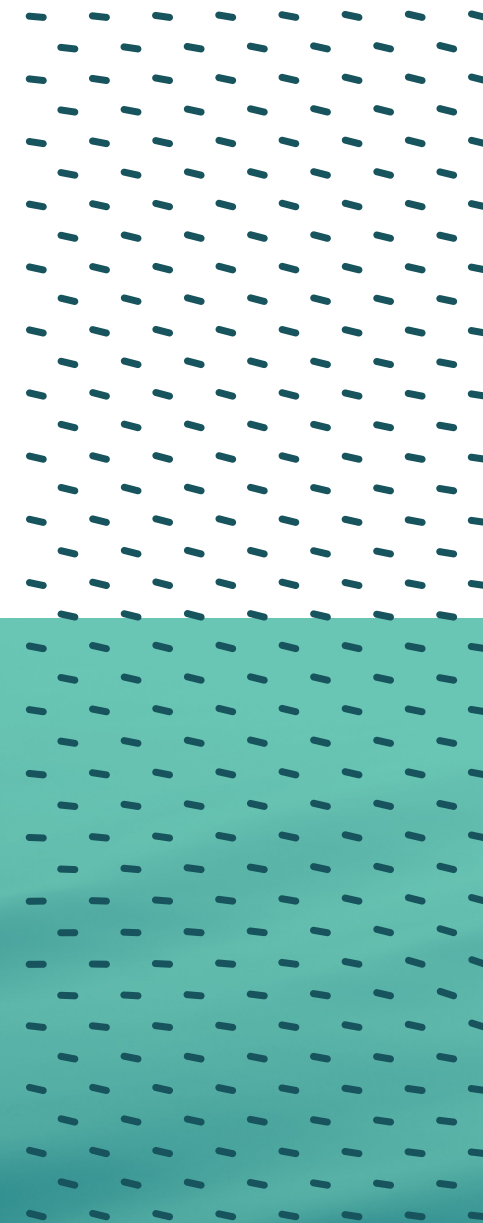




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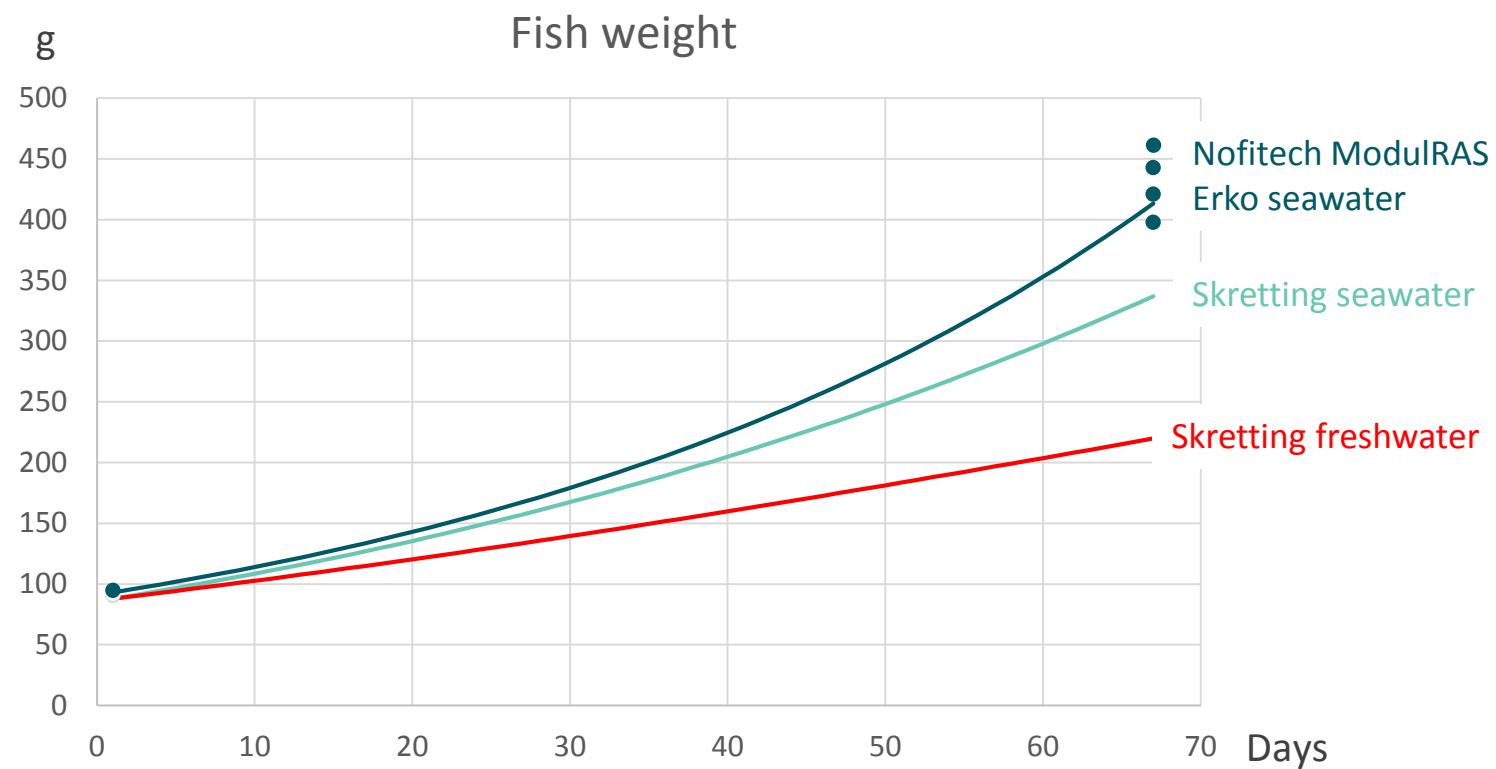
Monitoring microbes and handling H₂S-
challenges in Norwegian postsmolt RAS



Large postsmolt RAS trending in Norway



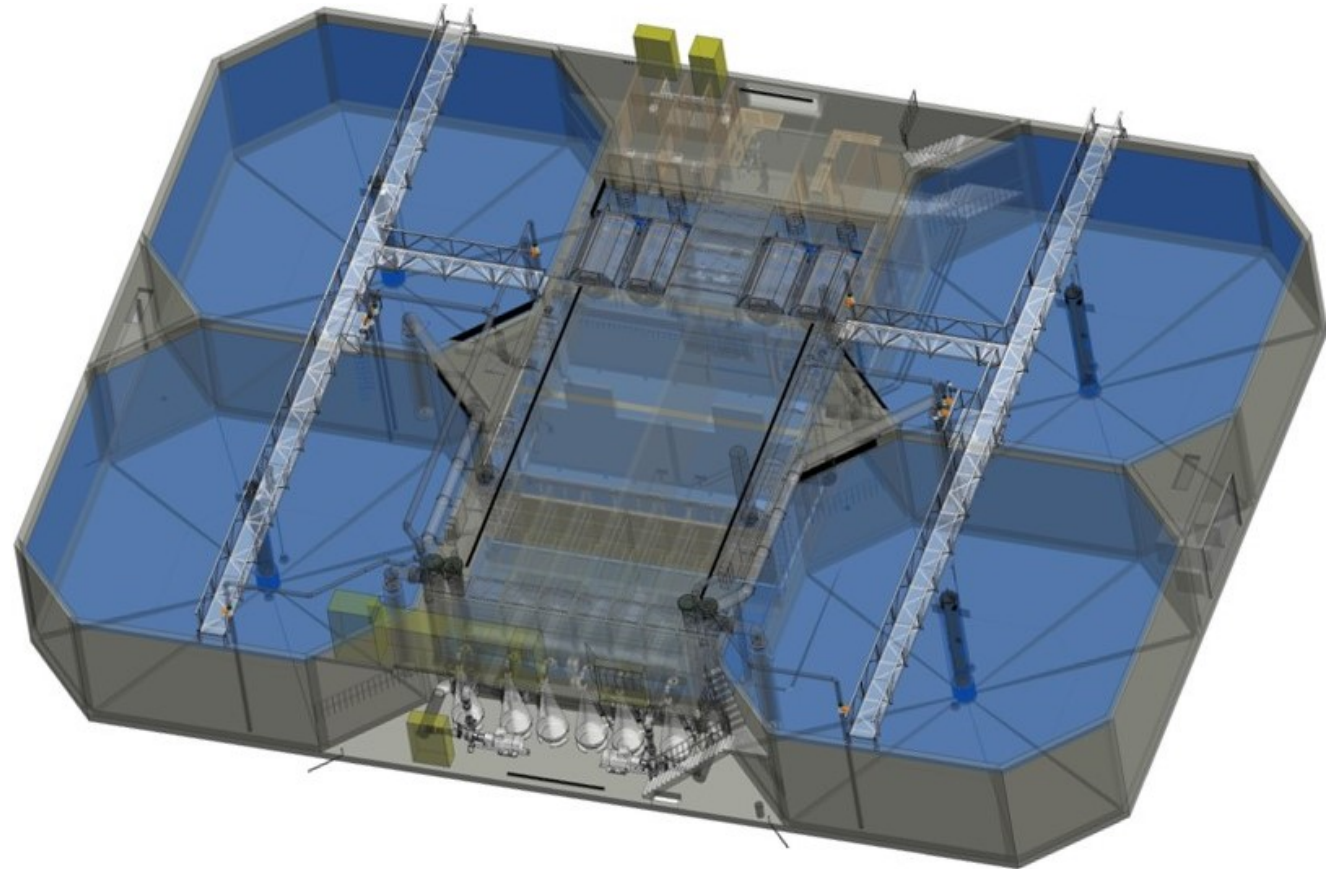
Seawater gives great postsmolt growth



Winter generation 2017-2018 Modul1
14°C
Average SGR: 2.28
Mortality: 0.5 %

Seawater systems have different challenges than freshwater systems

- CO₂ degasser efficiency
- Biofilter efficiency
- Ozonation and rest oxidants
- Biosecurity from seawater source
- Corrosion
- H₂S
- Sea water systems require more!



Living in seawater

< 1 bacteria mL^{-1}

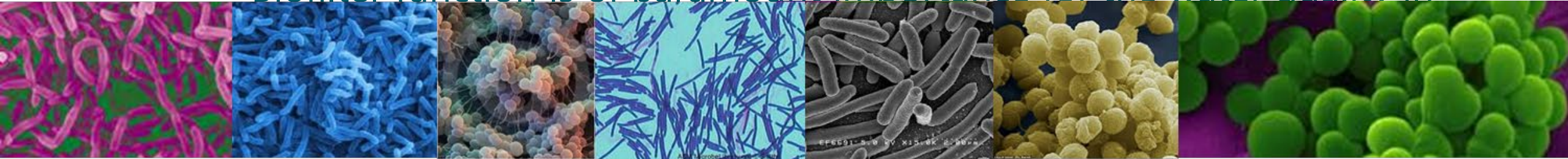


10^6 bacteria mL^{-1}

10^7 virus mL^{-1}

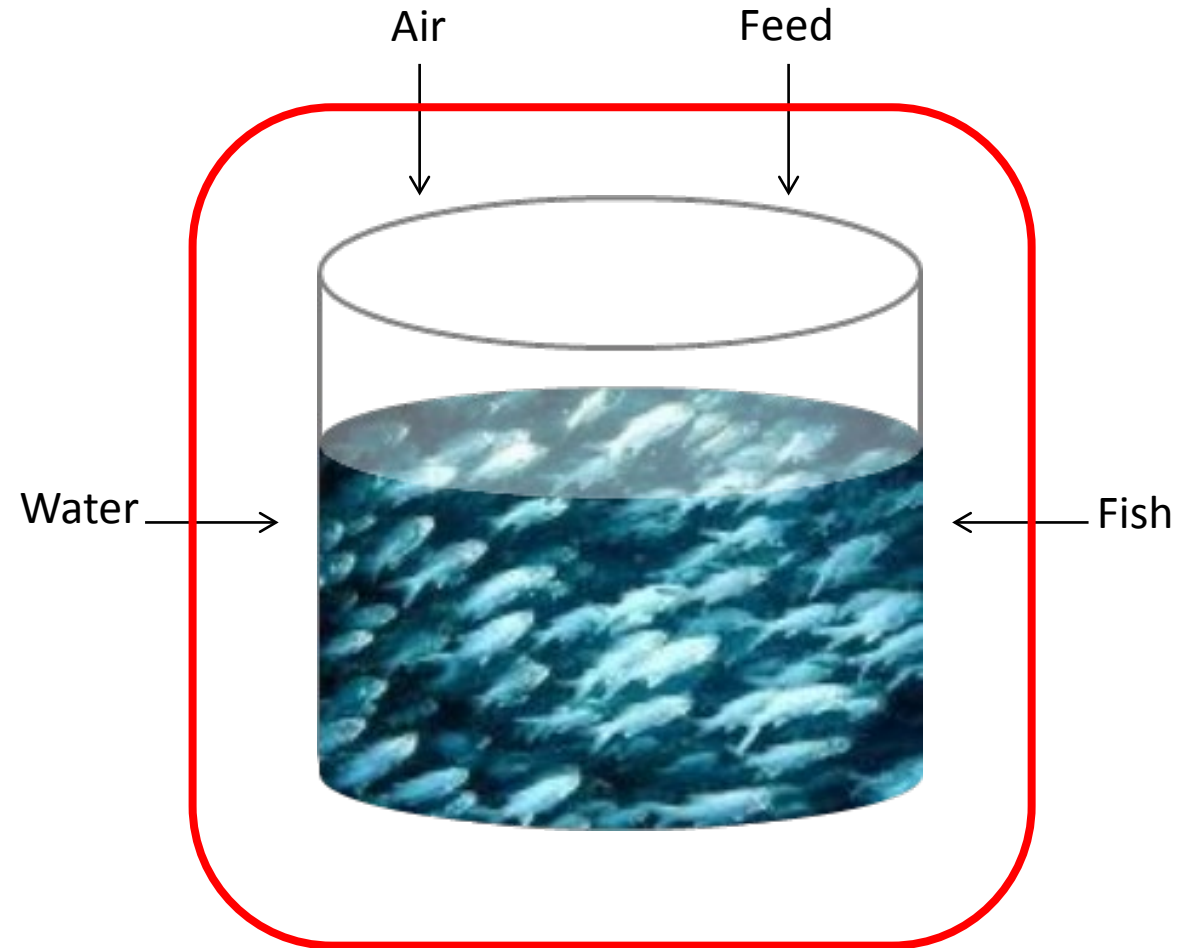
Good bacteria, specific pathogens and opportunists

- Good bacteria give normal development and protection against disease
- Specific pathogens must be kept out
- Unstable conditions select for domination by opportunistic bacteria
- A microbial environment dominated by opportunistic bacteria increases the risk of infection of weakened, wounded and stressed fish
- Biofilter function is of paramount importance for the water quality in

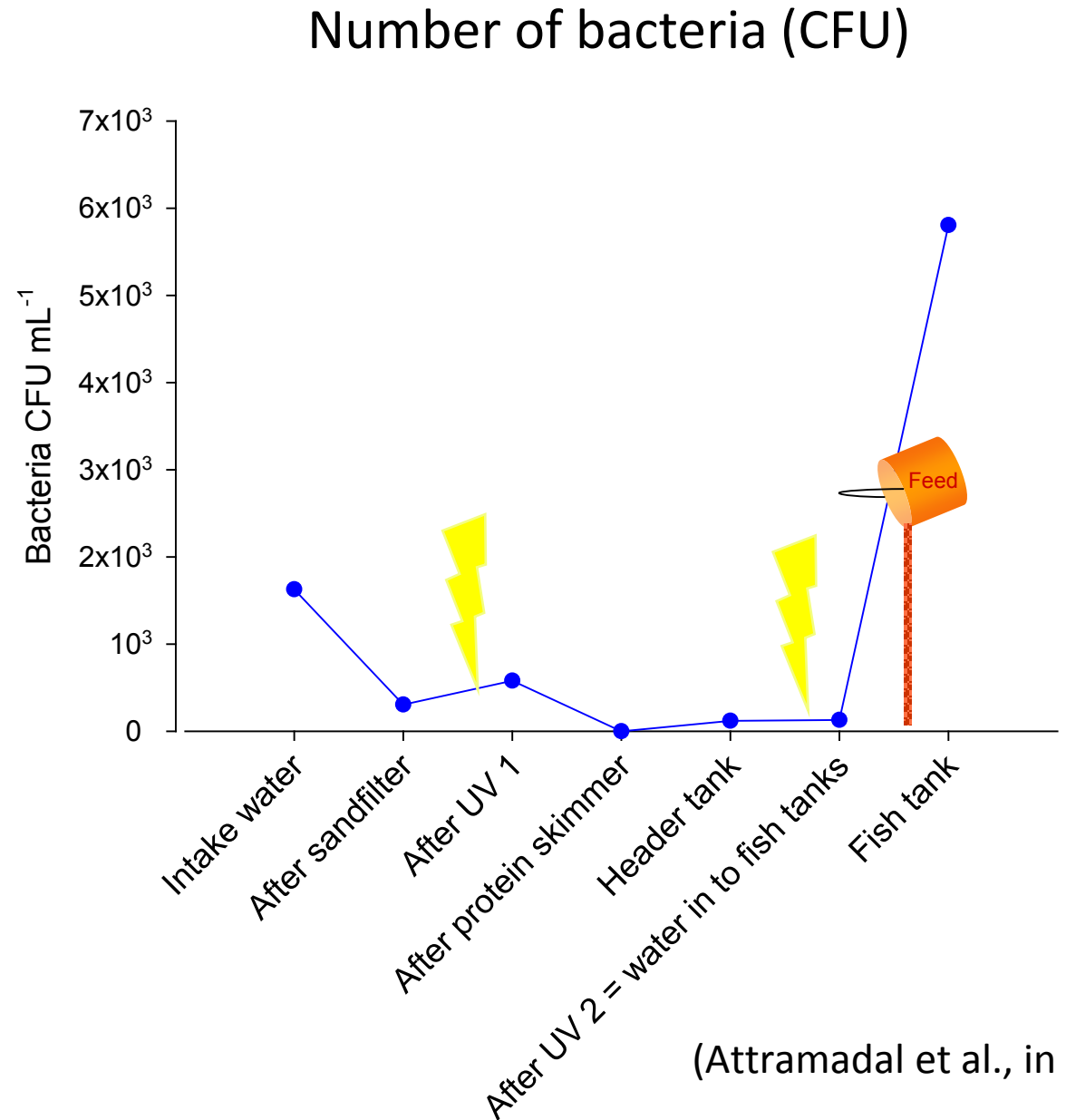


Biosecurity is important

- Stop specific pathogens from entering
- Multiple barriers provide good security
- Typically solids removal (200 μm) and UV (Norway)
- Ozonation (>700 mV) of seawater give toxic rest oxidants that must not reach the fish
- Combining membrane filtering and UV secures double barrier against contamination, as well as solids removal and optimal efficiency of UV

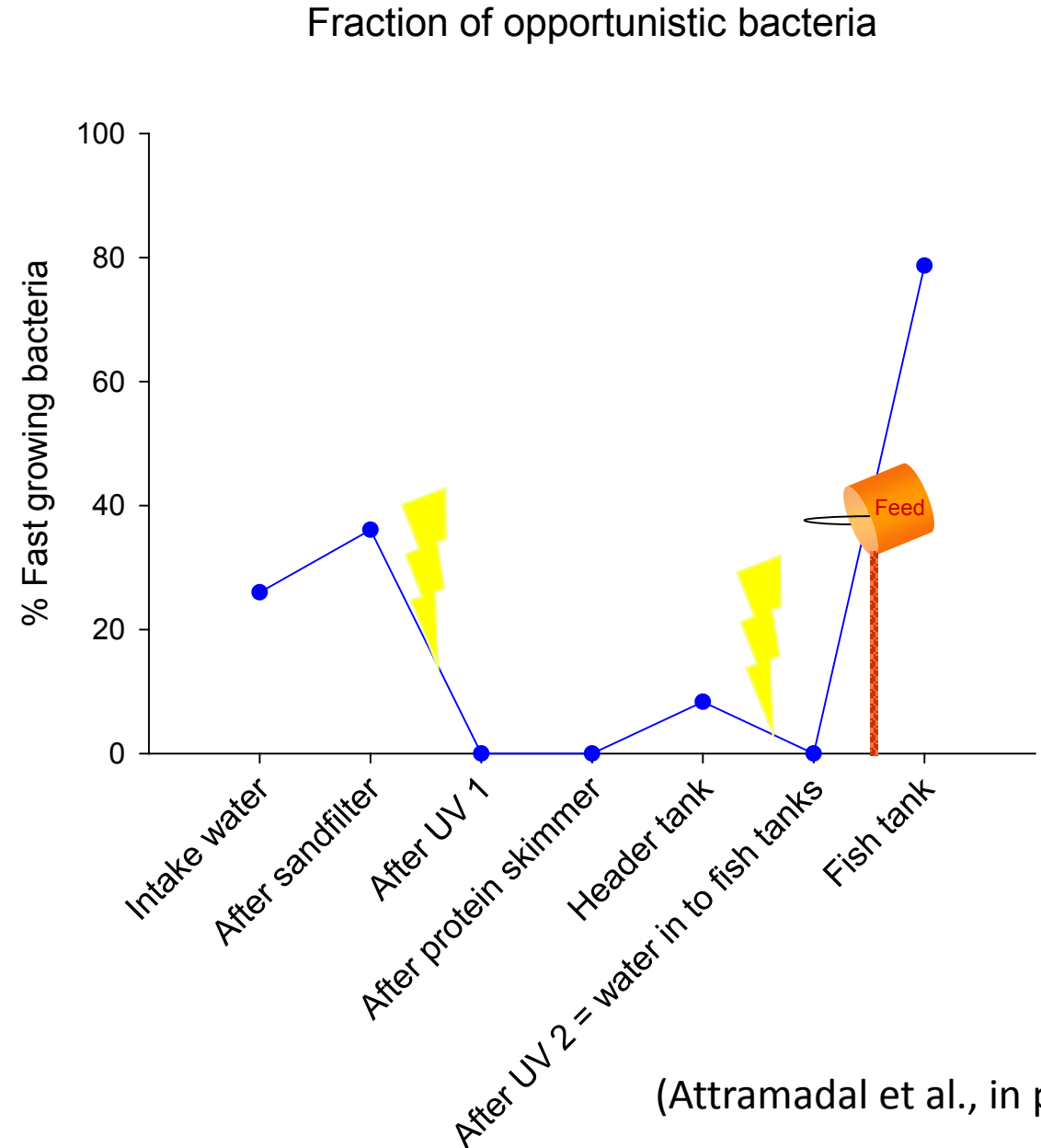


Even with optimal biosecurity, bacteria grow inside the system



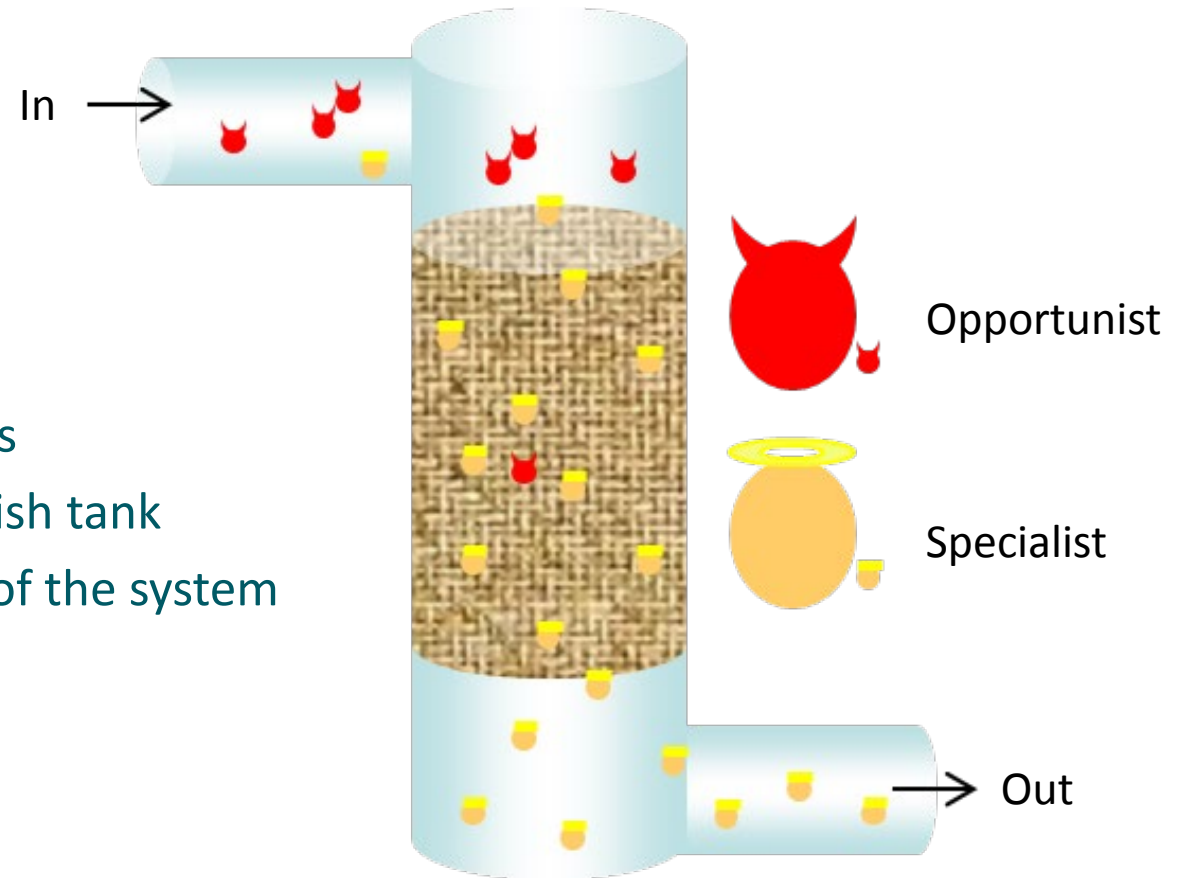
(Attramadal et al., in prep.)

Regrowth favors the opportunistic bacteria that can grow fast when competition for food is low

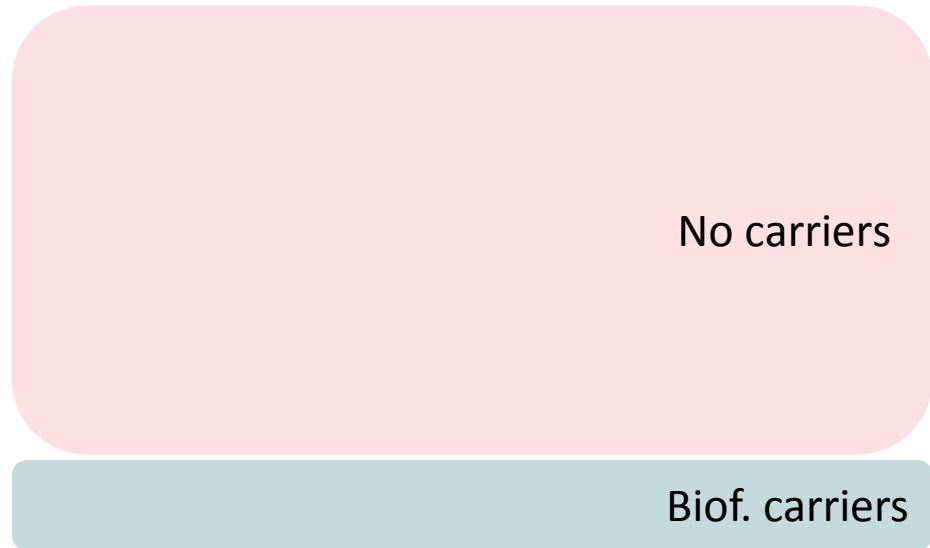


The biofilter influences the regrowth of bacteria

- Large surface area
- Many bacteria competing for the food
- Unfavorable conditions for opportunists
- Reduce the number of bacteria in the fish tank
- Increases the hydraulic retention time of the system



The biofilter reduces the number of bacteria going to tanks



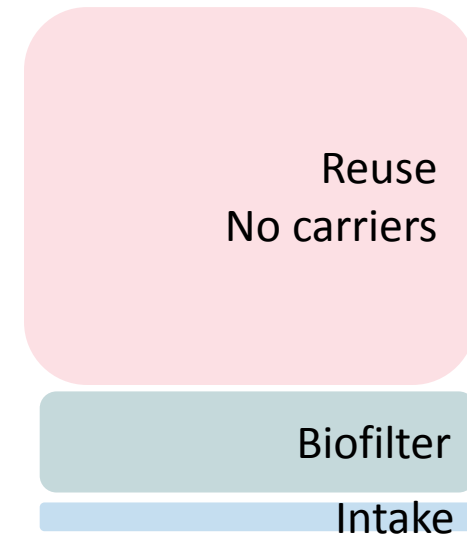
Biofilm carriers 12°C

No carriers 12°C

Biofilm carriers 20°C

No carriers 20°C

The biofilter reduces the number of bacteria in tanks



Reuse with no biofilm carriers

RAS

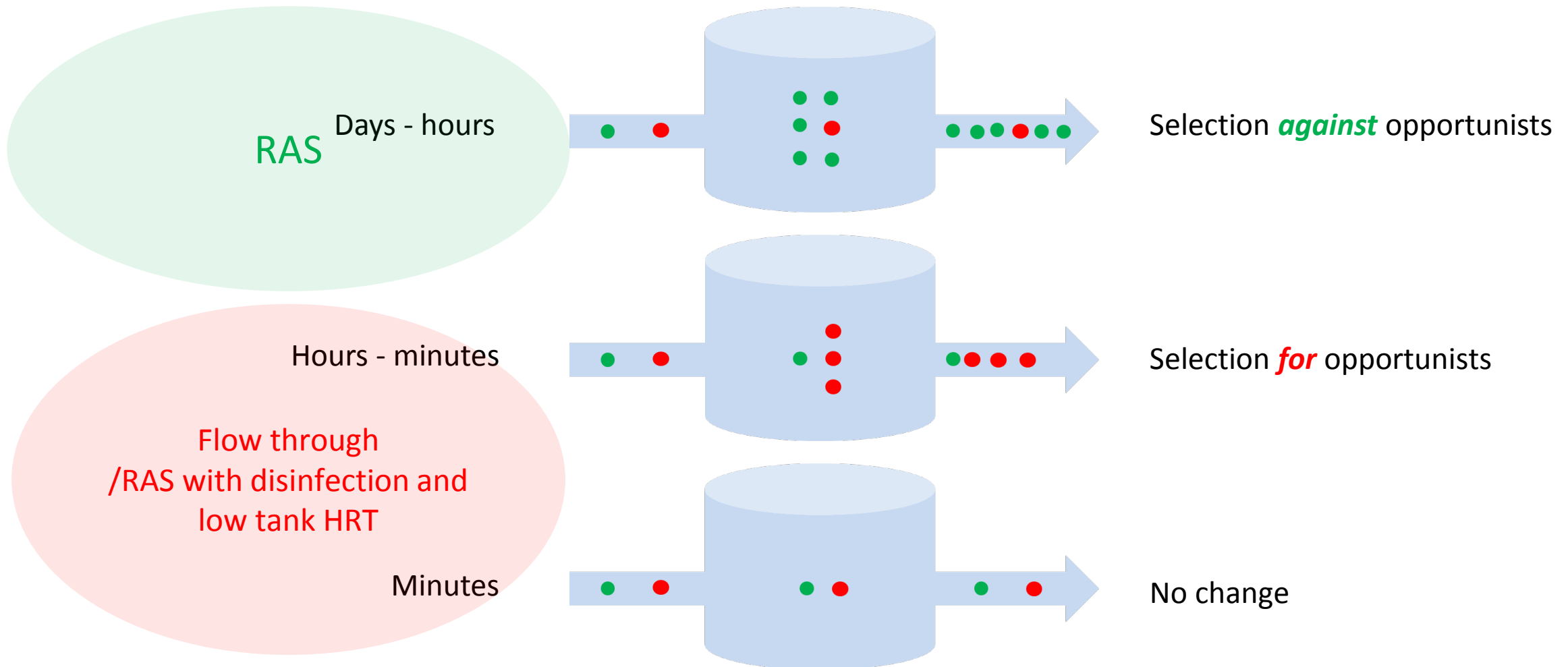
Intake water

HRT of total system and fish tank

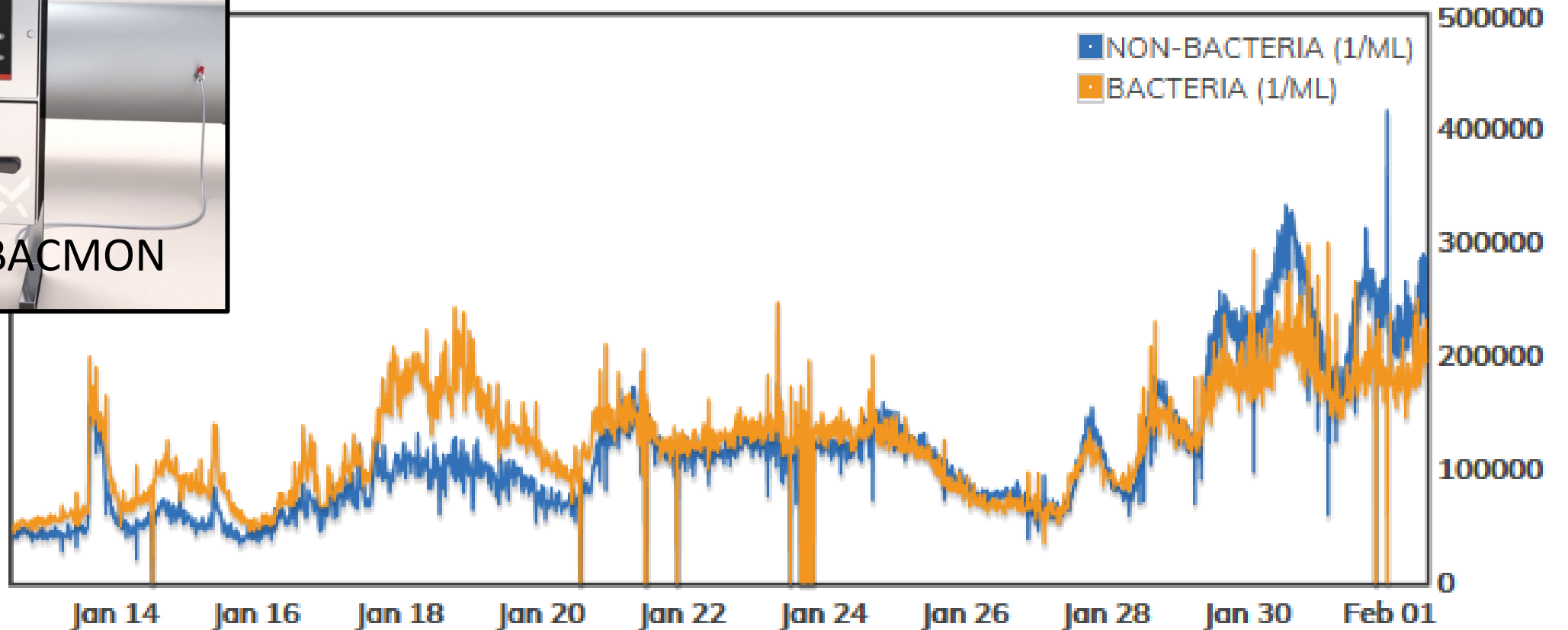
- In fish tanks, bacteria in the water growing slower than the HRT will be washed out
- In RAS, slower growing specialist bacteria can return to the tank through the water treatment loop
- Unless disinfected on the way...



HRT of total system and fish tank



Quantitative surveillance of bacteria

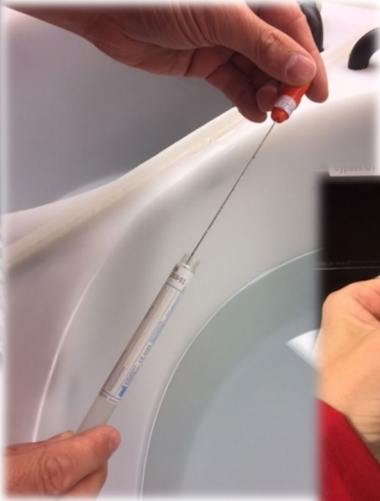


Quantitative surveillance of bacteria in RAS: SINTEF MonMic project

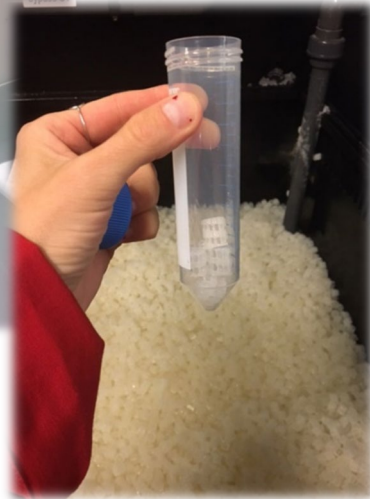
Sampling



Water



Biofilm



Biofilter

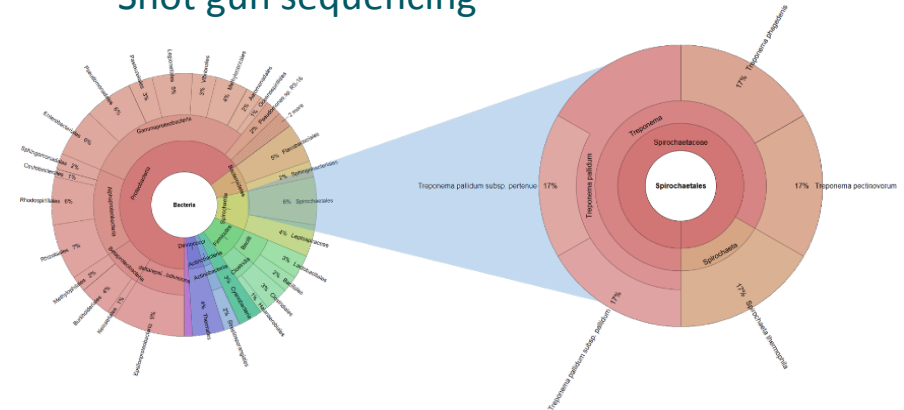
Every 2 weeks for 15 months
x 5 commercial RAS
~2700 samples

DNA extraction



Sequencing

-16S ribosomal RNA gen amplicon sequencing
-Shot gun sequencing



Systematic analysis of microbial community

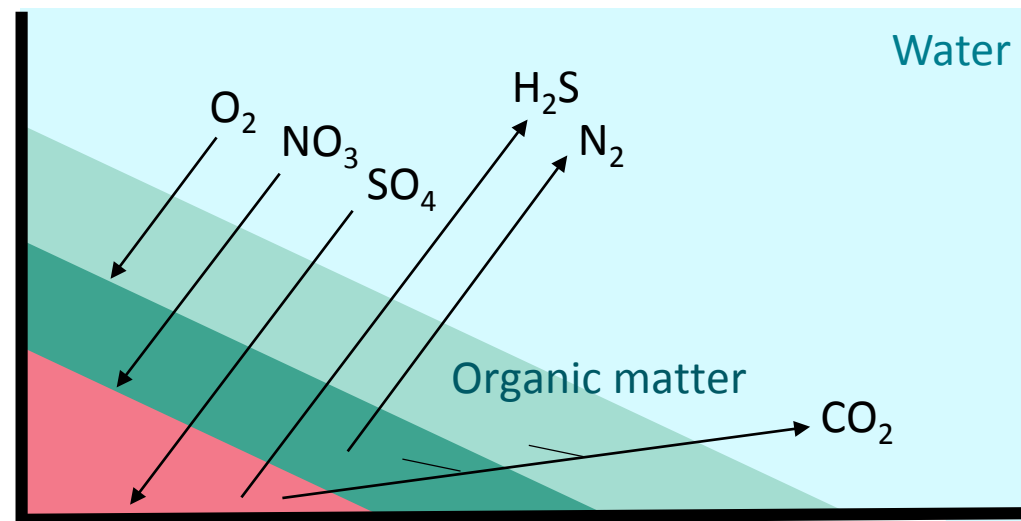


Correlation to:
- Water quality
- Operations and incidents
- Fish survival, FCR, growth

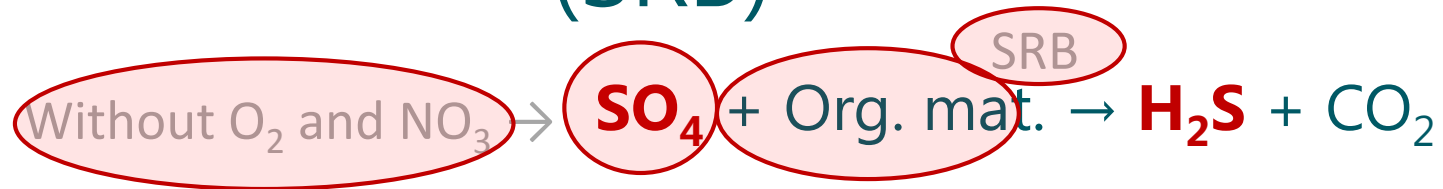
H₂S and sulphate reducing bacteria (SRB)

Preferred order of oxidant for bacteria consuming organic matter:

1. $\text{O}_2 + \text{Org. mat.} \rightarrow \text{H}_2\text{O} + \text{CO}_2$ (Respiration)
- No O₂ → 2. $\text{NO}_3 + \text{Org. mat.} \rightarrow \text{N}_2 + \text{CO}_2$ (Denitrification)
- No O₂ or NO₃ → 3. $\text{SO}_4 + \text{Org. mat.} \rightarrow \text{H}_2\text{S} + \text{CO}_2$ (Sulfate reduction)



H₂S and sulphate reducing bacteria (SRB)

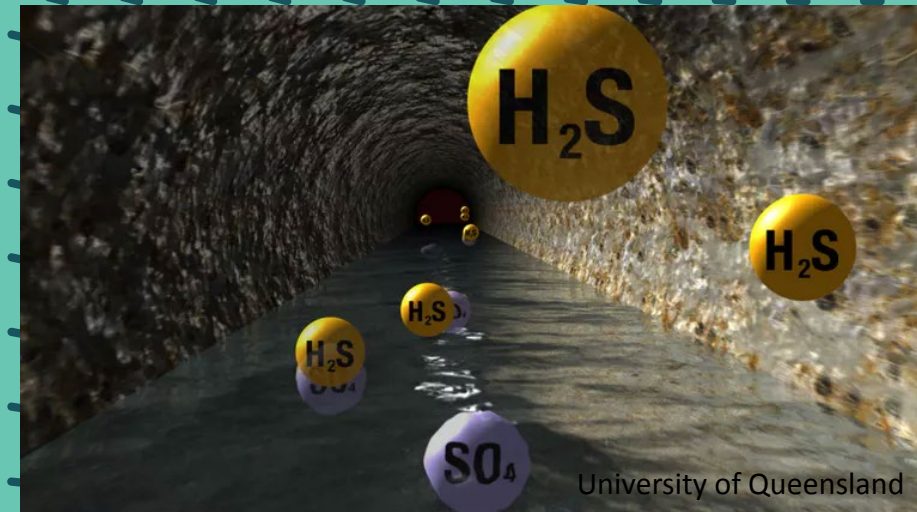


Possibilities to avoid the production of H₂S:

1. **Remove organic matter** (water velocity, hydraulics, remove particles)
2. **Secure access to oxygen or nitrate** (to the innermost layer of biofilm and sludge)
3. **Remove sulphate** (seawater 1000× more sulphate than fresh water, membranes possibility)
4. **Remove SRB** (hard since many different species and also survive in aerobic conditions)

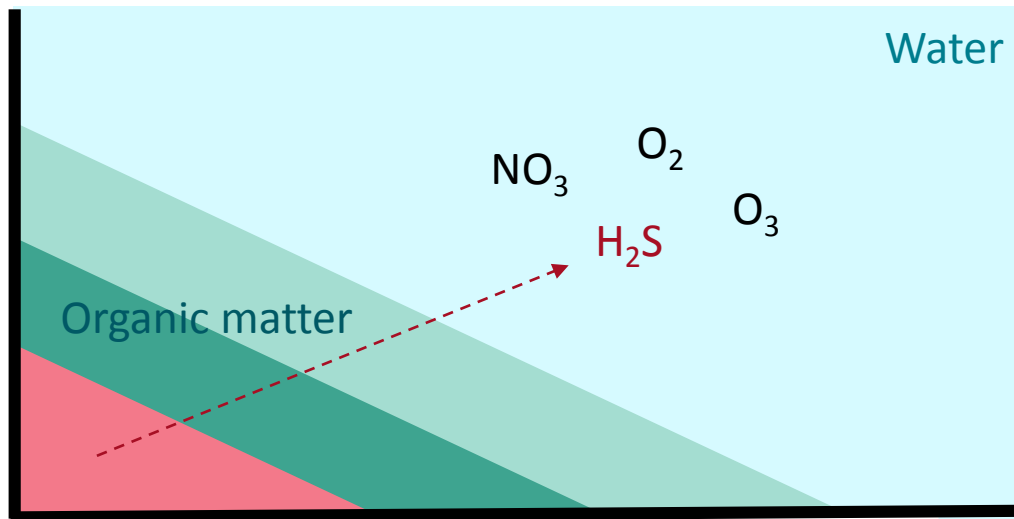
Typical H₂S accidents

- Both design- and management errors
- Dead zones, thresholds, corners, pipe bends
- Stagnant water, or sludge in contact with a stagnant volume of air
- Too low water flow
- Infrequent flushing of pipes
- Backlash of water or water not leaving where it is supposed to
- Too thick biofilm
- No washing of system between batches of fish

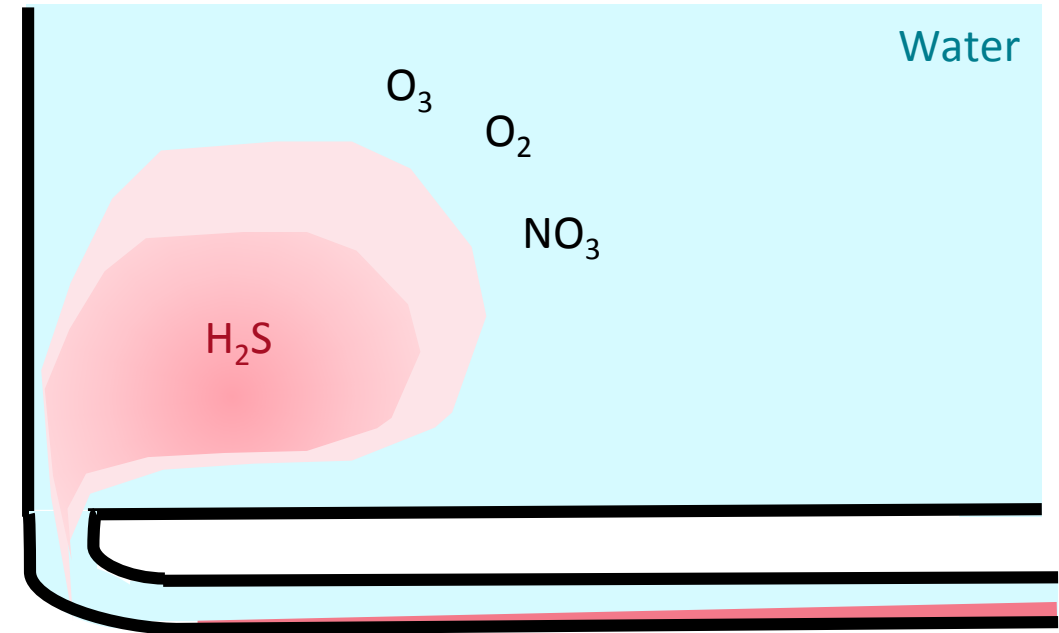


H₂S in the rearing water

Background level building up



Sudden release



Actions to avoid H₂S in RAS

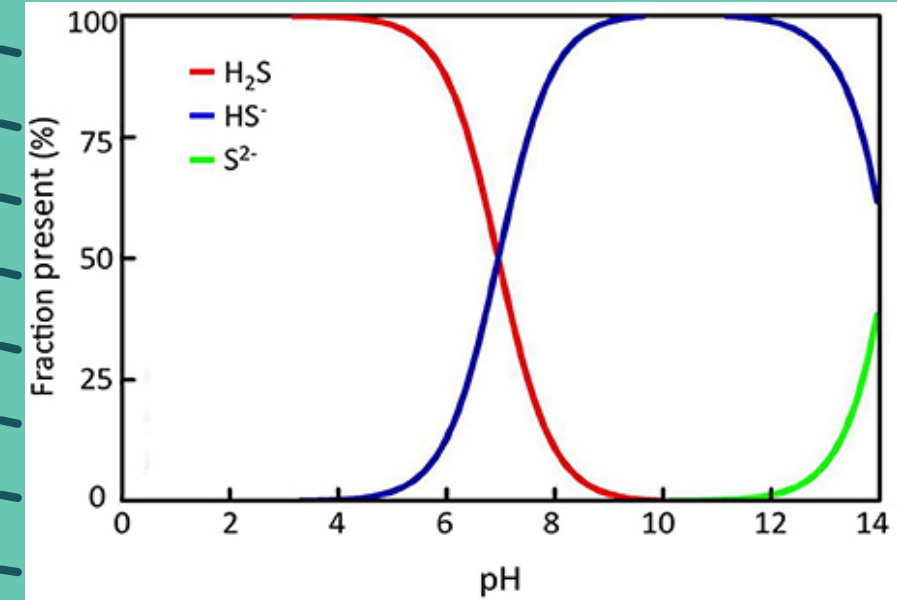
Design:

- *Coating of surfaces*
- *Good hydraulics and water velocities in pipes*
- *Short pipes, few pipes*
- *No dead zones or thresholds*
- Surveillance:
 - *Inline H₂S-sensors*
 - *Passive sensors (NIVA)*
- Management:
 - *Education of staff*
 - *Routines for cleaning and flushing*
 - *Action plans*



Actions to save fish from H_2S in the water

- Identify source and stop it (but don't wash and release more when fish is in the system)
- Bring in H_2S -free water from another source
- Maintain high level of nitrate ($> 40 \text{ mg/L}$)
 - *Feed biofilter when starving fish*
 - *Add calcium nitrate*
- Increase pH to reduce the fraction of the toxic form present (considering TAN levels)
- Ozone and oxygen
- Sample fish and water





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