DEMYSTIFYING CRITICISM OF HYDROGEN PEROXIDE UTILIZATION IN RECIRCULATING AQUACULTURE SYSTEMS

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aquaculture europe 19

Aquaculture

Production systems

- ✓ Pond farms
- ✓ Offshore aquaculture
- ✓ Capture based aquaculture
- ✓ Combination of different species (IMTA)
- ✓ Land-based RAS

Environmental impact

Nutrient input/ eutrophication   Fouling   Diseases

STOCKING DENSITY

Bio-Salmon
10 kg/m^3

Off-shore
20 kg/m^3

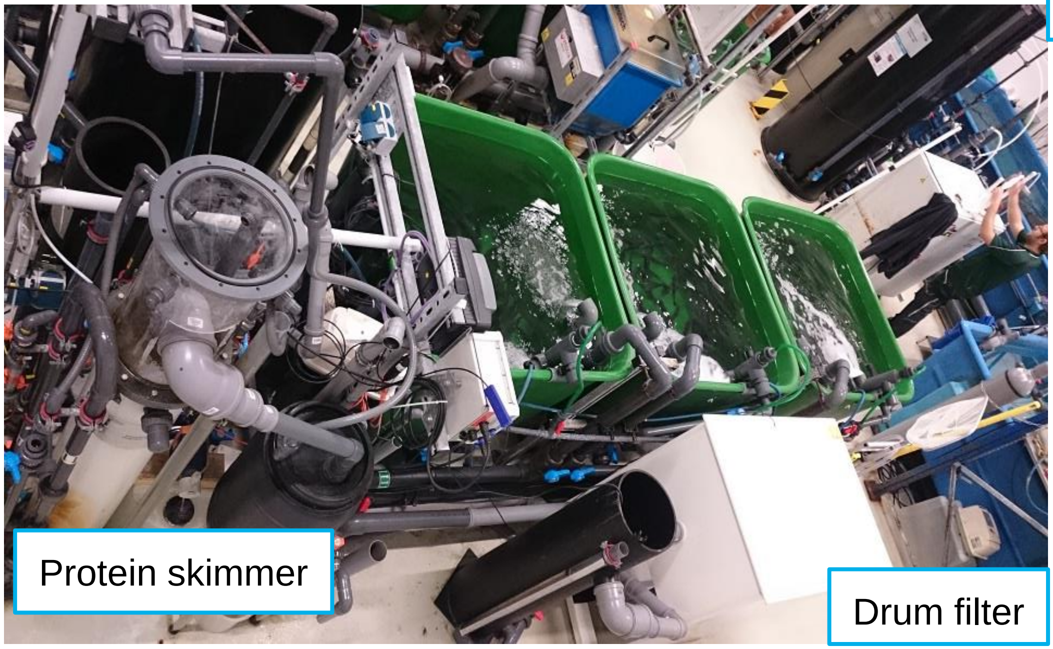
Conventional
farm/juveniles
20-50 kg/m^3

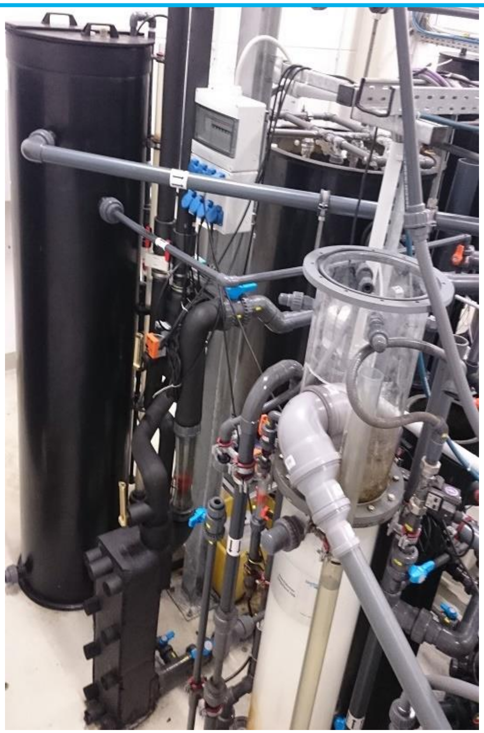
Estimated $8-10 billion market investment for
water treatment improvement

Source: K. Lorkowski & State of the market report, members.luxresearchinc.com

Water treatment in RAS

Nitrification-Denitrification filters



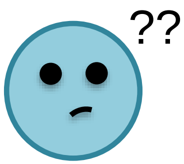


Additional improvements:

- **Ozone**
- **UV-light**
- **Alternative methods e.g. Ultrasound**
- **Chemical disinfectants**

Pictures: D. Bögner

Water treatment in RAS



Methods	Pros and Contras
Ozone: requires investment in equipment, maintenance, and control units	Highly reactive, non-selective
	Produced in situ, with energy costs
	May be highly toxic at very low concentrations
	Costs between 7-16 €/day (without power supply) depending on generator size
UV: requires Investment in equipment and maintenance	Reactive, non-selective
	Produced in situ, with high energy costs
	Requires clear water for effectiveness
	Frequent costs due to lamp replacements
Alternative methods e.g. ultrasound requires investment in equipment, control units and maintenance	Not tested at commercial scales
	Produces heat
	Effectiveness depends on target
	High costs for power supply

Chemical disinfectants used in aquaculture

- ✓ Formalin and Glutaraldehyde
- ✓ Iodophors => Iodine
- ✓ Sodium or Calcium hypochlorite => Chlorine
- ✓ Sodium or Calcium hydroxide / Hydrochloric acid / Calcium oxide / Hydrochloric or Phosphoric acids => pH changes
- ✓ Commercial disinfectants e.g. Vikron (with potassium peroxymonosulfate (21.41%) and sodium chloride (1.5%))
- ✓ Cationic surfactant disinfectants e.g. Quartenary ammonium compounds (benzalkonium and benzethonium chlorides)
- ✓ Alcohols (isopropyl alcohol or ethanol at concentrations of 60 to 90%)
- ✓ Phenol derivatives

Hydrogen peroxide (H_2O_2) application

Decomposition via dismutation reaction

Green oxidant

$$H_2O_2 + HO_2^- \rightarrow 2H_2O + O_2 + HO^\bullet$$

Improve oxygenation and water disinfection

100 % Oxygen saturation

0 °C → 14.6 mg O_2/L

17 °C → 9.7 mg O_2/L= 20.6 mg/L H_2O_2

1g H_2O_2 → 0.47 g O_2

FDA approved dosages for 35% H_2O_2 treatment: **250-500mg/L**

SMR(Min. O_2 requirement): 75 mg / kg Fish / h → **160 mg H_2O_2**

AMR(Max. O_2 requirement): 250 mg / kg Fish / h → **532 mg H_2O_2**

RAS 60kg (Real O_2 requirement): 333 mg / kg Fish / h → **709 mg H_2O_2**

Source: Pardieck et al 1992; Hâncu et al., 2002; Yao et al., 2017; Claireaux and Lagardère, 1999; Schmidt et al 2006; Fredricks, 2015

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Hydrogen peroxide (H_2O_2) application

Species	Application	Response	Reference
Salmonids	70-100 mg/L for 2 h	Affects the gills	Schmidt et al., 2006
Atlantic Salmon	1500 mg/L 4 times a day for 20 min	↑Glucose/Lactate/Cortisol with circadian stress responses	Vera and Migaud, 2016
Scophthalmus maximus	240/480 mg/L for 30min	↑Ventilation, erratic swimming and escape behavior	Avendaño-Herrera et al., 2006
Dicentrarchus labrax	**50 mg/L** for 1h	↑Stress response: Disorders in Na^+, Mg^{2+} and Ca^{2+} ions in blood	Roque et al., 2010
RAS rearing Oncorhynchus mykiss	10-20 mg/L for 30 min-3h	High intensity RAS: NO_2-N and TAN were unaffected. Low intensity RAS: ↑NO_2-N (1mg/L) after 2 days, safe level: →**5mg/L**	Pedersen and Pedersen, 2012
Biofloc rearing Litopenaeus vannamei	29-348 µl/L for 2h	↑oxygenation - ↑toxicity (melanoses) -↑NH_4-N and NO_2-N safe level: →14.3µl/L	Furtado et al., 2014

RAS-Hydrogen peroxide -Trail

Fish stock: 21.6 kg/m^3
Initial mean weight: ~500 g
Final weight: 710 g
Temperature: 17-18°C
pH=7.8-8.2
Salinity: 32 ‰
Smart Digital S-DDA pump (Grundfos)
Dosage capacity: 2.5 ml/h- 7.5L/h

Dicentrarchus labrax

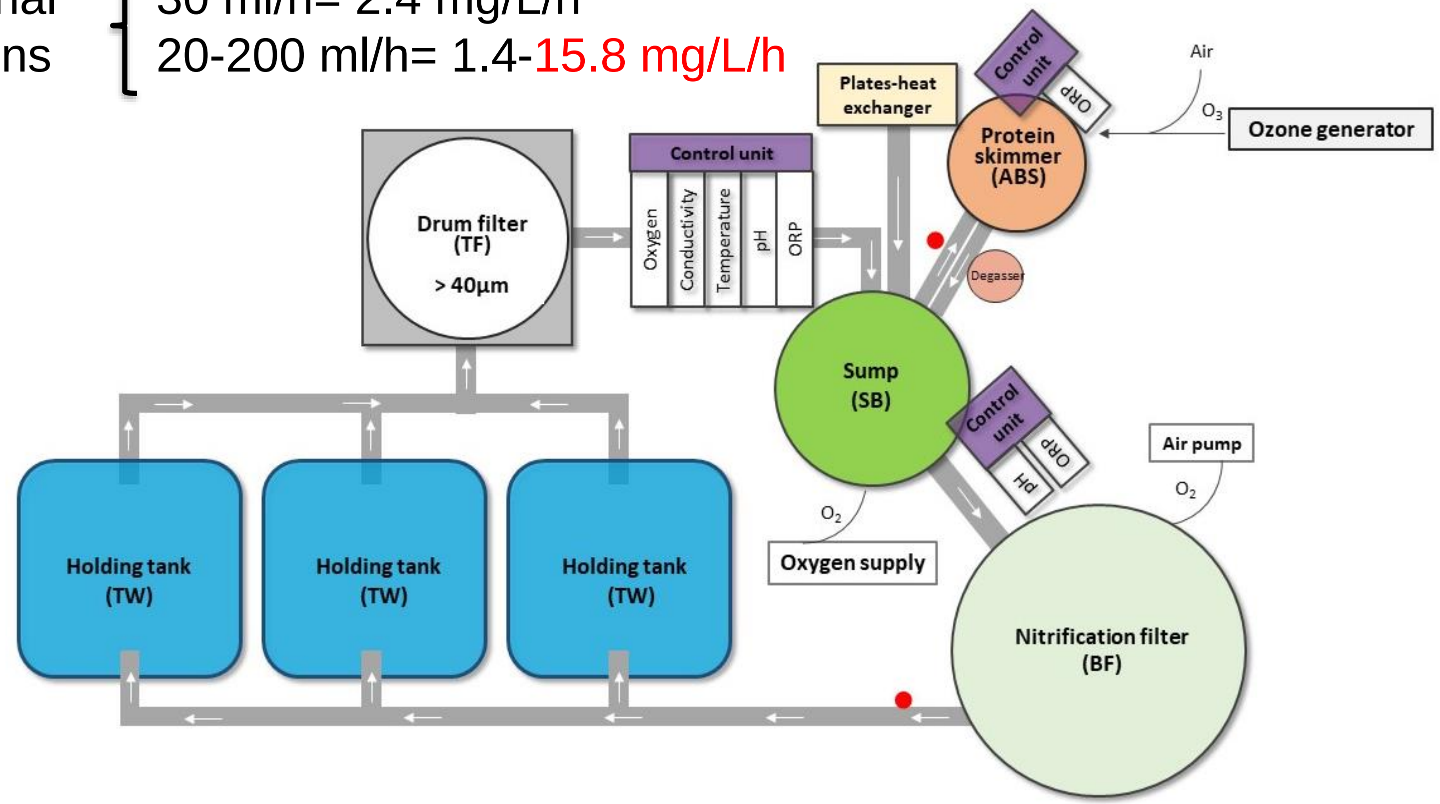
Aim of the project

Determine **where** in RAS H_2O_2 should be applied to achieve the **best oxygenation and disinfection**

Pictures: M. and D. Bögner

RAS-Hydrogen peroxide -Trail

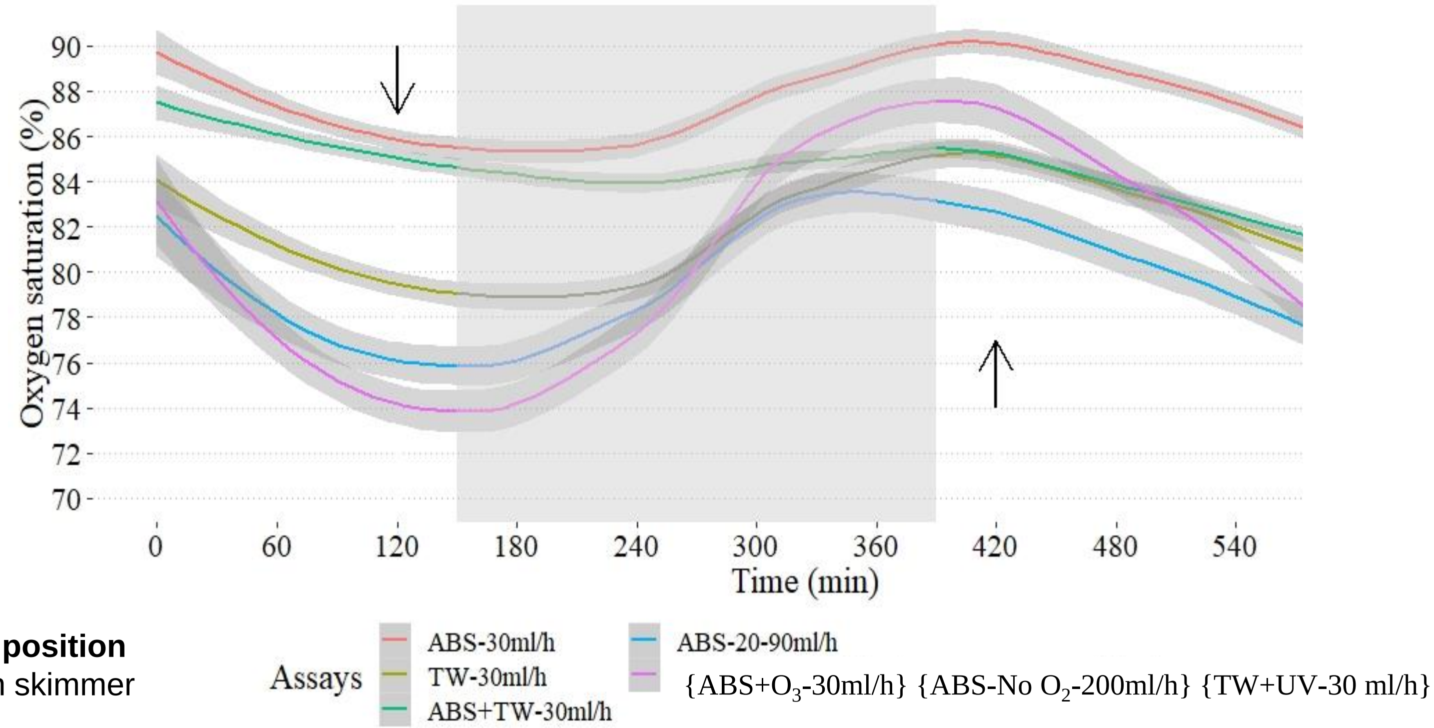
Tested nominal concentrations
- 30 ml/h= 2.4 mg/L/h
- 20-200 ml/h= 1.4-15.8 mg/L/h



Different system performance depending on the position of the dosage pumps (red dots) and system configuration

RAS-Hydrogen peroxide -Trail

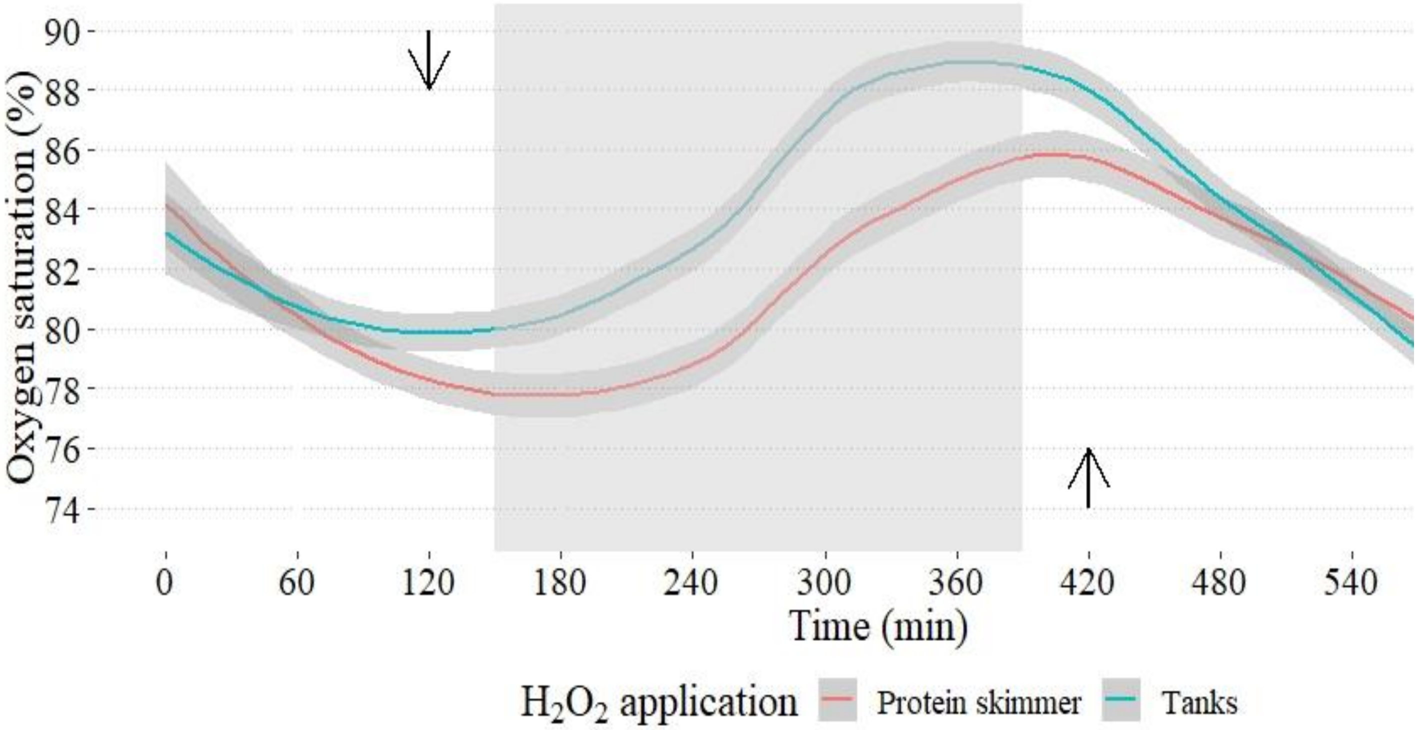
Oxygenation



Application position
ABS=Protein skimmer
TW=Tanks

RAS-Hydrogen peroxide -Trail

Oxygenation



Application in the protein skimmer offered the best position for reaction with organic load but had the highest impact on the biofilter.

RAS-Hydrogen peroxide -Trail

Measured H_2O_2 concentrations

Assay 1 (ABS)
TW→Max: 0.5 mg/L
SB→Max: 2 mg/L
ABS→Max: 5 mg/L
BF →Max: 0.8mg/L

Assay 2 (TW)
TW→Max: 3.5 mg/L
SB→Max: 1.5 mg/L
ABS→Max: 1.5 mg/L
BF→Max: 0.5 mg/L

Assay 3 (ABS-TW)
TW→Max: 2.3 mg/L
SB→Max: 1.5 mg/L
ABS→Max: 4 mg/L
BF→Max: 1 mg/L

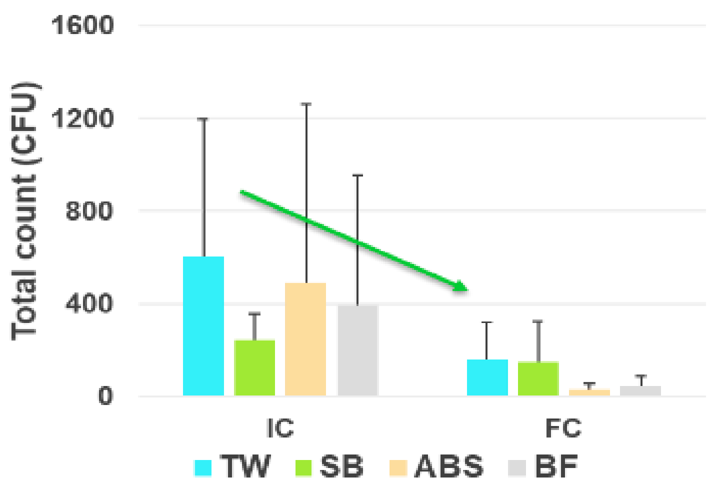
Assays 4 and 5 (with increasing doses and with or without combination with other disinfection methods)

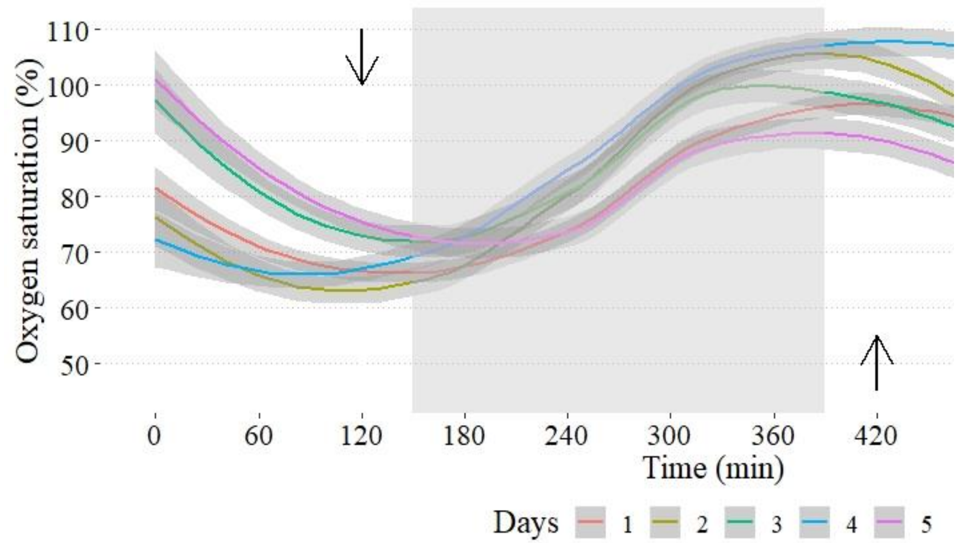
mg/L/h Appl.	1.4mg/L/h ABS	2.4mg/L/h TW	2.4mg/L/h ABS	7mg/L/h ABS	15.8mg/L/h ABS
TW	0.4	3.8	0.5	2	4.4
SB	2	1.2	1.8	4	6.8-10
ABS	3.7	1.3	4.4	17	10-25
BF	0.4	1.3	0.8	3	3-5

Equilibration in **1h**
Depletion to initial conditions after stopping dosage in **~1-1.5h**

RAS-Hydrogen peroxide -Trail

Hydrogen peroxide based oxygenation with 15.8 mg/L/h

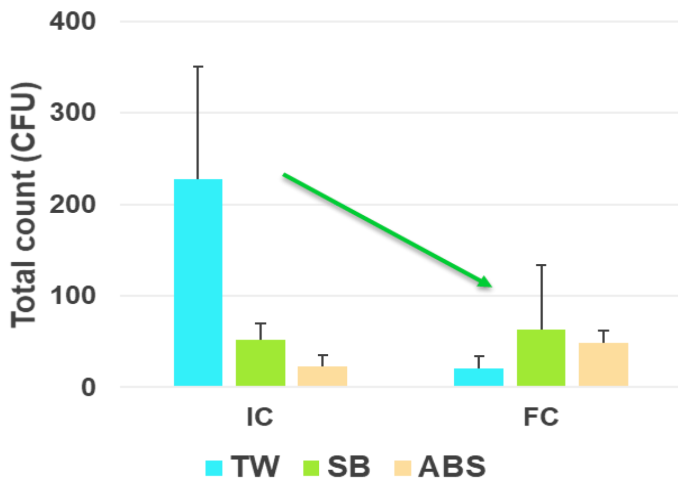


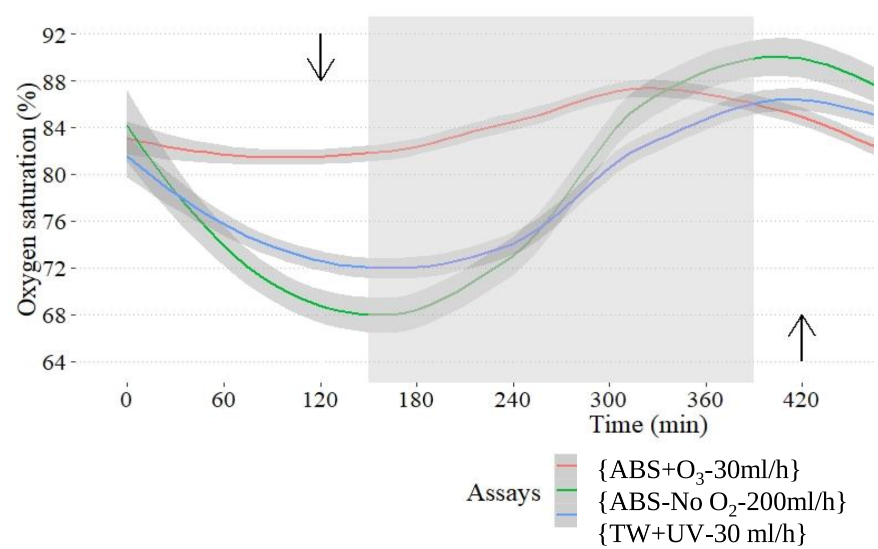


Strong reduction of the bacterial load within each compartment with high variability at initial conditions due to application pattern (4h on/ 16 off, no additional disinfection in between)

RAS-Hydrogen peroxide -Trail

2.4 mg/L/h H_2O_2 in the protein skimmer combined with ozone

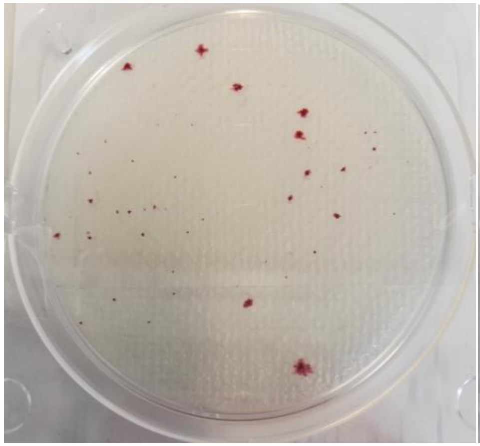


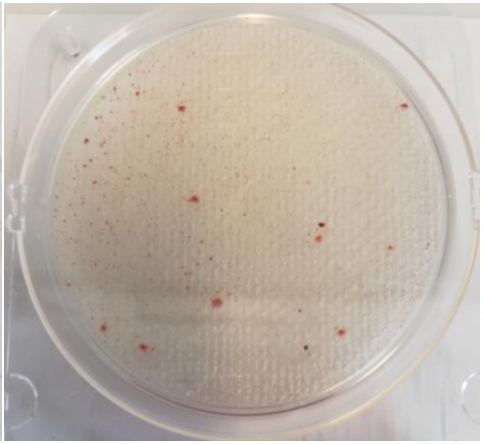


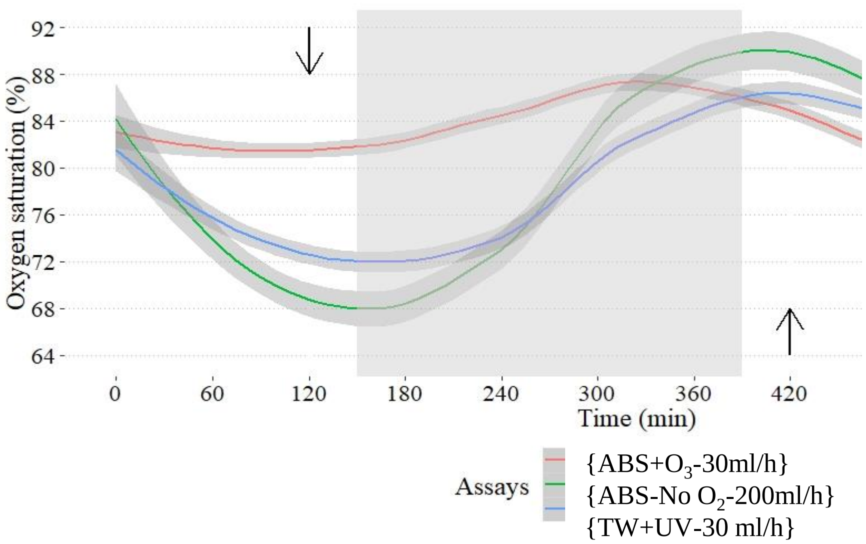
With Ozone: Strong reduction of bacterial load in the tanks but **application required adjustment** of the control parameters (ozonization was subdued by redox values achieved with H_2O_2).

RAS-Hydrogen peroxide -Trail

2.4 mg/L/h H_2O_2 to the tanks combined with UV







With UV: had better oxygenation of the tanks but lower disinfection effect and **possible change on bacterial composition**.

RAS-Hydrogen peroxide -Trail

Impact on the reared species

<u>Cortisol</u> Control: 59.8±49.5
Treatment: 62.2±43.5

<u>Glucose</u> Control: 131.0±34.9
Treatment: 155.1±45.3

- Stress in *D. labrax* is **genetically driven** and **dependent on size/age**. Individuals may have consistent high (**439.2 ± 31.1 µg/dl**) or low (**247 ± 85.1 µg/dl**) cortisol levels. Natural daily Glucose levels may vary between **100-170 mg/dl** [a,b,c]

- Levels of both markers **did not differ between control and treatment**; both tended to increase with increasing H_2O_2 doses and **showed high variability** more related to feeding stress response than to H_2O_2 dosage. The number of **fish showing conspicuously low cortisol levels increased**.

References for stress markers: a) Samaras, A., et al., 2016; b) Cerdá-Reverter, J.M., et al.,1998; c) Gutierrez, J., et al., 1984
Picture: D. Bögner

Benefit analysis

RAS by common operation

Costs for:
- Oxygenation: 0.20-0.61 €/day
- BF Oxygenation:0.78 €/day
- Disinfection via O_3 Generator:
  - 7.67 €/day (small)
  - 13.15 €/day (medium)
  - 15.07 €/day (large)

- 8.65-9.06 €/day (small)
- 14.13-14.54 €/day (medium)
- 16.05-16.46 €/day (large)

Without costs for power supply consumption, control units for O_3, and biosecurity related issues

RAS by H_2O_2 operation

Costs for:
- Oxygenation:
  - 0.50 €/day (30 ml/h)
  - 3.36 €/day (200 ml/h)
- BF Oxygenation:0.78 €/day
- Disinfection via O_3:0 €/day

- 1.28 €/day (30 ml/h)
- 4.14 €/day (200 ml/h)

Without costs for dosage units, their power consumption, maintenance and H_2O_2 storage

Take home message

- Toxicity risk and species-specific thresholds
- System knowledge and instructed staff
- Selectivity upon bacterial groups
- Handling and applicability
- Controls for effectivity
- Costs for investment, maintenance and operation

Some aspects apply for all kinds of disinfection method

H_2O_2 advantages and disadvantages

- Savings in terms of oxygenation possible even at low concentrations
- Disinfection without environmental impact
- Fast removal from the system, cleaning effect and reduction of turbidity
- Transportation (applies to most chemical methods) and storage/decay if incorrect storage (solved by close dosage systems)

Benefit analysis

1. Budget in terms of H_2O_2 production:

- 1L (50%)=1Euro
- 1L (35%)=0.7Euro + dosage costs

2. Budget in terms of Oxygen

- In the BF: air compressor pump 120L air/h with 130W power consumption (0.78 Euro/day by 0.25 Euro/kWh)
- Oxygenation in tanks: pure oxygen=by 1-2 L/min

- In this study: 1-2 L/min were used for 65 Kg fish kept over the critical oxygen level

→ 1440-2880L/day=1.44-2.88 m^3 oxygen/day

- 12 bottles of 200 bar oxygen=157 Euro + 60 Euro shipment each 2 weeks

→ 2400 bar =1540.8 m^3 oxygen = 217 Euro

- 1.44 m^3 oxygen/day = 0.20 Euro/day
- 2.88 m^3 oxygen/day = 0.40 Euro/day

Benefit analysis

3. Budget in terms of Ozone production and disinfection

Ozone generator:
Price: 20000 Euro (5g Ozone/h)
40000 Euro (60g/h)
47000Euro (100g/h for 25-50 Kg feeds/day)
Maintenance: 800 Euro/year
Lifetime: 10 years
Additional power consumption

5 g Ozone/h are required for 1.25-2.5 Kg feeds/day
↓
62.5-125 kg Fish with 2% BW feeding

Investment: 5.48 Euro /day
Maintenance: 2.19 Euro /day
Power consumption

No other disinfection method is used in our system but under real conditions by higher stocking density

- Formaldehyde
- Hypochlorite
- UV (if clear water)

Thank you!   Vielen Dank!