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Aquaculture Innovation Workshop,
26-27.09.2011, Campbell River, BC

Research on Atlantic salmon welfare and performance in RAS at Nofima Centre for Recirculation in Aquaculture

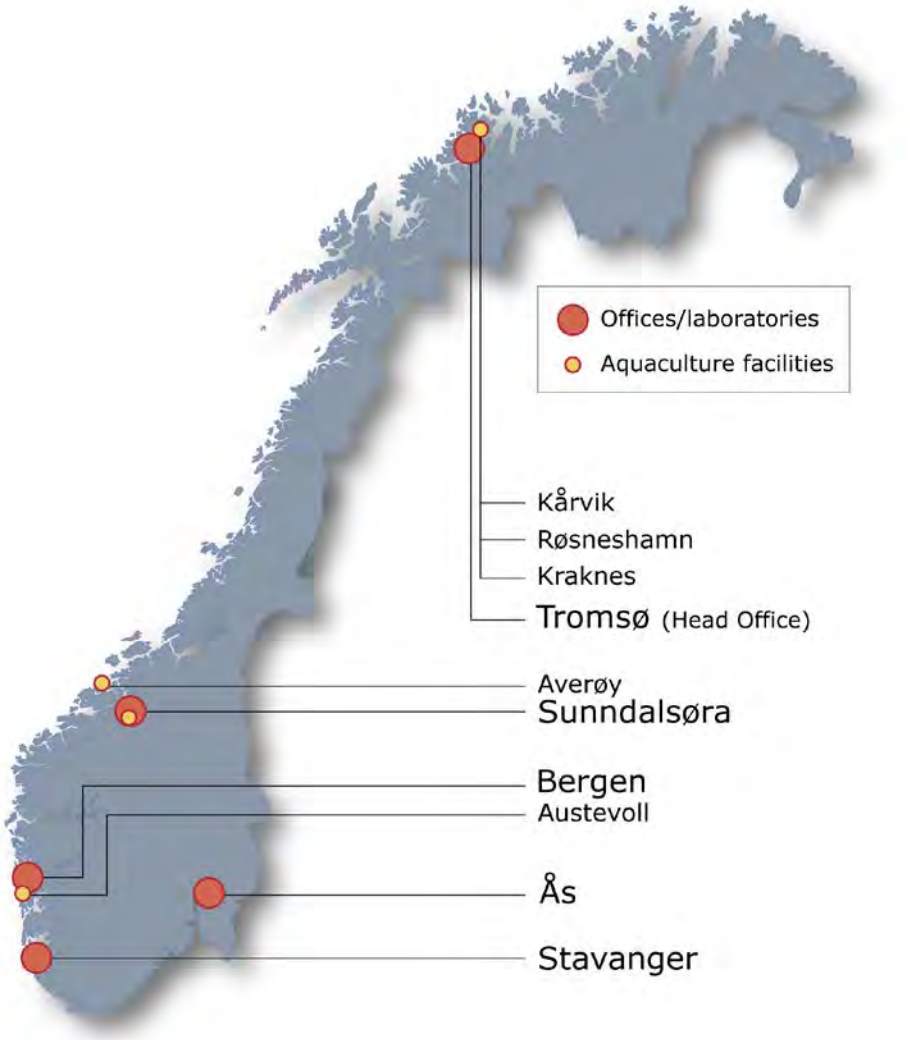
Jelena Kolarevic & Bendik F. Terjesen

Overview

- Nofima and Nofima Centre for Recirculation in Aquaculture (NCRA)
- Water quality requirements for Atlantic salmon and its relevance for recirculating systems
- Effects of ammonia and nitrite exposure on Atlantic salmon parr growth and welfare
- Why and how water quality requirements affect dimensioning, investments and running costs of aquaculture recirculating systems
- Effects of sudden changes in pH and alkalinity on biofilter microbial community
- Assessment of fish welfare

Facts about Nofima

- Established January 1st 2008
- A merge between Akvaforsk, Fiskeriforskning, Matforsk and Norconserv
- Target sectors: Aquaculture, Food & Seafood Industry
- Employees: 450
- Turnover 2009: 470 mill NOK (US $ 80 mill. +)
- Head office in Tromsø



Infrastrcture

Our toolbox:

- Fish trials – sea and land. Feed- and environmental control, specialised locations (Averøy, Sunndalsøra, Tromsø):
  - Feed & Nutrition; Digestive trials, New ingredients
  - Product quality
  - Breeding trials
  - Preventive health measures
  - Vaccine testing
  - Controlled infection trials with lice (approved by the Norwegian Food Safety Authority)
  - Trace elements, pigmentation etc
  - Technical testing
- Well-equipped fish health laboratory in Tromsø approved for challenge experiments and GMO experiments
- Feed technology centre located in Bergen

Nofima Centre for Recirculation in Aquaculture (NCRA)

- Opened by the minister of Fisheries and Coastal Affairs, Lisbeth Berg-Hansen 23/11/10

- Vision: Contribute to increased knowledge about nutrition, physiology, health and welfare in cultured fish species in RAS

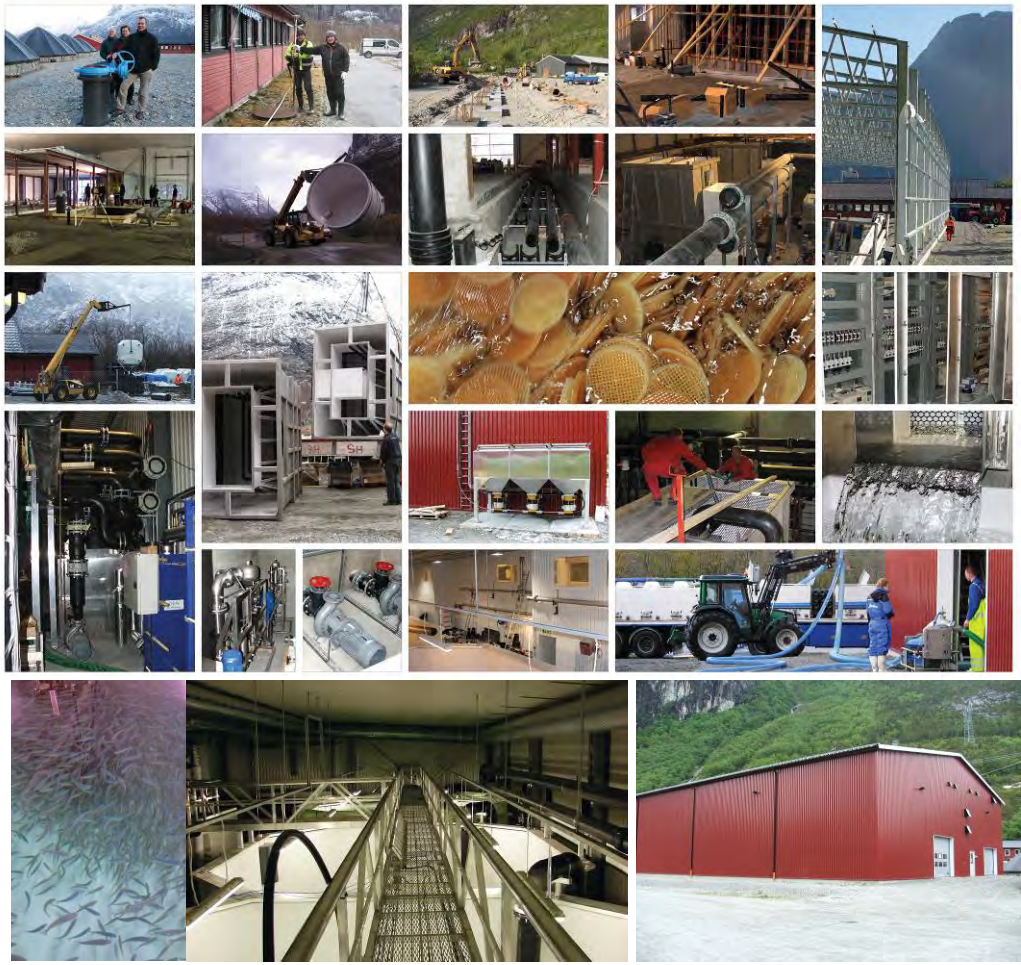


Foto: Nofima & Jelena Kolarevic

Legislation on water quality (Norwegian Food Safety Authority)

How does this relate to RAS?

Last updated on 16/11/2010

Parameter	Value
pH (tank inlet)	6.2 - 7.8
Oxygen partial pressure	not over 100%
Oxygen (tank outlet)	> 80%
Total gass pressure in the tank water	not over 100%
CO2	<15 and 10 mg l^{-1}, salmon and trout
Aluminium (free)	< 5 µg l^{-1}
Aluminium (gills)	< 15 µg g^{-1} gill tissue (dry mass)
Nitrite (fresh water)	< 0.1 mg l^{-1}
Nitrite (sea water)	< 0.5 mg l^{-1}
Total ammonia nitrogen (TAN)	< 2 mg l^{-1}
Ammonia	< 2 µg l^{-1}

At pH 7.8, optimum nitrification., can have a maximum 0.2 mg / L TAN to keep the 2 µg / L NH3-N

<85% negative effect on FI

Why higher for salmon than trout?

No information about Cl- requirements

At pH=6.8 and 12° C

NH_3-N?

http://www.kystlab.no/nedlasting/Settefisk/Mattilsynet%20-%20Aktuelt.pdf

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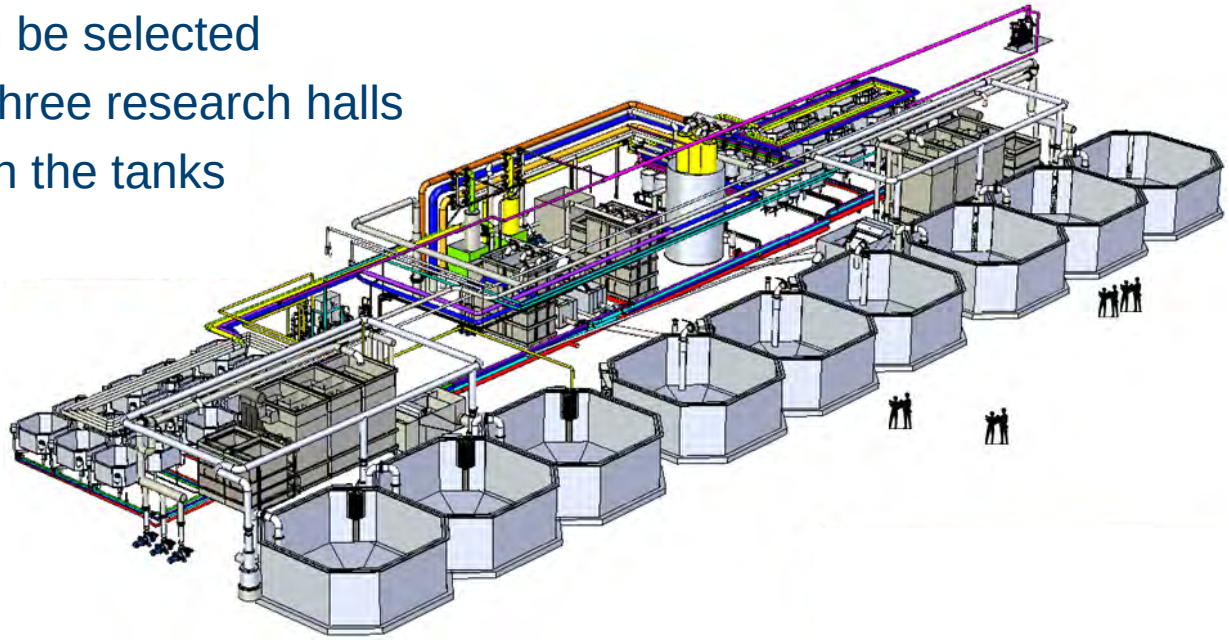
Nofima Centre for Recirculation in Aquaculture (NCRA)

- 1750 m^2, 500 m^2 2nd floor
- Four separate RAS
- Six separate research halls with 57 tanks in total
- Tank size from 0.8 m to 7m
- 1100 m^3 of rearing capacity

- NCRA offers flexibility in experimental design and scale:
  - Water from RAS1 og 2 can be selected randomly for 48 tanks and three research halls
  - Triple outlet configuration in the tanks
  - Three-chambered MBBR



”Fish welfare and performance in recirculating aquaculture systems”

Research Council of Norway Strategic Institute Program
”RASALMO” 2008-2012

Forskningsrådet

Main goal:

”To improve the knowledge of how welfare and performance of Atlantic salmon relate to environmental conditions in recirculating aquaculture systems”

Nofima scientists:
Grete Bæverfjord
Harald Takle
Jelena Kolarevic
Bendik Fyhn Terjesen (PL)

External collaborators:
Steve Summerfelt & Chris Good
(Freshwater Institute, WV, USA)
Yngve Ulgenes (SINTEF Byggforsk)
Liv Torunn Mydland (UMB/APC)

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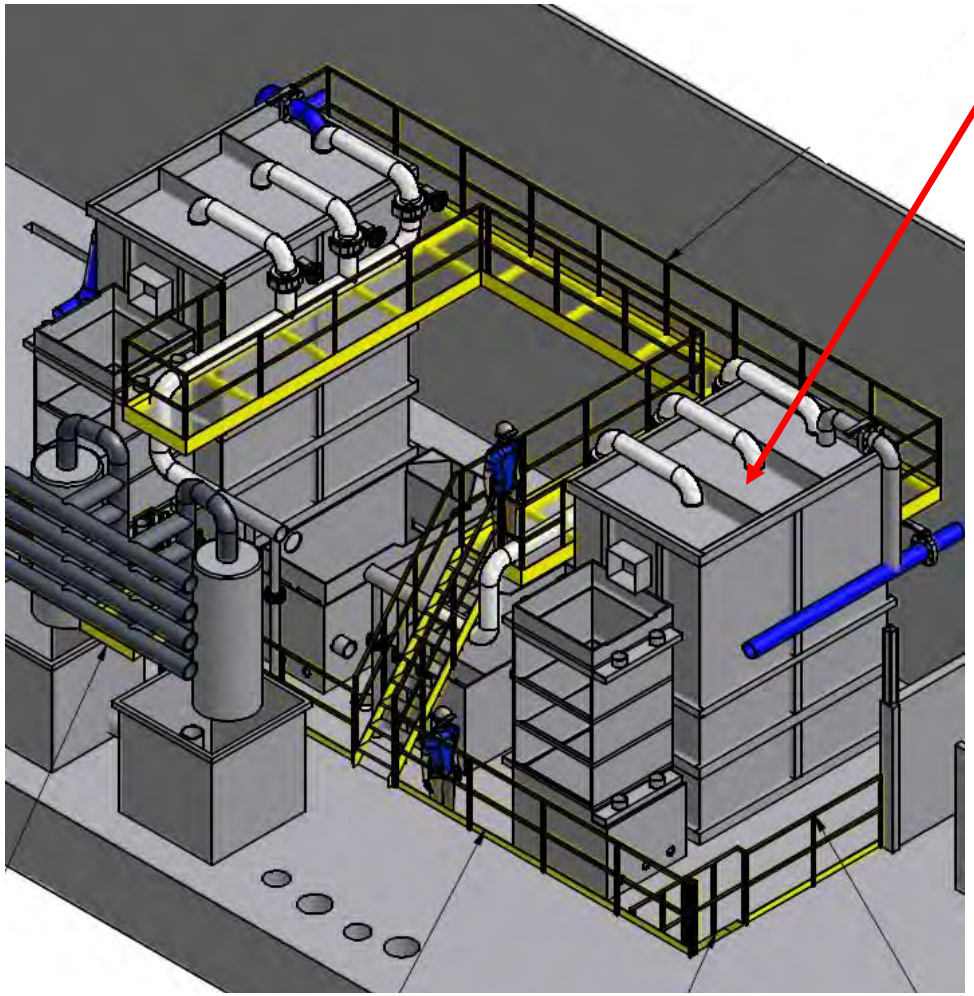
Why are ammonia and nitrite thresholds critical for recirculating systems?



Drawing of central treatment in Nofima Centre for Recirculation in Aquaculture (Storvik, Nofima)

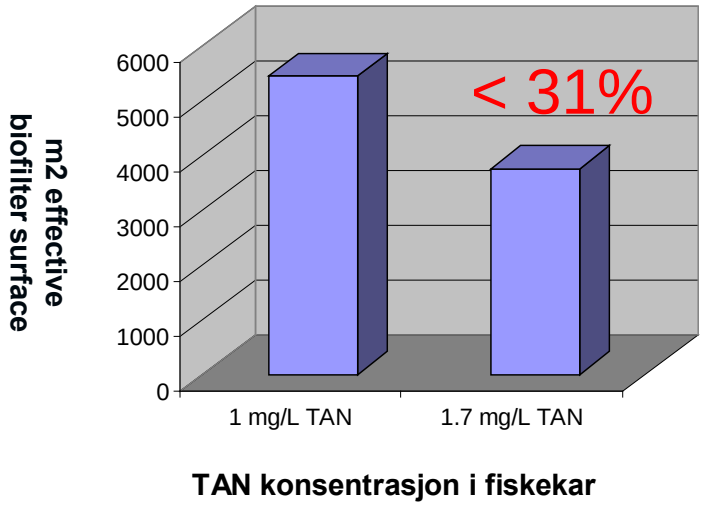
Biofilter:

$$NH_4^+ \Rightarrow NO_2 \Rightarrow NO_3$$

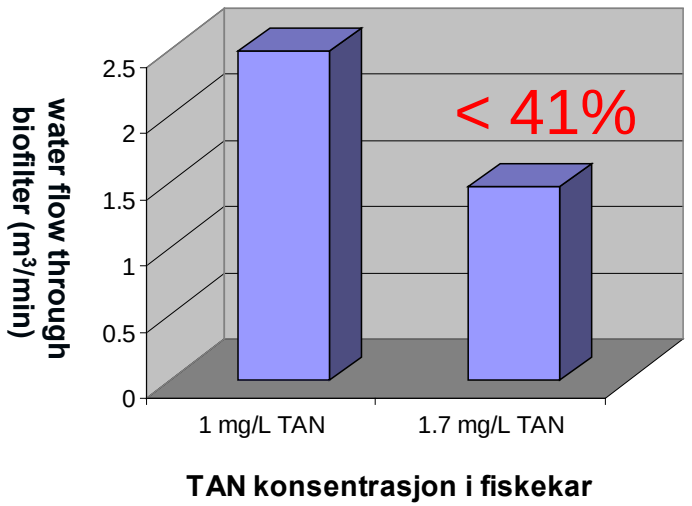
- At low substrate concs. biofilter efficiency is low
- Low ammonia & nitrite thresholds for the fish thus dictate large biofilter size and/or flow requirements
- Fish health and welfare, NH_3/NO_2 incompletely studied in salmon in FW
- Biofilter size and flow dictate investments, running costs, and ecological footprint
- Thus, industry should know what the fish require to accurately invest in and operate reuse systems

The relationships between fish tank ammonia concentration and biofilter size or waterflow

Biofilter area for TAN removal at 60% efficiency



Water flow requirement for TAN removal at 60% efficiency and same load



Research into correct water quality criteria for fish in RAS may result in lowered running costs due to less water pumping

(TAN = Total ammonium nitrogen = NH_3-N + NH_4-N)

Terjesen et al. (unpublished 2011)

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Why is ammonia toxic (acute effects)?

- NH_4^+ stimulates the breakdown of glucose and disrupts energy metabolism
- NH_3 increases the consumption of amino acids to the point of amino acid deficiency
- NH_4^+ disrupts nerve impulses through changes in neuron membrane potential
- Leads to increased oxidative levels and decreased function of antioxidant systems
- NH_3/ NH_4^+ leads to an imbalance in the acid-base and salt regulation

The acclimatory responses of fish to increased environmental ammonia

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Factors that influence NH_3-N toxicity

- Species and developmental stage
- Low temperature increase toxicity of NH_3-N in salmon
- Feed intake increases plasma NH_4^+, but at the same time up-regulates defence systems
- High water/swimming speed increases toxicity of NH_3-N in trout
- Low O_2 concentration increases toxicity of NH_3-N in salmon smolt
- Increased CO_2 decreases TAN toxicity (as long as pH drops)

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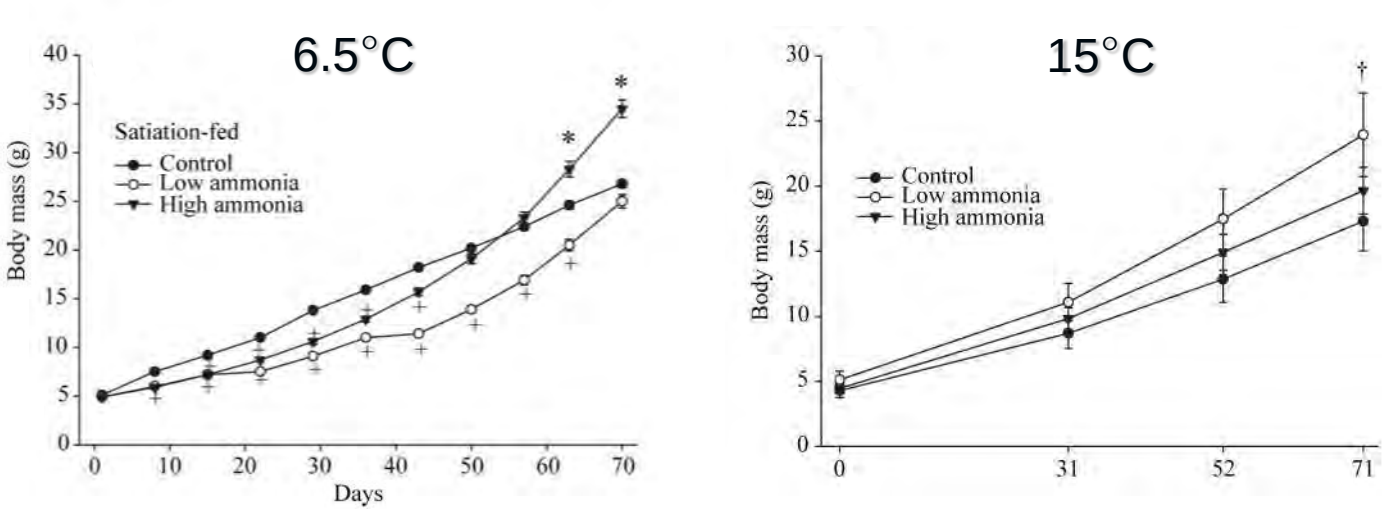
Is there an optimal window of ammonia concentration for fish?

Wood, C.M., 2004. Dogmas and controversies in the handling of nitrogenous wastes: Is exogenous ammonia a growth stimulant In fish? J Exp Biol 207, 2043-2054.

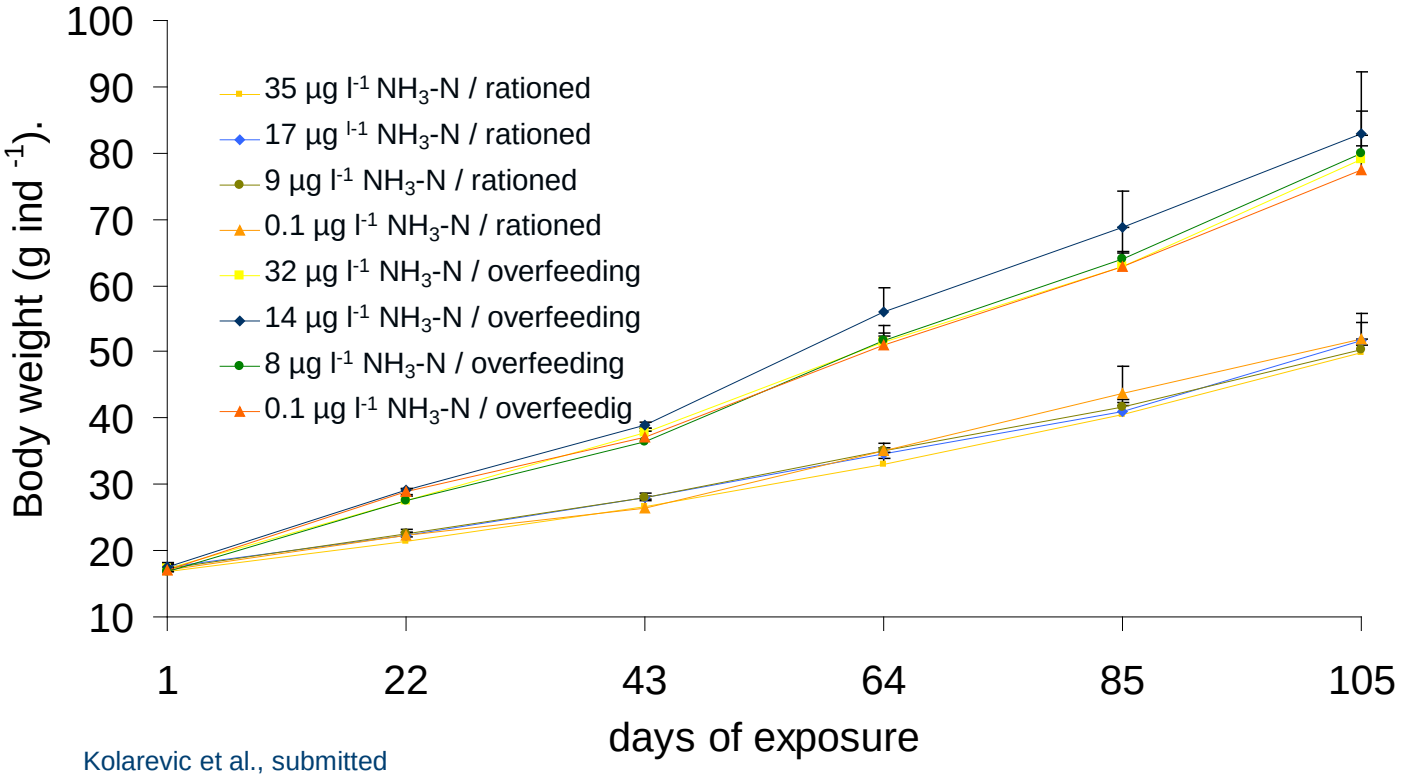
Rainbow trout



P_{NH_3}~ 23µtorr

Feed intake and growth of Atlantic salmon parr during chronic ammonia exposure (105 days)

- 4 NH_3-N levels X 2 feeding rations; 12^0C; pH = 6.8; oxygen >85%; FT
- Atlantic salmon parr grew well up to 35 µg/L NH_3-N
- Best growth at NH_3-N 14 µg/L, full feed ration
- No difference in feed intake and FCR



Kolarevic et al., submitted

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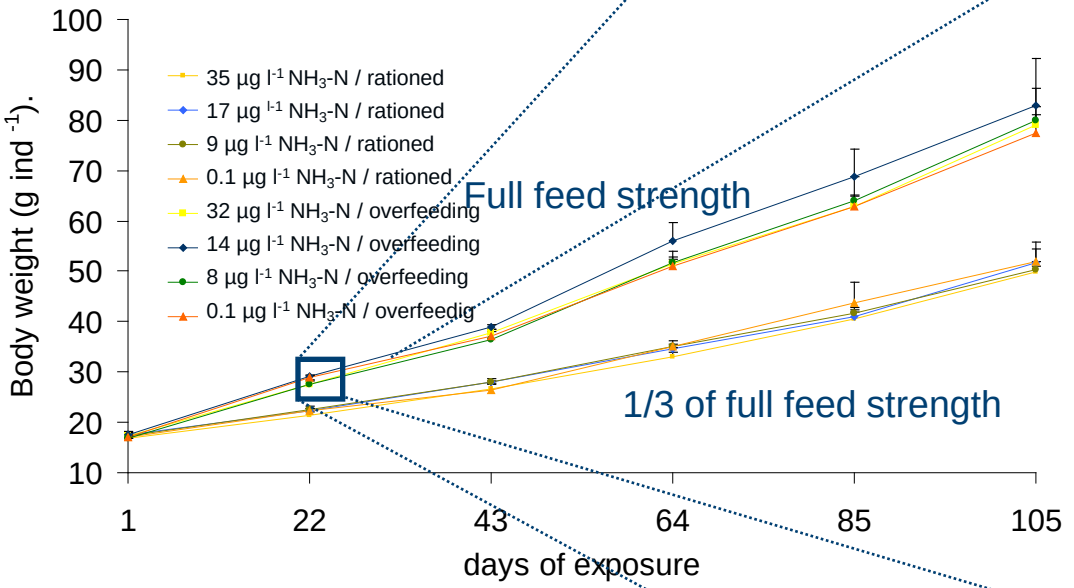
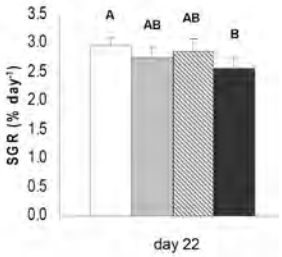
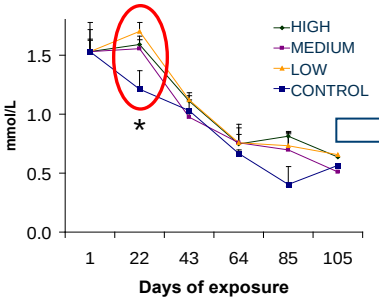
Early response of Atlantic salmon parr to ammonia exposure (day 22)



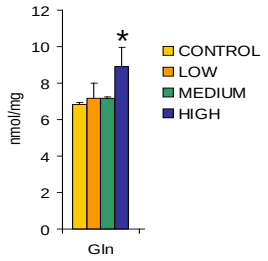


Reduced growth rate

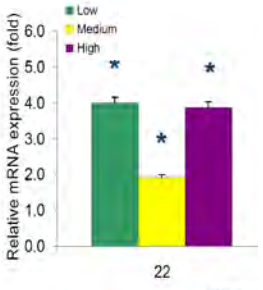
Small but significant gill changes



Ammonia accumulation in plasma



Detoxification to amino acids in brain tissue



Increase in gene expression of ammonia transporter in the gills

(Kolarevic et al, submitted 2011)

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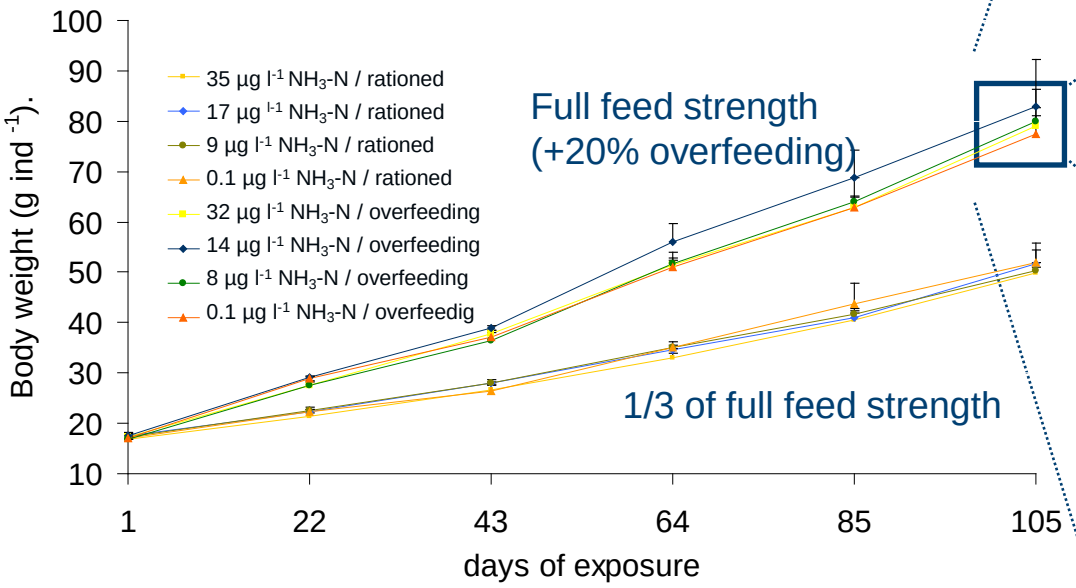
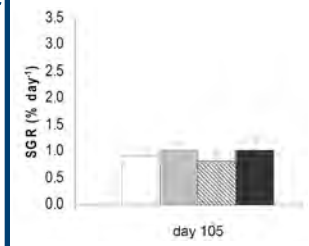
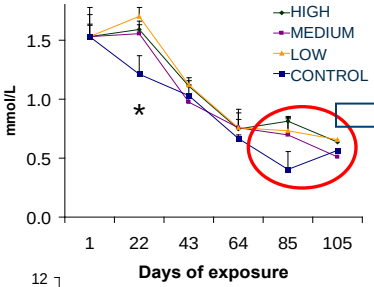
Atlantic salmon parr status at the end of the long-term ammonia exposure



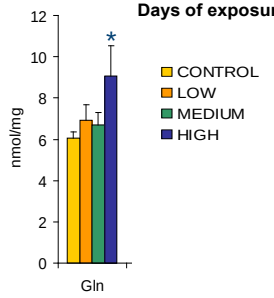


No detectable effect on growth

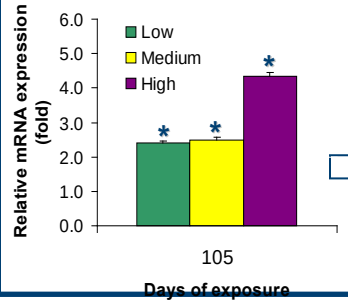
No detectable gill damage



No significant accumulation in plasma



Detoxification to amino acids in brain tissue



Increase in gene expression of ammonia transporter in the gills

(Kolarevic et al, submitted 2011)

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What do these results mean for the design and operation of RAS?

- Repeat the experiment in RAS at higher nitrite/CO_2/TSS levels than in previous exposure study
- Aquaculture operation regulations: 2 mg/L TAN at pH 6.8-7.2 = 3-7 µg/L NH_3-N (v/12ºC)
- After a period of adaptation, Atlantic salmon parr showed tolerance up to 35 µg/L NH_3-N
- No significant effect of elevated ammonia on salmon parr growth and welfare
- No effects of exposure on smoltification and development of sea water tolerance (PIT tag)
- If verified in RAS these results can affect the sizing and flow in biofilters

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Nitrite, a potentially problematic compound in RAS

- Can accumulate in RAS after rapid changes in for example pH,alkalinity,feeding
- Caused by slower cell division in nitrite oxidizing versus ammonia oxidizing bacteria
- Cl^- addition (as NaCl) is often used to reduce the effect of nitrite by outcompeting NO_2 in the transport of HCO_3^-
- In general, it is said that a 20:1 ratio between the Cl and NO_2-N is optimal for fish, but is this valid for Atlantic salmon parr?

Brit Tørud (2009). From the presentation «Praktisk fiskehelsetjeneste i resirkuleringsanlegg»

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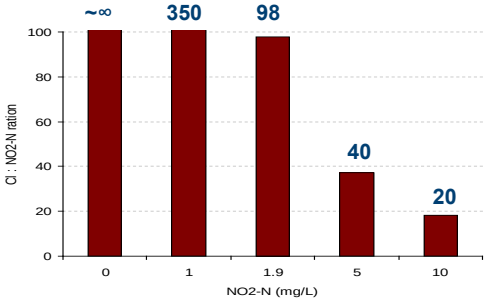
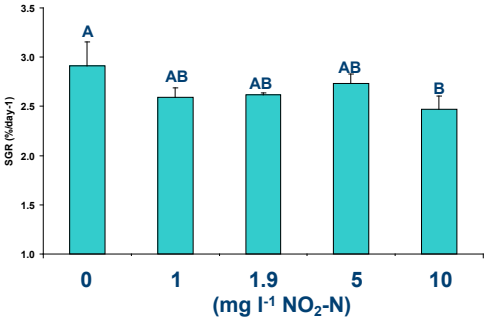
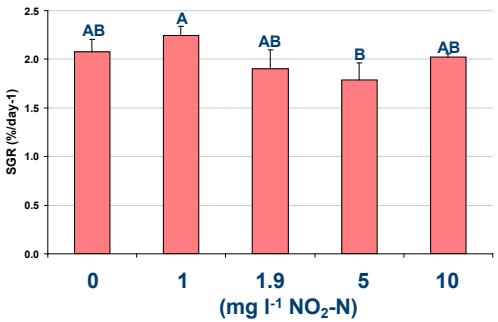
Chronic nitrite exposure of Atlantic salmon at different Cl: NO_2-N ratios



- Up to 10 mg/l NO_2-N with 20:1 to 350:1 and ~ infinite Cl:NO_2-N rations, 85 days of exposure





Significant effect on SGR Day 0-21-49,
(n=3 tanks, mean +SD. ANOVA, p<0.02-0.03)
No mortalities related to nitrite exposure

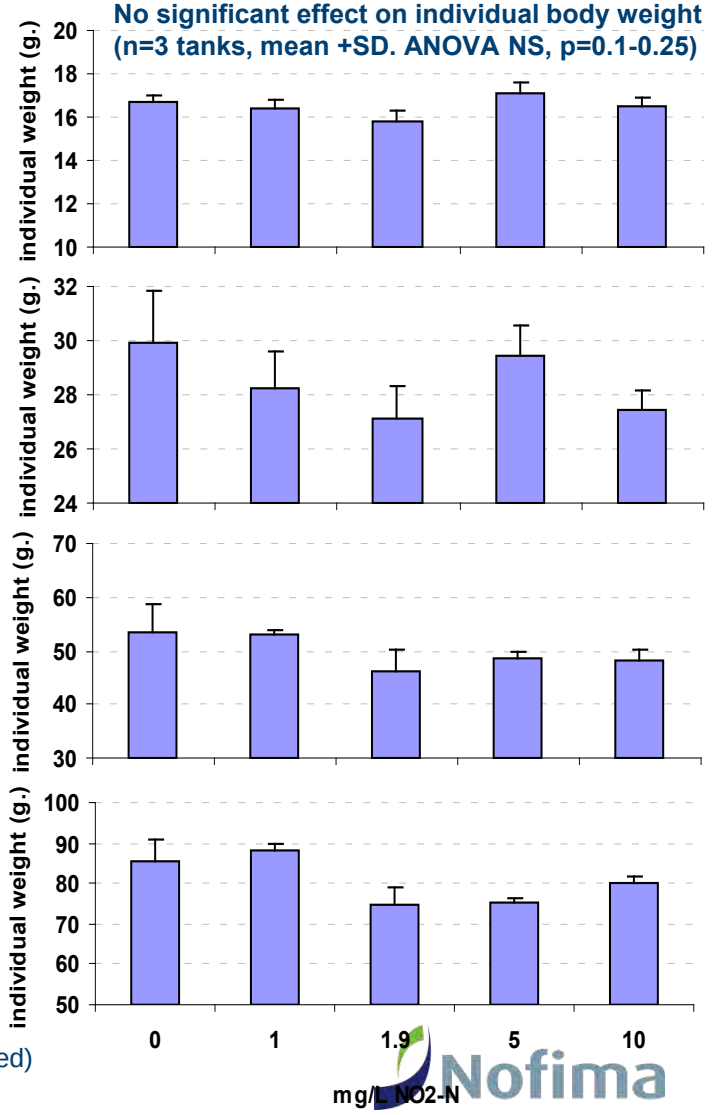
Day 0

Day 21

Day 49

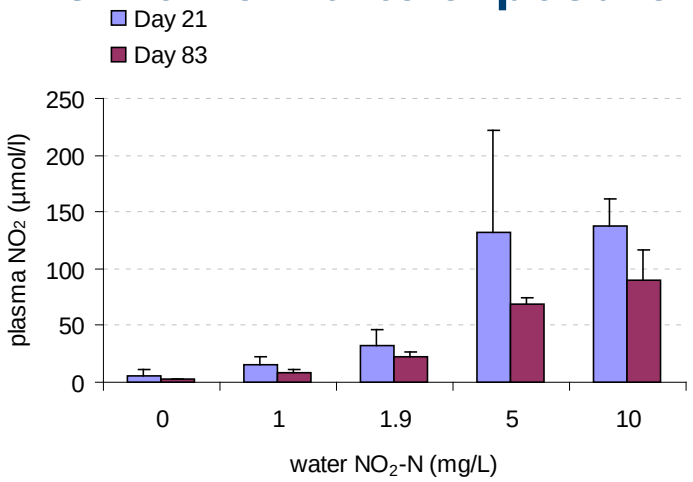
Day 83

No significant effect on individual body weight (n=3 tanks, mean +SD. ANOVA NS, p=0.1-0.25)

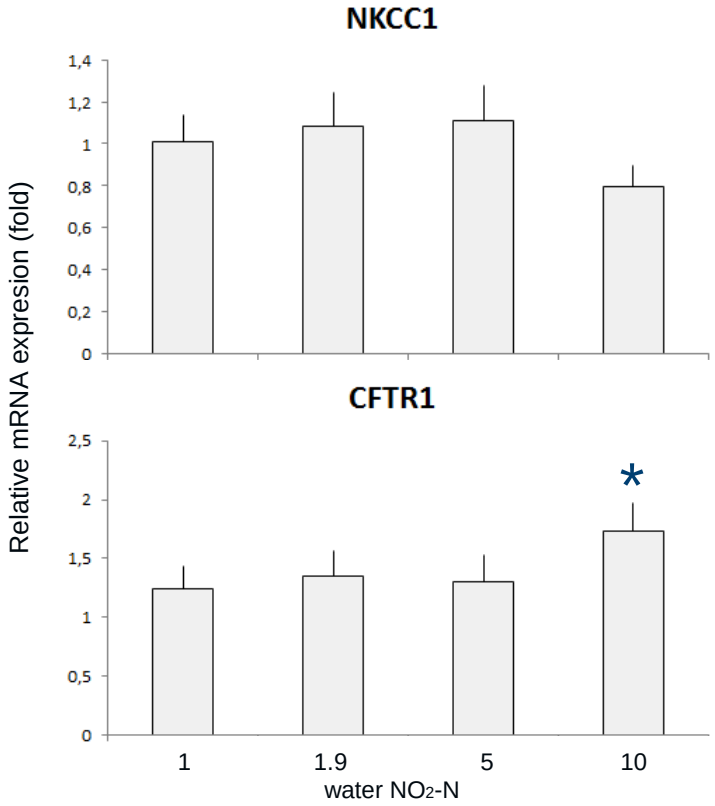


Gutierrez et al. 2010 (unpublished)

Chronic nitrite exposure of Atlantic salmonat different Cl: NO_2-N ratios



- Accumulation of nitrite in plasma during the exposure (days 21 and 83)



- NKCC1down regulation in the gills at 10 mg/l NO_2-N that could be related to uptake of Cl^- or NO_2^- (day 21)

- Upregulation of apical transporter CFTR1 in the gills at 10 mg/l NO_2-N (day 21)

Gutierrez et al. 2010 (unpublished)

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What can these results mean for the monitoring of nitrite in RAS ?

- Aquaculture Operations Regulations: under 0.1 mg / L nitrite in freshwater
- As shown many times before, and here, nitrite concentrations must be considered in relation to chloride concentration
- Exposure to nitrite had negative effect on growth early in the experiment, but the effect was not significant at the end of the experiment
- However, there was a tendency of reduced individual weight at the end of exposure,suggesting that Cl: NO_2-N ratio should be well over 20
- Continuing with molecular, biochemical and histological analyses

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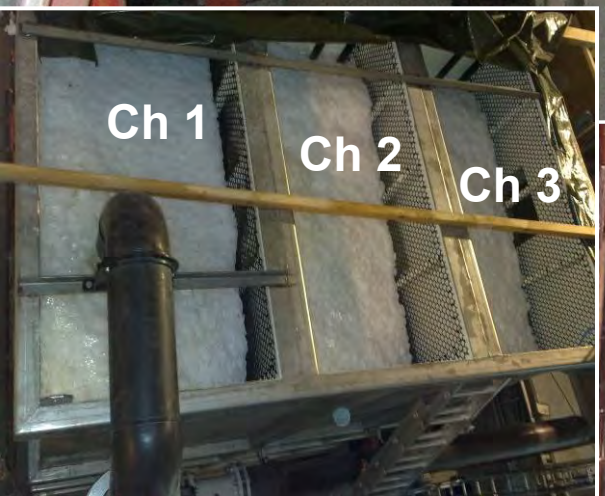
Water quality and bacterial development during start-up, steady-state and disturbances in a new, unused moving bed bioreactor (MBBR)

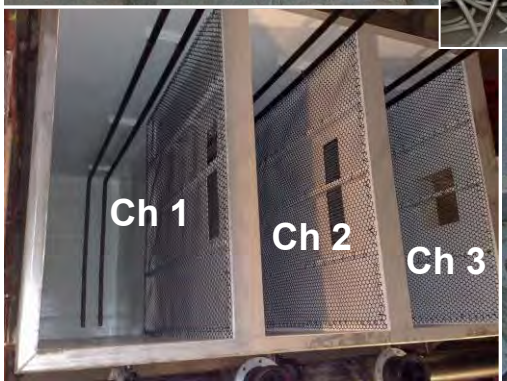
- Substrate was added to RAS1 and the system was running in closed circuit with continuous dosing.
- Fish tanks were not connected to the system.
- From day 87, 20% of make-up water was added daily.

MBBR

CO2-degasser

Iwaki dosage





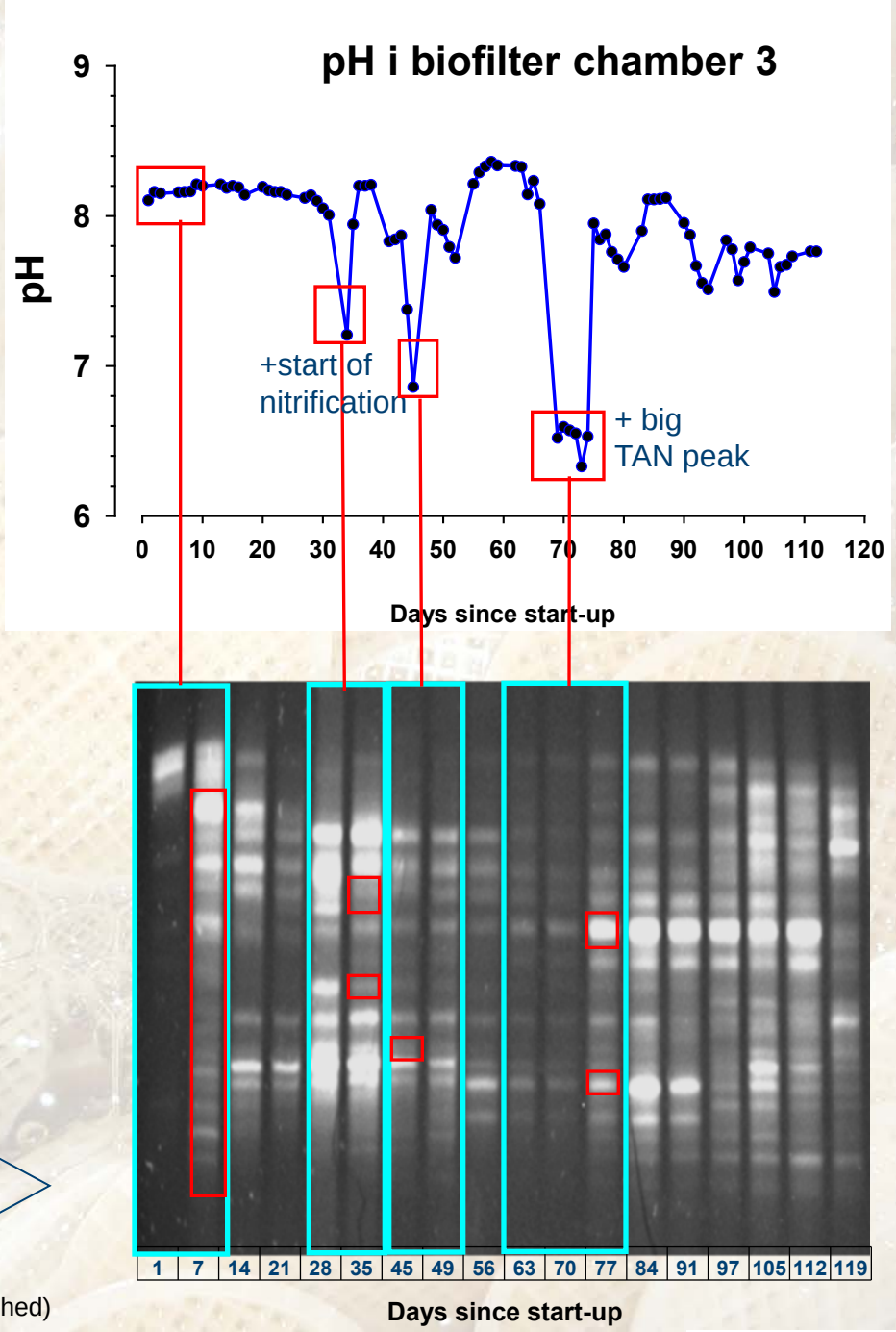
Pumpesump

Microbial community development

Short changes in the environment cause long-term changes in the microbial community

Good pH and dosage control can be beneficial also during start-up phase

DGGE (denaturing gradient gel electrophoresis) of V3 region genes in 16S rRNA isolated from biofilm



Mydland et al. 2010 (unpublished)

Welfare

- Many definitions (feeling-, function- and nature-based definitions)
- Ways of measuring fish welfare:

  - Direct welfare indicators (health, physiology, behaviour)

  - Indirect welfare indicators (water quality, handling and transport)

- Welfare in RAS
- ”Real-time” welfare assessment

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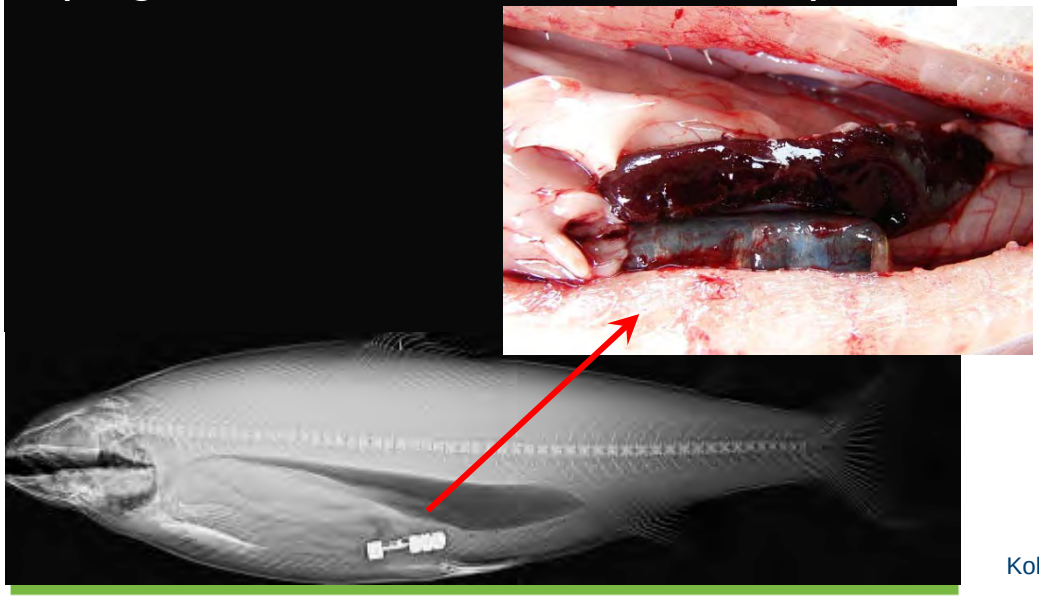
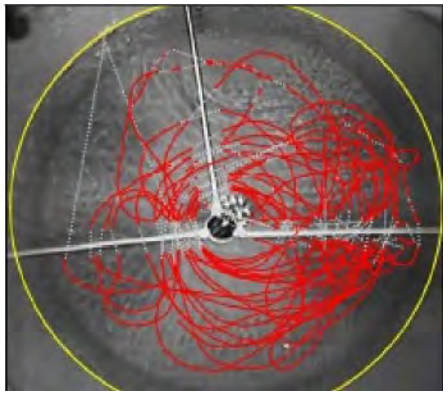
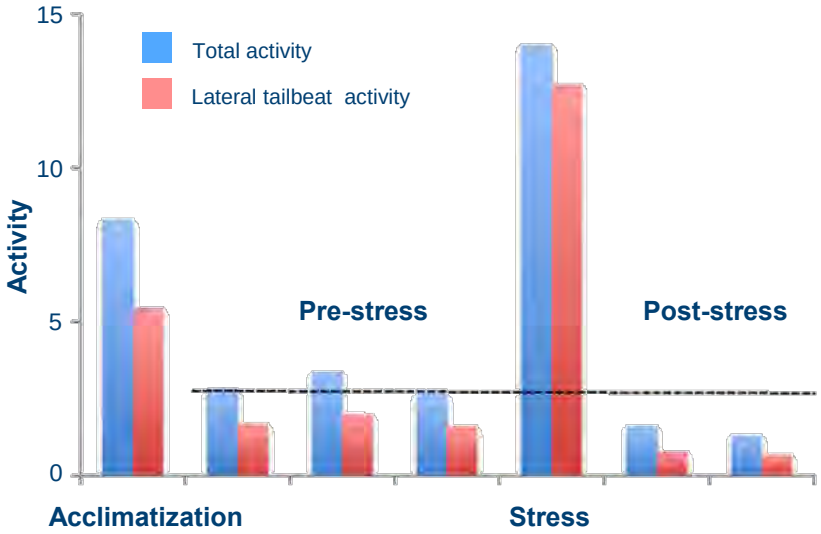
”Real-time” welfare assessment

- Stress can cause increase in fish activity
- Use of 3D acceleration taggs (ThelmaBiotel) for welfare assessment in RAS
- Effects of altered oxygen and water flow conditions at the tank level (1kg Atlantic salmon in FW-RAS)





3D AccelTag (ThelmaBiotel)



Kolarevic et al, 2011. Unpublished

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Thank you for your attention!

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