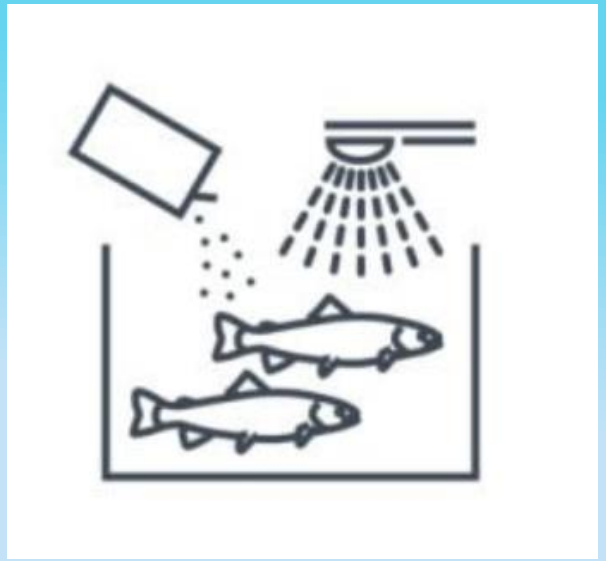
Gill Health Initiative Oct 2023

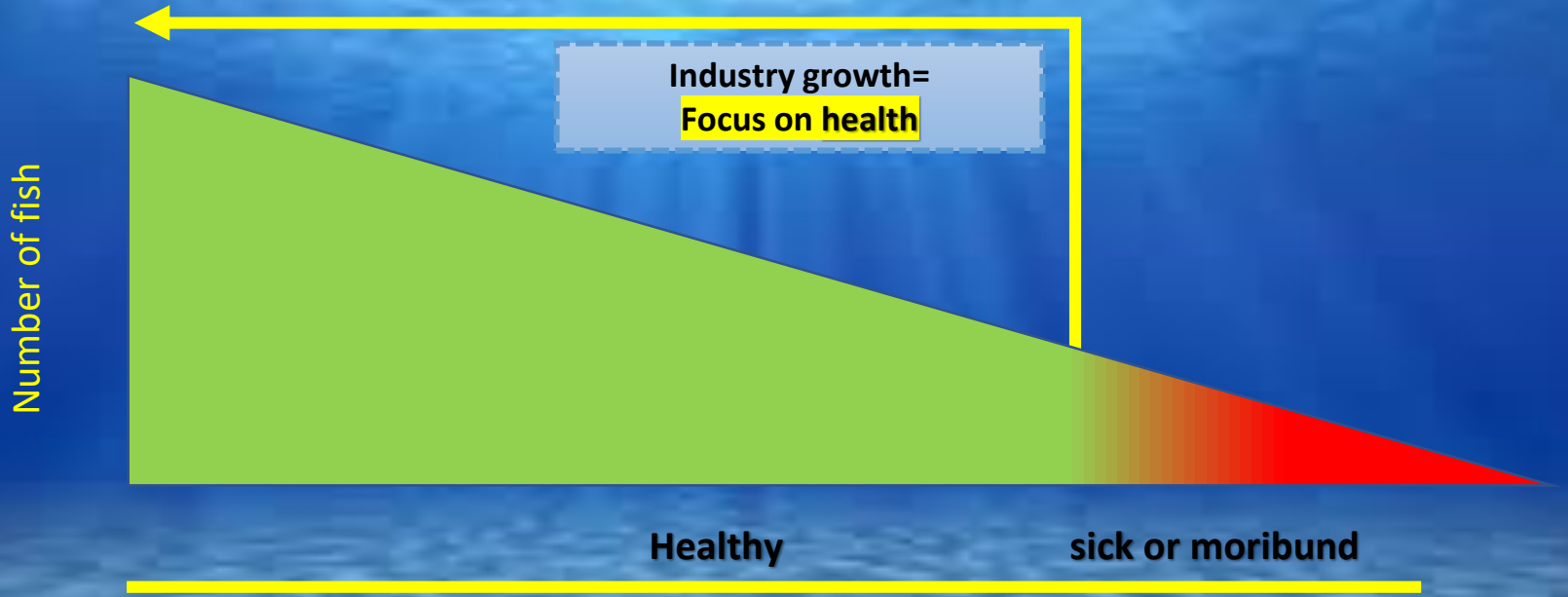
CONTENT

1. CHANGING YOUR POINT OF VIEW
2. MULTIPLES SPECIES WITH 2-PART GILL MUCOSA
3. DON'T JUST COUNT
4. REQUIREMENTS OF STANDARDS
5. FISHMOM



How do you know your stock is <u>healthy</u>?

INDUSTRIAL ANIMAL PRODUCTION VS INDUSTRIAL FISH PRODUCTION



Veribarr™ designed as an industrially scalable metric for fish barrier health

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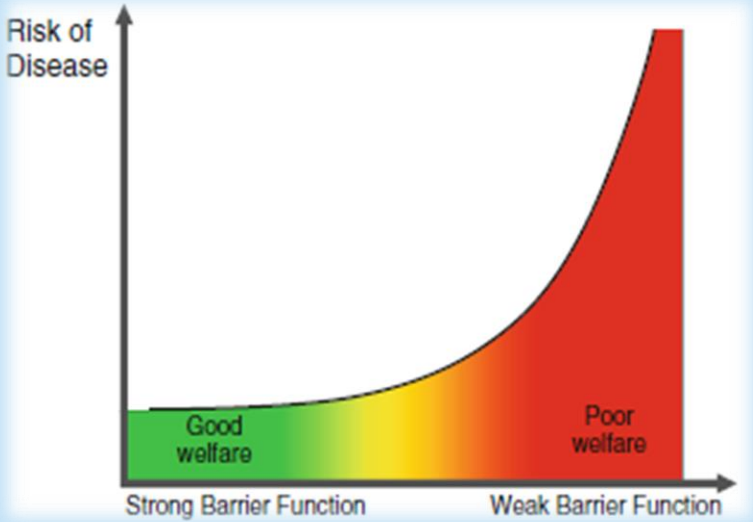
MUCOSAL EPITHELIUM = SKIN, GILLS, GUTS = BARRIER FUNCTIONS



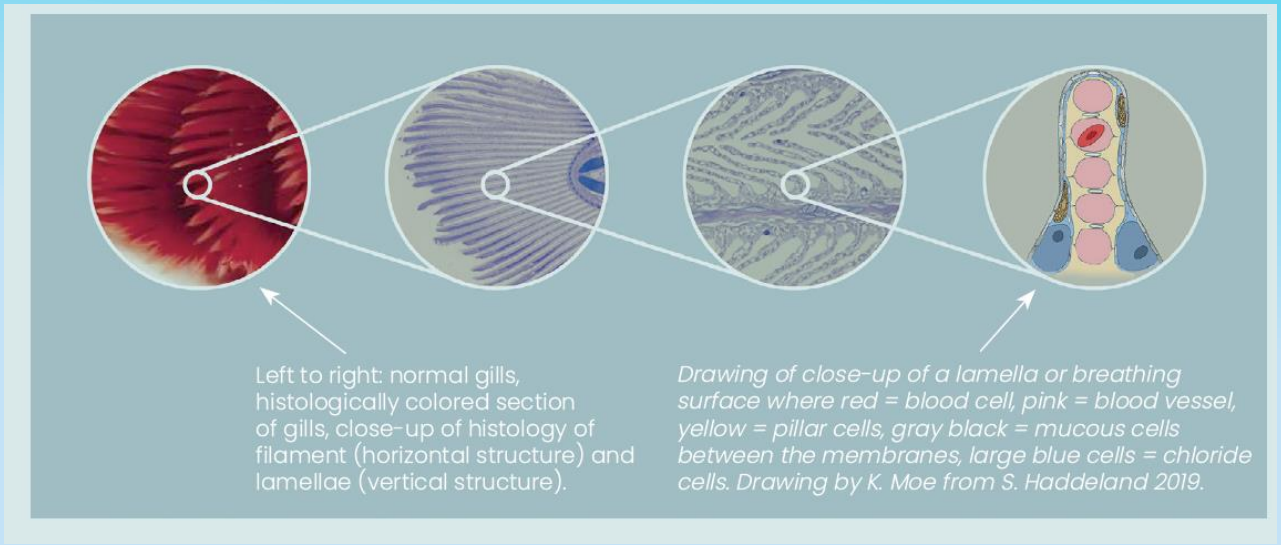
Redrawn from Segner et al.

Mucous cells

scales

Mucous barrier epithelium wraps over and around the scales, in guts and around gills - Nice layers, poor statistics

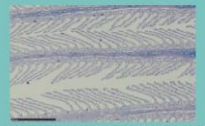
Good barriers = good health



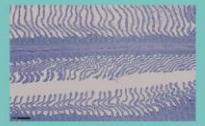
Left to right: normal gills, histologically colored section of gills, close-up of histology of filament (horizontal structure) and lamellae (vertical structure).

Drawing of close-up of a lamella or breathing surface where red = blood cell, pink = blood vessel, yellow = pillar cells, gray black = mucous cells between the membranes, large blue cells = chloride cells. Drawing by K. Moe from S. Haddeland 2019.

Gill breathing surfaces and the variation in mucous cell density.



0.1% mucous cell density
Healthy and fine



5.1%

11 %

14 %

19 %

Dark blue spots = mucous cells
Light blue tissue= epithelial cells

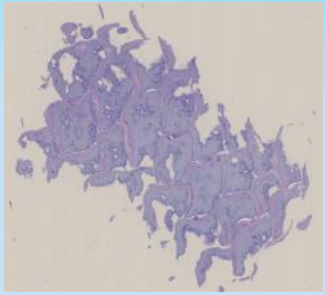
Gill lamellae and *volumetric* density of mucous cells

Change your point of view by 90º

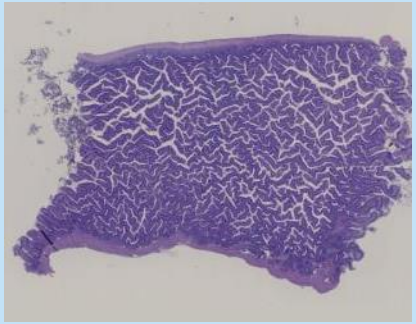
Tangential sections for Mucosal Mapping (Veribarr™) analyses = statistically robust results and *millions* of times more surface representation

Gill (lamellae, filament)

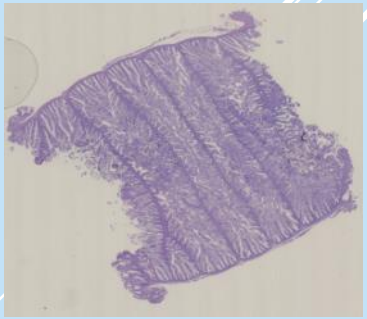
Dorsal hud



Fore gut



Hind gut



Quantitive Analysis

directly comparable across

- Species
- Tissue
- Treatment
- Time
- System

Veribarr vs traditional histology

Comparison	Histological quantification of mucous cells	Veribarr™ on mucous cells (design-based stereology, 3D from 2D)
Length or area	1-2 mm running length	1-2 cm^2 surface area
Unit of measure	Relative to existing structures	Universally applicable
Orientation of section	Very important	Not important
Standardization	- No standardized units - Not directly comparable across treatment and organs	- Standardized reporting - Comparable across treatment and organs
Qualitative or quantitative	Qualitative and quantitative	Quantitative
Method	Manual	Semi-automated
Bias	Biased unless random rules applied	Unbiased

(Table modified from Dang *et al.* 2020, Table 4).

GENERATION STUDY

GILLS

Freshwater

Origin differences

Seawater

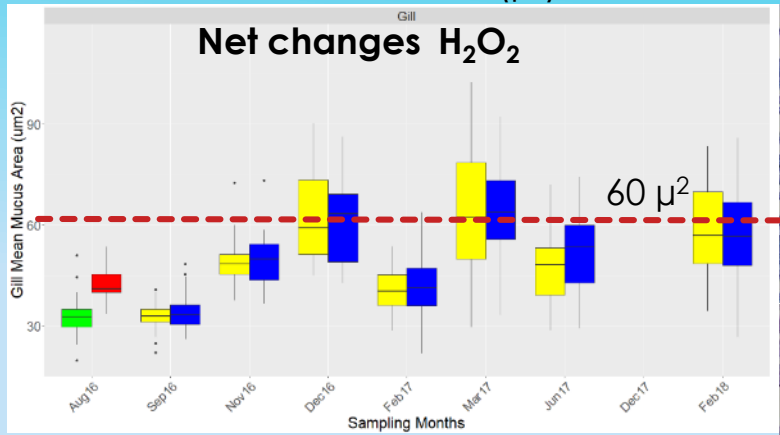
Reacts to net changes, particles, delousing

Significant differences within gill score 0-1

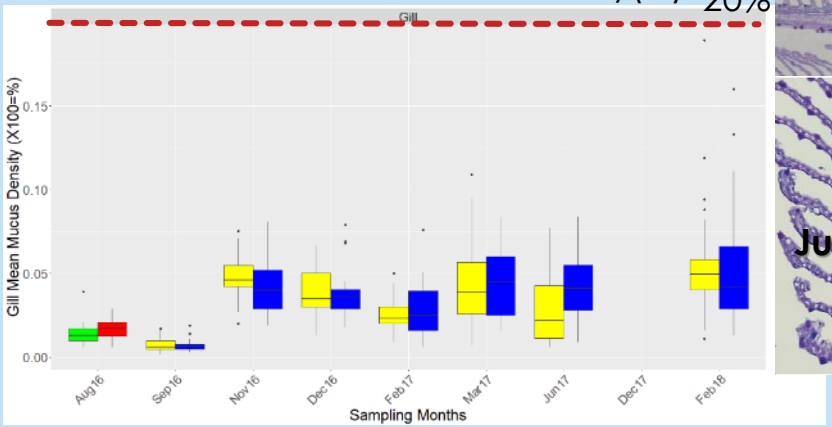
n=30-45 under each box
2 treatments in triplicate

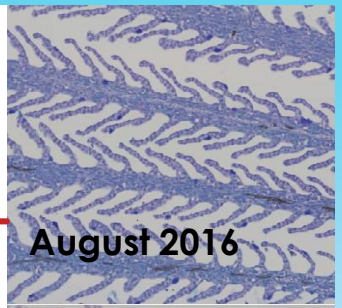
WITH MARINE HARVEST, SKRETTING, FHF, IMR, NIFES OG QUANTIDOC

Lamellar mucous cell size (μ²)



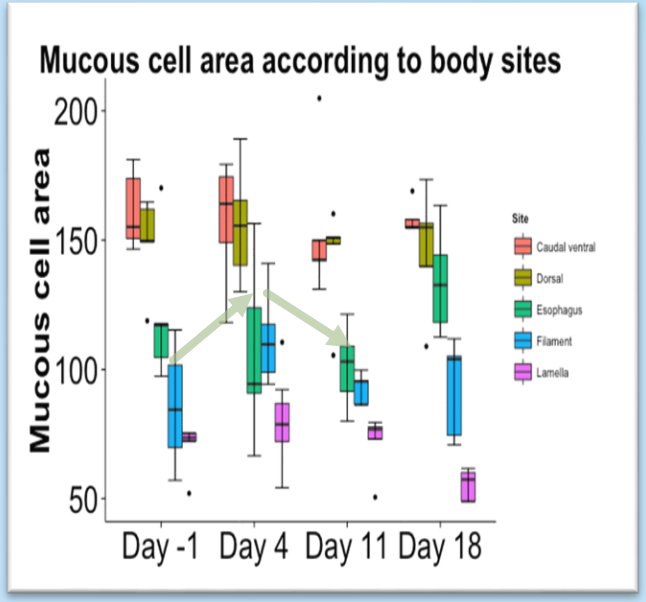
Lamellar mucous cell vol. density(%)





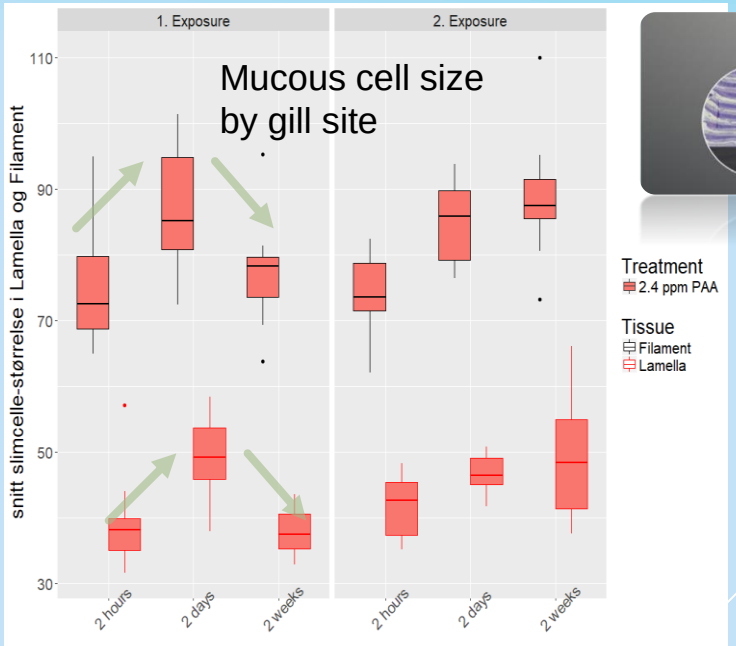
Standards allow Comparable Responses across time, treatment and trials:

1. Gills show delayed reaction, peaking a few days after stressor
2. Require 2-3 weeks to return to «normal»
3. Gill lamellae and gill filament are **2 distinct mucous barriers**



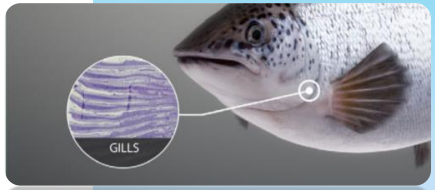
From Rantty I, 2016

- Commercial delousing **with hydrogen peroxide**
- Seacage n=22 fish



From Haddeland S, 2019

- **2.4 ppm PAA** in seawater –RAS
- n=60 fish



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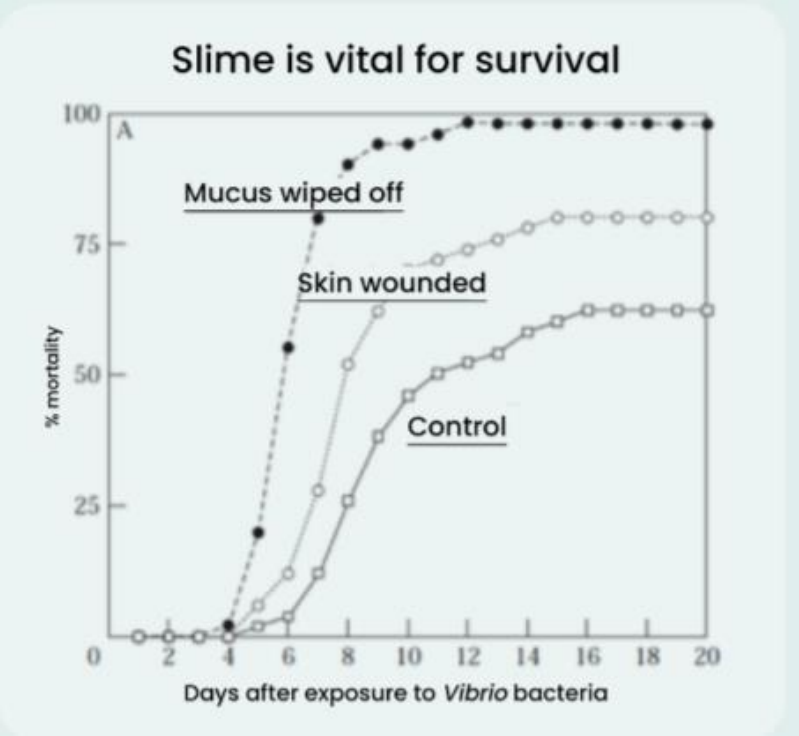
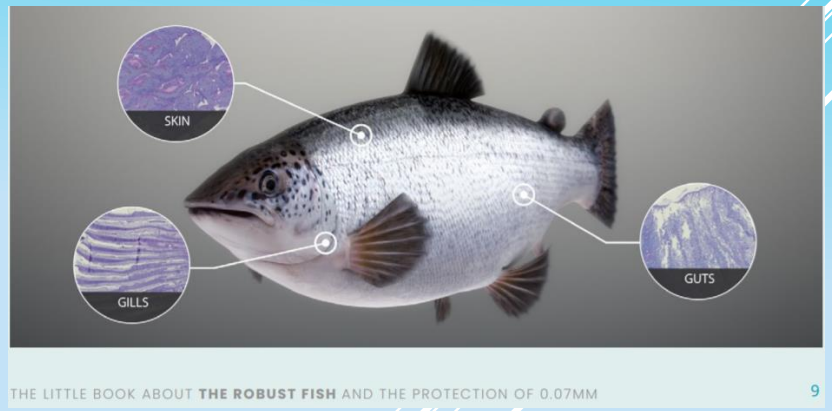
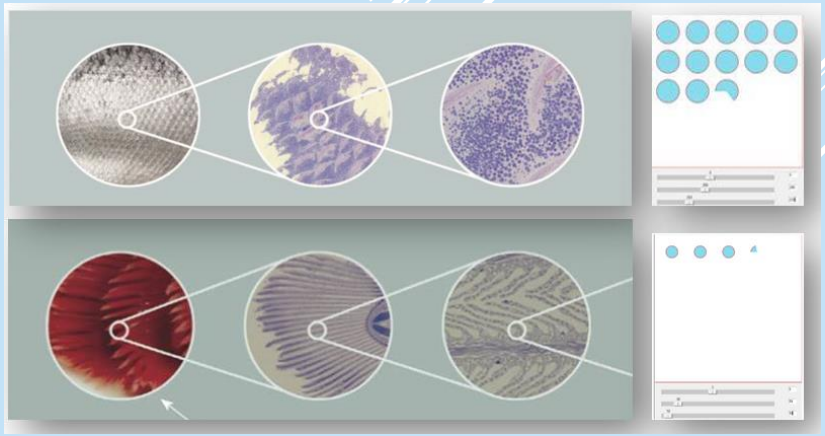
Mucosal barrier health – the Dicer v2 to compensate



Svendsen & Bøgwald 1997

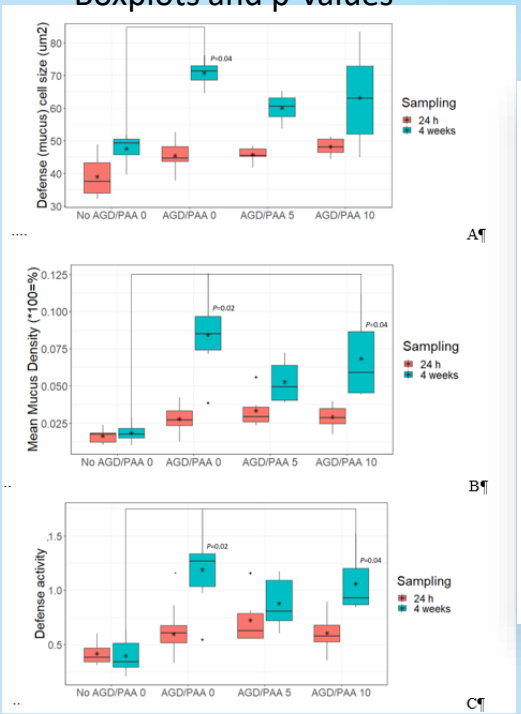




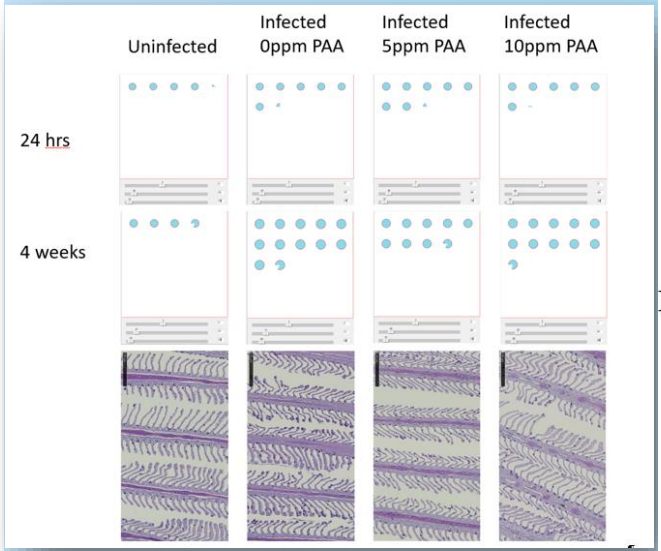
Amoebic Gill Disease increases mucous production within 24 hrs

- Treating with twice the dose Peracetic Acid (PAA) is half as good at 4 weeks

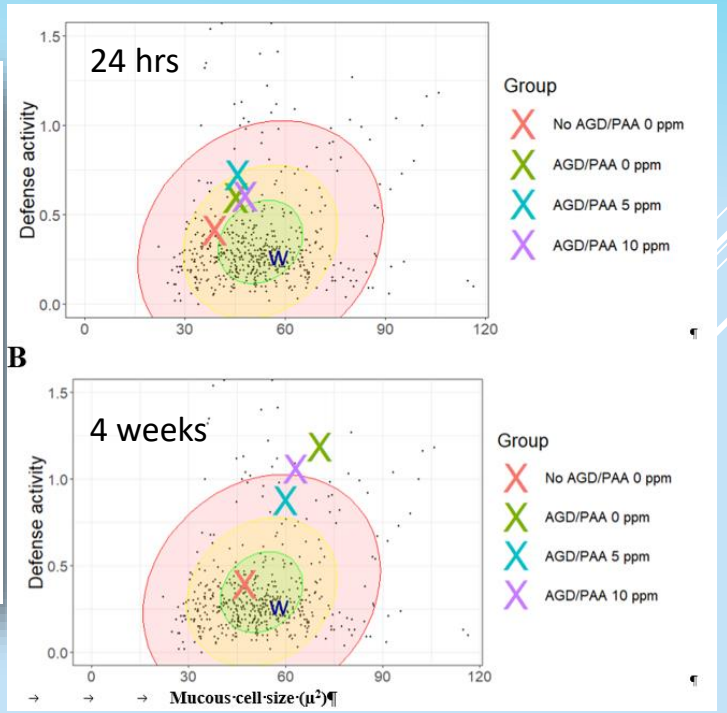
Boxplots and p-values



Dicer illustrations of gill lamellar mucous cells and histological sections



Mean results against Veribarr database

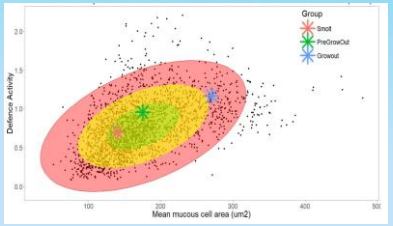


24 hrs 4 weeks

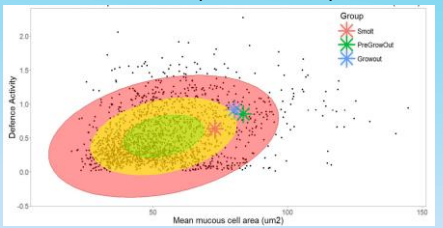
from Lazado et al. 2022. **Immunity and stress associated with the infestation of the parasite *Neoparamoeba perurans* are differentially shaped by a potent oxidant in Atlantic salmon (*Salmo salar*)**

Mucosal responses in multiple tissues for 3 stages in RAS
Dicer images and Veribarr database comparison

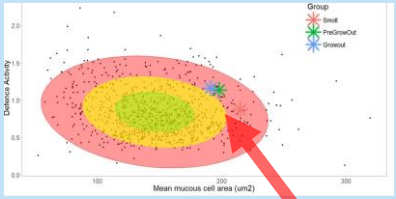
Skin (DB N=2161)



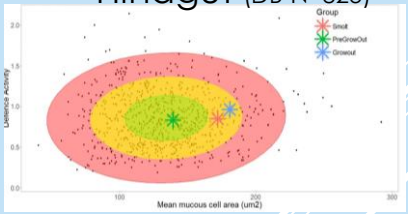
Gills (DB N=1887)



Foregut (DB N=879)

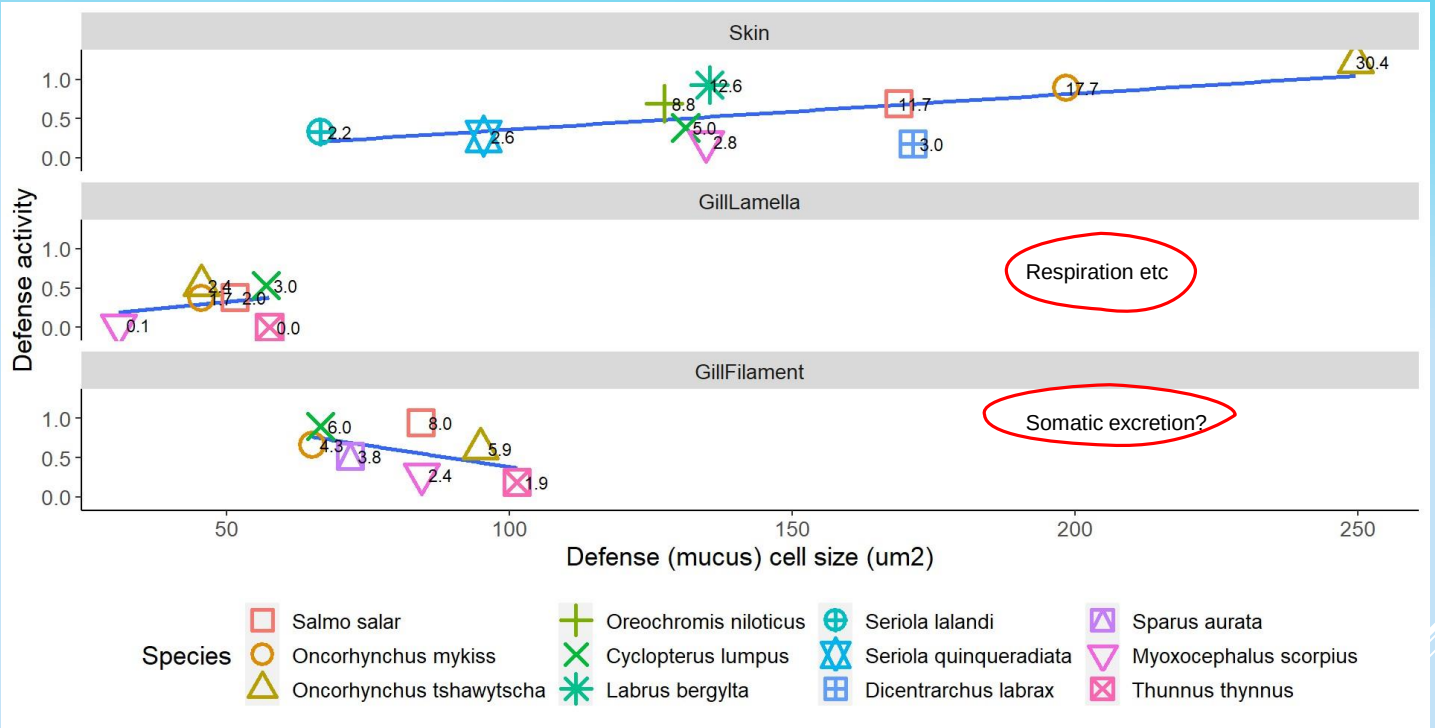


Hindgut (DB N=823)



Diarrhea – revised feed recipe

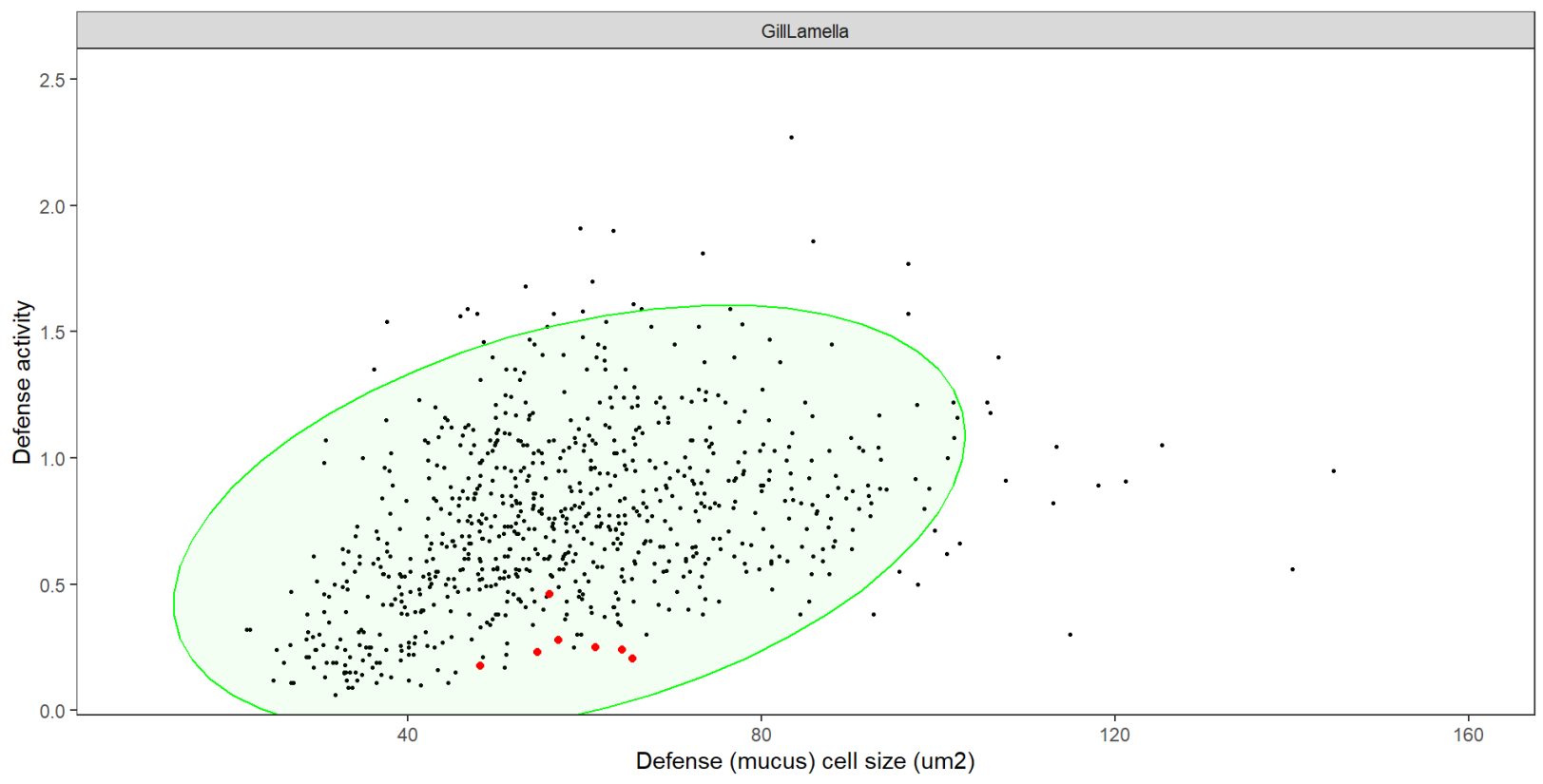
Results across 12 species



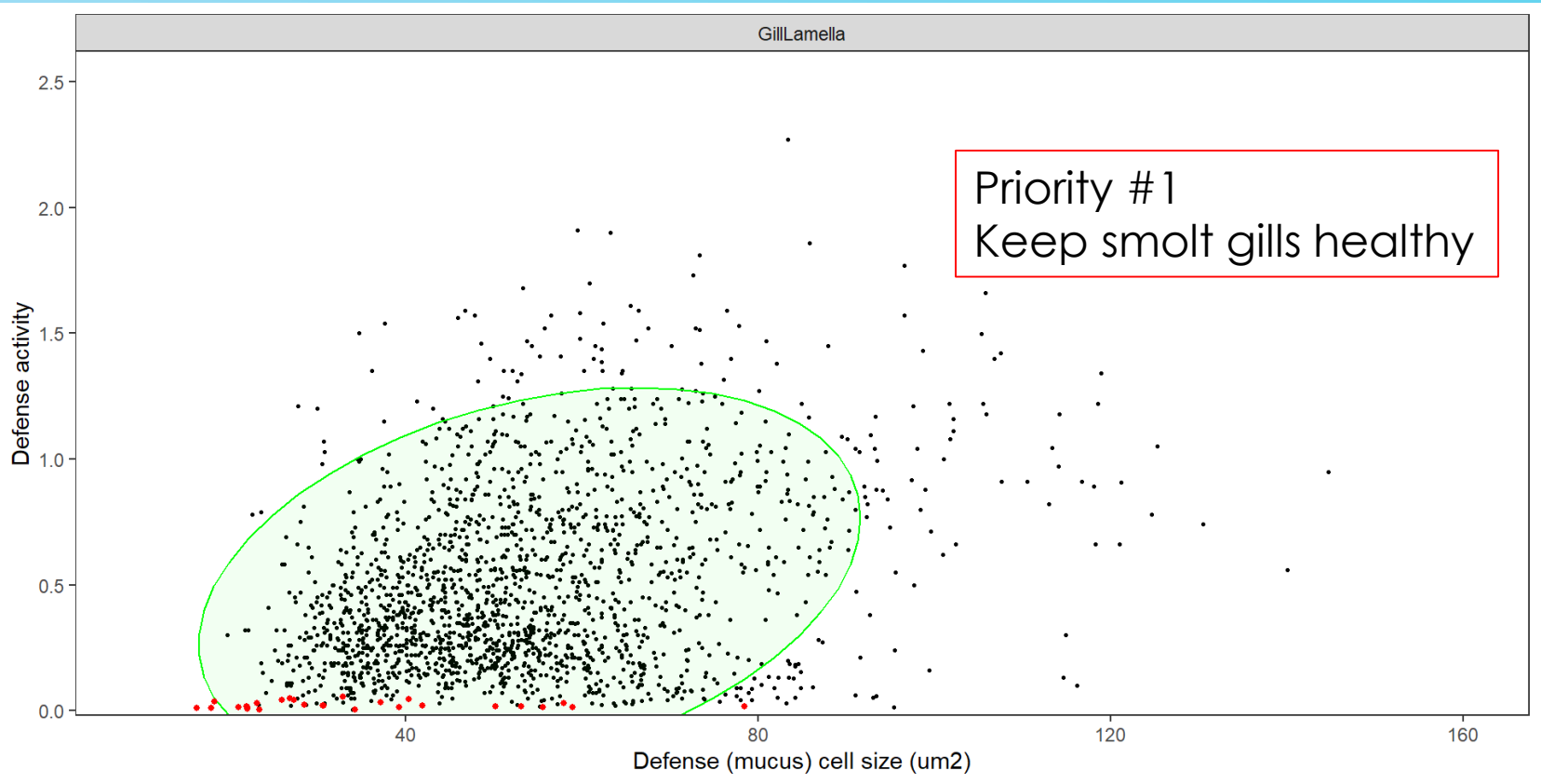
The variation in MC size (μm^2) in epithelia and calculated MC defense activity of the tissue (1000/(MC size / MC volumetric density) for 12 fish species. MC volumetric densities in the epithelia (%) are presented as labels.

From: Merkin et al (in prep) Revising current understanding of the mucosal interface in fish based on the quantitative pattern of mucous cell production in the skin and gills of Atlantic salmon and 11 other fish species

Wild adult salmon gill lamellae vs seabased farmed salmon



Wild salmon smolt gill lamellae vs farmed salmon gill lamellae



DON'T JUST COUNT: CELL SIZE MATTERS!

5 cells with radius 9.77 microns vs
5 cells with radius 2.5 microns

= **58 times more volume (cytosol)** and **15 times more cell membrane**

Gills

Guts

Skin

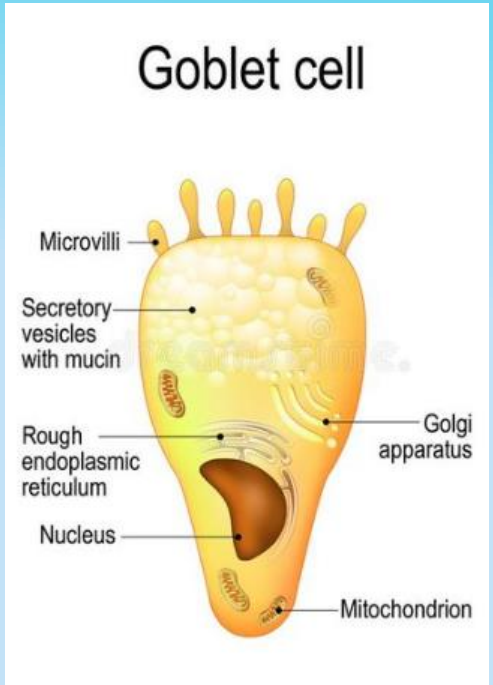
AREA (μ^2)	RADIUS µ	Surface area sphere (μ^2)	Volume sphere (μ^3)	NUMBER OF CELLS	Surface area in unit (µ2)	Volume in unit (µ3)
20	2.52313252	80	67,2835339	5	400	336,41767
		80	67,2835339	10	800	672,83534
50	3,9894228	200	265,96152	5	1000	1329,8076
		200	265,96152	10	2000	2659,6152
100	5,64189584	400	752,252778	5	2000	3761,2639
		400	752,252778	10	4000	7522,5278
150	6,90988299	600	1381,9766	5	3000	6909,883
		600	1381,9766	10	6000	13819,766
200	7,97884561	800	2127,69216	5	4000	10638,461
		800	2127,69216	10	8000	21276,922
250	8,92062058	1000	2973,54019	5	5000	14867,701
		1000	2973,54019	10	10000	29735,402
300	9,77205024	1200	3908,8201	5	6000	19544,101
		1200	3908,8201	10	12000	39088,201

CELL SIZE MATTERS:

5 cells with radius 9.77 microns vs
5 cells with radius 2.5 microns

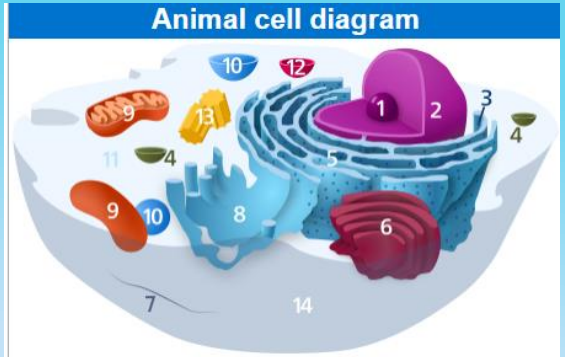
= **58 times more volume (cytosol)**

= **15 times more cell membrane**



Major metabolic pathways
that occur in the **cytosol** in animals:
protein biosynthesis,
pentose phosphate pathway,
glycolysis and gluconeogenesis

3 mucin types:
trans-membrane MUC1, MUC4, MUC16
secreted (gel-forming) MUC2, MUC5AC, MUC6
soluble (non-gel-forming) MUC7, MUC8, MUC9, MUC20
- critical in maintaining cellular functions, particularly those of epithelial surfaces.



Components of a typical animal cell:

1. Nucleolus
2. Nucleus
3. Ribosome (dots as part of 5)
4. Vesicle
5. Rough endoplasmic reticulum
6. Golgi apparatus (or, Golgi body)
7. Cytoskeleton
8. Smooth endoplasmic reticulum
9. Mitochondrion
10. Vacuole
11. **Cytosol** (fluid that contains organelles; with which, comprises cytoplasm)
12. Lysosome
13. Centrosome
14. Cell membrane

Requirements of standards

1. Early detection of infectious and non-infectious disease depends on routine surveillance and knowledge of **fish's biology** (aquaculture ≠ agriculture underwater)

2. Account for: risk factors, frequency of surveillance, number of samples

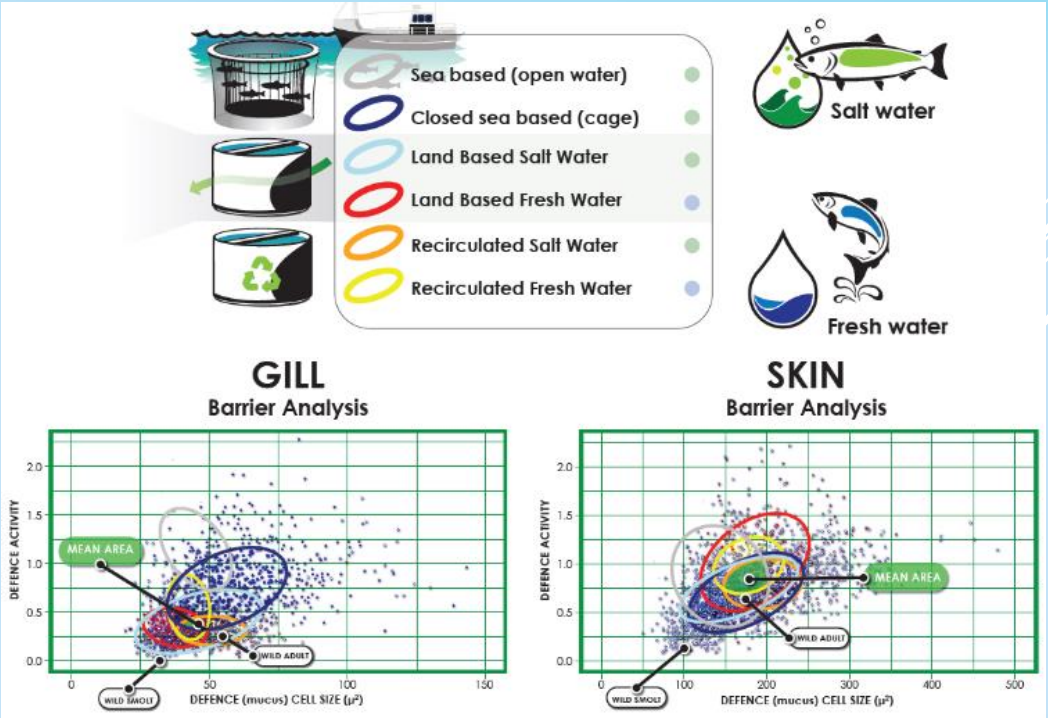
3. Acceptable limiting values: vary by life stage, environment, production activities

4. Industrially scalable and comparable

5. Important factors: sampling strategy and detection tools

Standardization increases understanding and control of barrier health

Veribarr™ salmon database from 2018 (Skin N=~2000 Gills N=~1000)



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The FISH DETECTIVE using Veribarr™

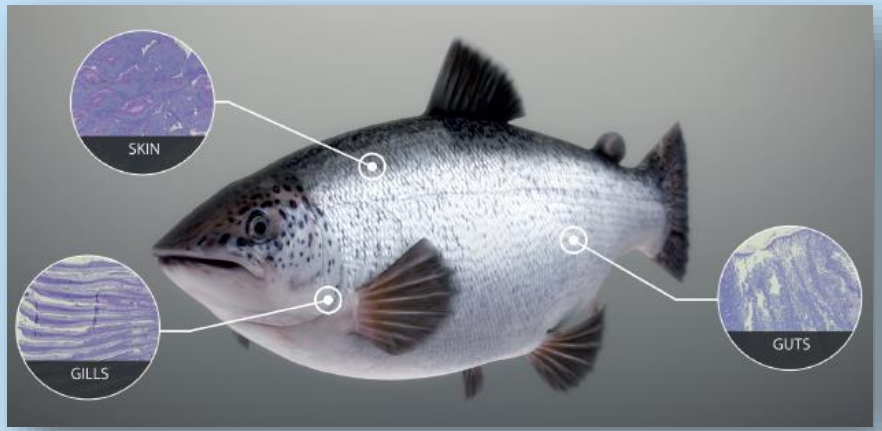
An industrial standard

Database on 13 arter:
Salmon, trout, yellowtail, seabass, seabream, tilapia, lumpfish, wrasse, tuna, barramundi

Works on:
All fish species
All systems

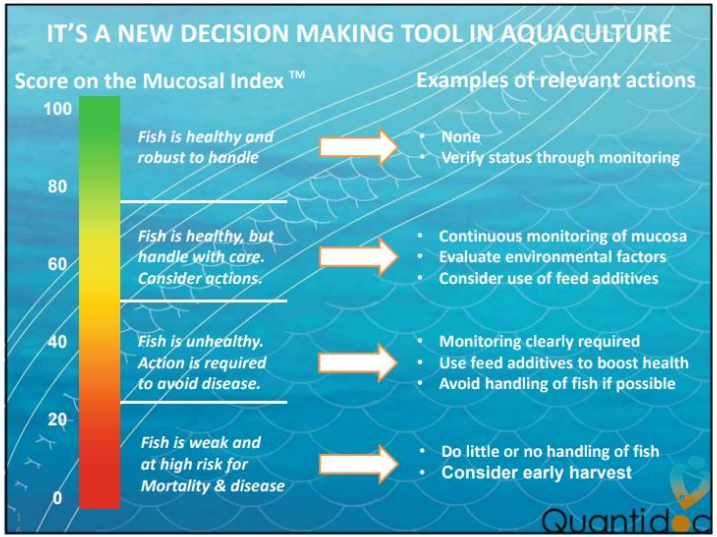
Database on 7 systems
RAS and Flowthrough; open and closed cages; SW and FW, Wild fish in nature

SKIN = SHIELD
Reflects surface contacts: tank and cage walls, crowding, handling, stocking density



GILLS = SENTINEL GUARD
Reflects stress, water quality, particles, pathogens
50% of fish surface area – earliest warning!

GUT = FOUNDATION
Responds to stress, feeds and water



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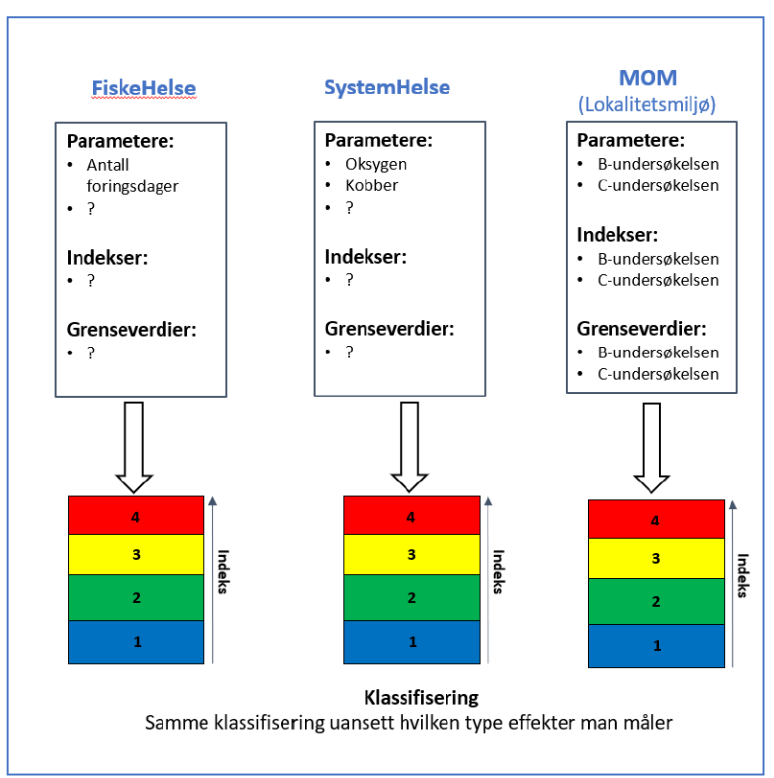
Announcing FishMOM- a framework for decision-making in aquaculture



Sketch of the FishMOM concept
for concept development, anchoring and consortium creation.

The MOM column is **M**iljø**o**vervåking av **m**atfisk (**NS9410**),
a risk-based system for site environment which has been **in use on ALL Norwegian fish farms since 2005.**

By applying the principles and concepts from MOM to the 2 other columns, the fragmented knowledge of fish- and system health (farm system &technology) we develop a **tool** with **flexible monitoring frequency and variable mitigations** depending on exploitation level.

The FishMOM consortium will identify parameters, indexes and suggest limiting values for the columns
Fish Health and System Health

Join us! Focus groups spring 2024!

Proposers: Aquacloud, HVL, NCE Seafood, IMR, UiB, UiS, VetInst

An external membrane 0.07 mm thick protects fish health: the mucous membranes of skin, guts and gills