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in the field of use and protection of water bodies and water quality*

## Treating gill diseases in freshwater – water chemistry in tarpaulins and well boats

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# Bathing

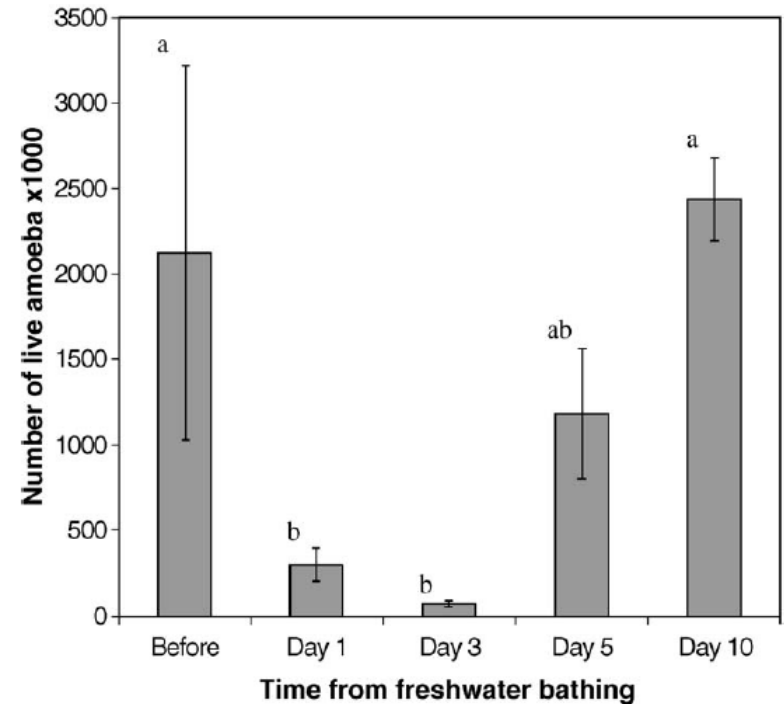
Table 1  
Definition of gross AGD gill scores as used by TASSAL

Infection level	Score	Gross signs
Clear	0	gills appear clean, healthy red colour
Very light	1	1 mucoid spot, light mucus
Light	2	2–3 mucous spots, some paling of colour
Medium	3	established thickened mucous patch
Heavy	4	> 3 mucous patches or single large patch

Table 2  
Difference in the gross signs, gill smear results, presence of live *Paramoeba* on smears and presence of gill lesions and *Paramoeba* in histological sections before and after freshwater bathing

Variable measured	Before	After
Prevalence of mucoid patches (%)	93.3 (1.9)	55 (7.9)
Gross appearance—clear (%)	6.7 (1.9)	44.9 (7.9)
Gross appearance—very light (%)	18.9 (4.8)	18.0 (5.7)
Gross appearance—light (%)	26.7 (1.9)	24.4 (1.7)
Gross appearance—medium (%)	24.4 (1.7)	9.1 (0.9)
Gross appearance—heavy (%)	23.4 (1.7)	3.5 (1.9)
DiffQuick positive gill smears (%)	77.6 (5.4)	27.1 (2.6)
<i>Paramoeba</i> live on fresh smear (%)	86 (n = 50)	27 (n = 11)
Number of <i>Paramoeba</i> /lesion	0.4 (0.11)	0.07 (0.02)
Number of AGD lesions/filament	0.23 (0.04)	0.21 (0.02)
<i>Paramoeba</i> within cysts (%)	31.9 (5.6)	71.2 (15.8)

Parsons et al. 2001 Aquaculture 195:205-210



Clark et al. 2003 Aquaculture 219: 135-142

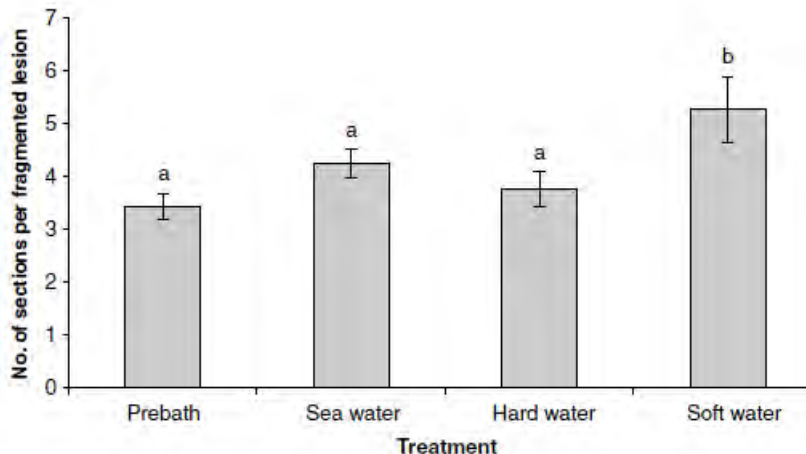
# Effects of freshwater bathing of AGD affected Atlantic salmon

Effects of experimental freshwater exposure on mean ( $\pm$  SE) respiratory variables of Atlantic salmon under normoxic (100% saturation) or hyperoxic (200% saturation) conditions. Like symbols are significantly different from each other

	O <sub>2</sub> saturation		O <sub>2</sub> saturation	
	100% Before	100% After	100% Before	200% After
PO <sub>2</sub> (mm Hg)	110.2 (5.9)	118.5 (5.0)*	123.3 (14.1)	154.2 (13.9)*
pH	7.756 (0.017)	7.799 (0.039)	7.706 (0.015)	7.716 (0.043)
CO <sub>2</sub> (ml/100 ml)	11.19 (1.36)	8.22 (0.96)	9.34 (1.38)	9.61 (1.02)
Hct (%)	25.2 (1.9)	26.3 (1.2)	23.3 (0.98)	23.6 (1.7)
Hb (g/100 ml)	7.16 (0.63)	6.72 (0.51)	7.23 (0.47)	5.98 (0.90)
MCHC	0.28 (0.03)	0.25 (0.02)	0.31 (0.02)	0.25 (0.03)
O <sub>2</sub> /Hb (ml/g)	1.25 (0.16)	1.01 (0.15)	1.01 (0.18)	1.32 (0.16)



Powell et al. 2001 Aquaculture 199: 259-266



Roberts and Powell 2003 Journal of Fish Diseases 26: 591-599

# Comparison of treatment approaches



## Tarpaulin

## Wellboat

Tow infected cage from production to bathing site

Pump fish from Production cage into bathing cage lined with tarpaulin

Treatment period

Pull away tarpaulin fish in «new» cage

Tow «clean» cage back to production site

Infected cage in situ

Tarpaulin slung under cage or skirt around cage

H<sub>2</sub>O<sub>2</sub> added to water

Treatment period

Tarpaulin/skirt pulled away

Well boat loads with FW

WB pull along side infected cage

Fish pumped from cage into wellboat well

H<sub>2</sub>O<sub>2</sub> added to water

Treatment period

Fish pumped back into cage

Water «treated/disinfected» and discharged

# Factors affecting freshwater abstraction and use

- Legal frameworks
  - SEPA
  - Environmental impact assessment legislation
  - Water framework directive
  - Conservation legislation
- River type
- Season
- Prevailing flow
- Abstraction intake points
- Storage reservoirs
- Pipelines
- Road/boat transport
- **Water chemistry**

# Freshwater availability

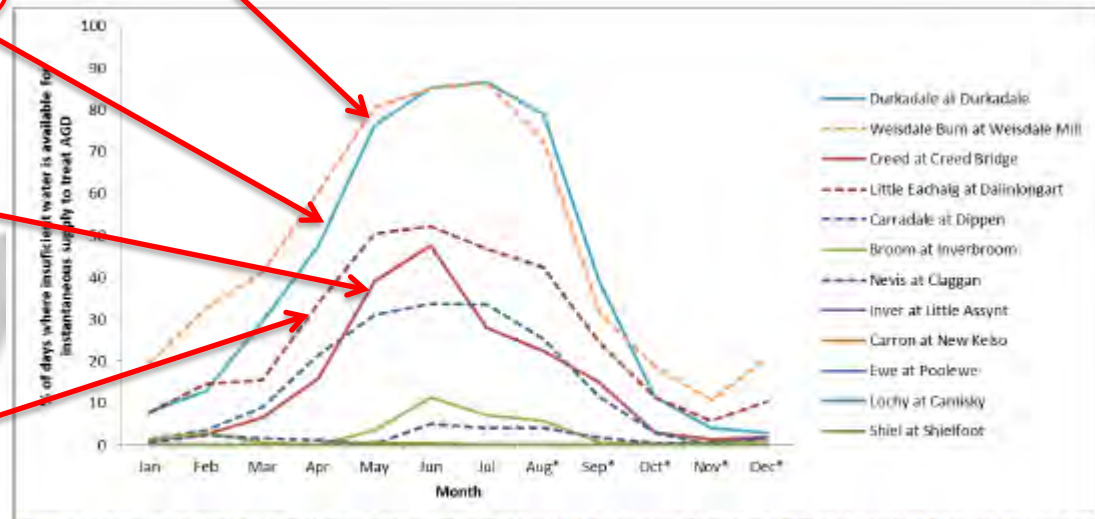
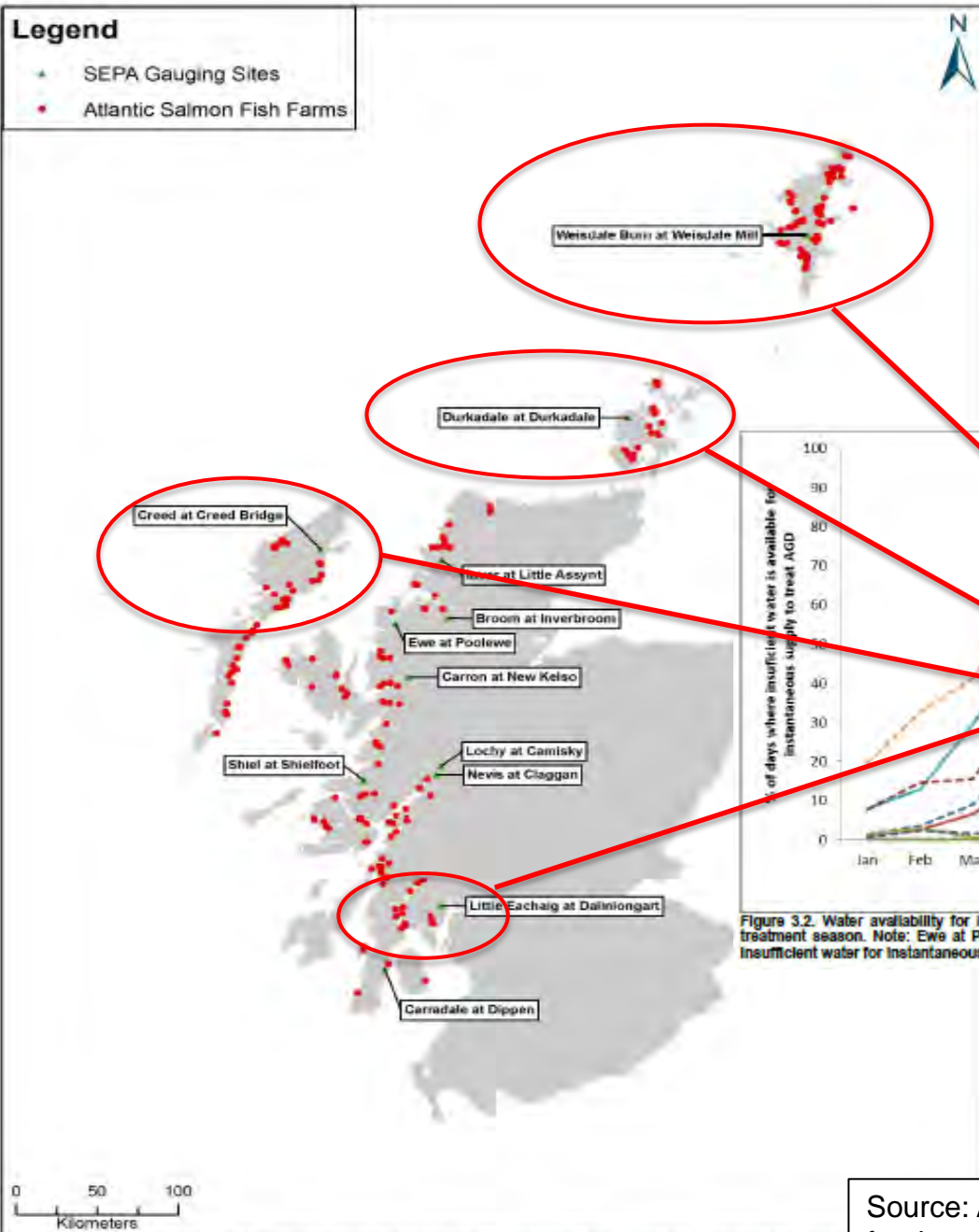
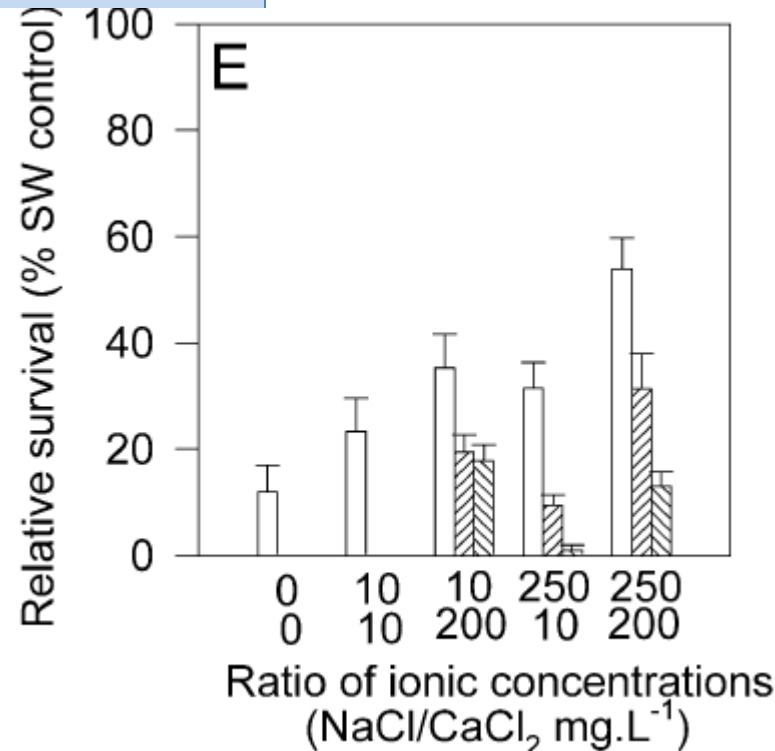
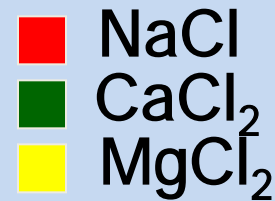
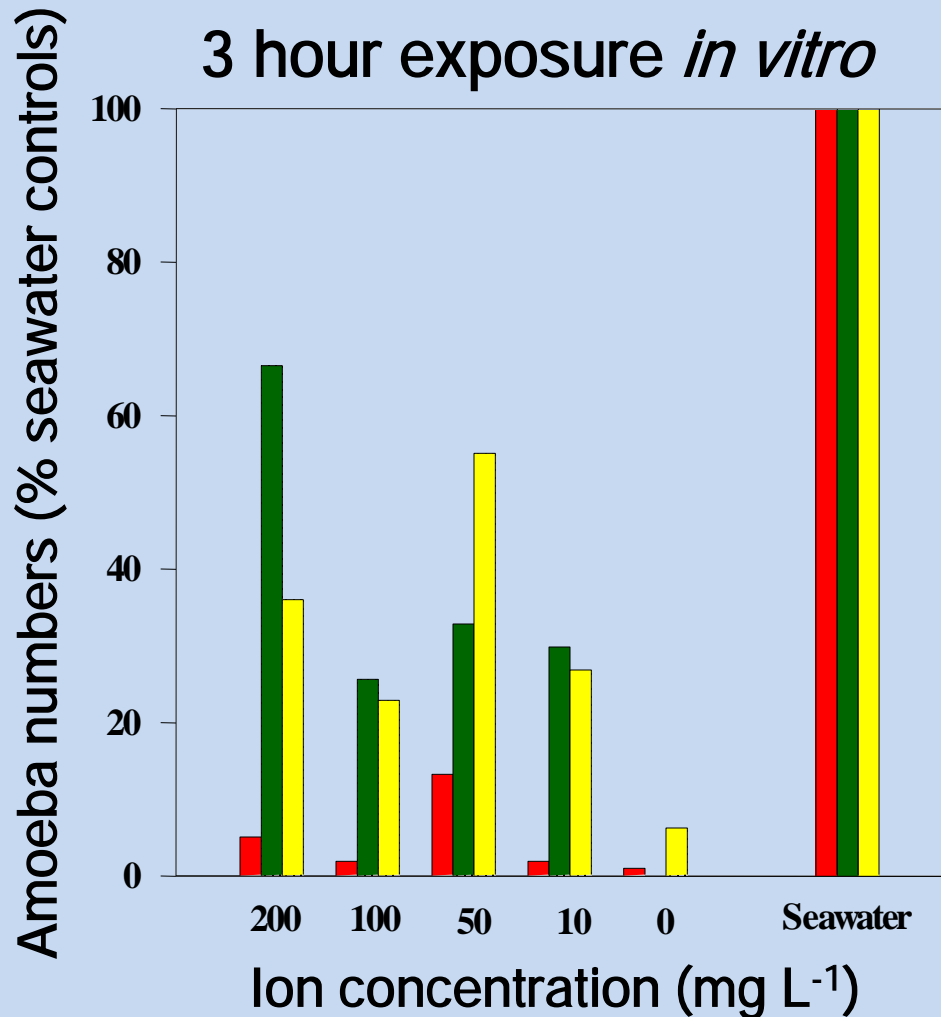


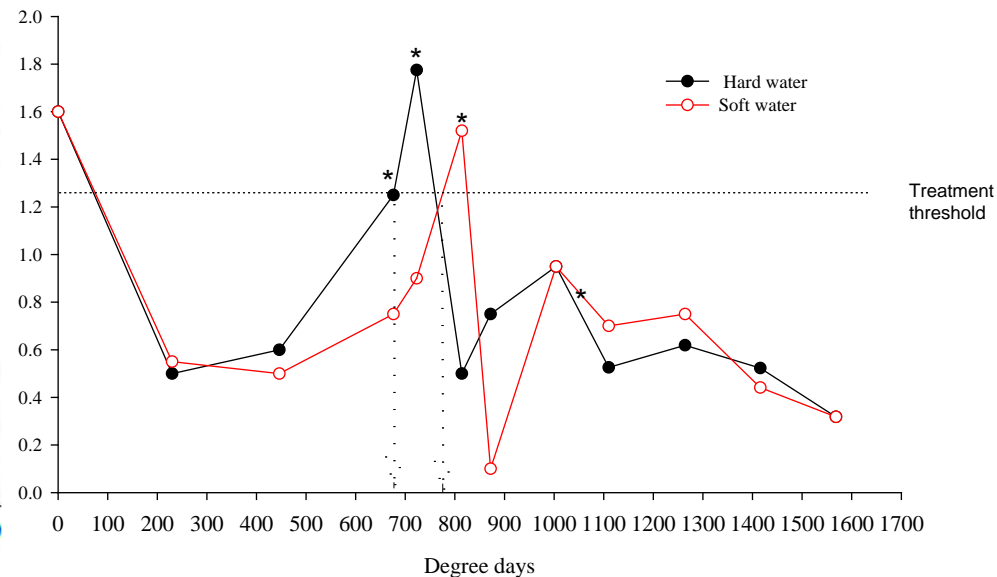
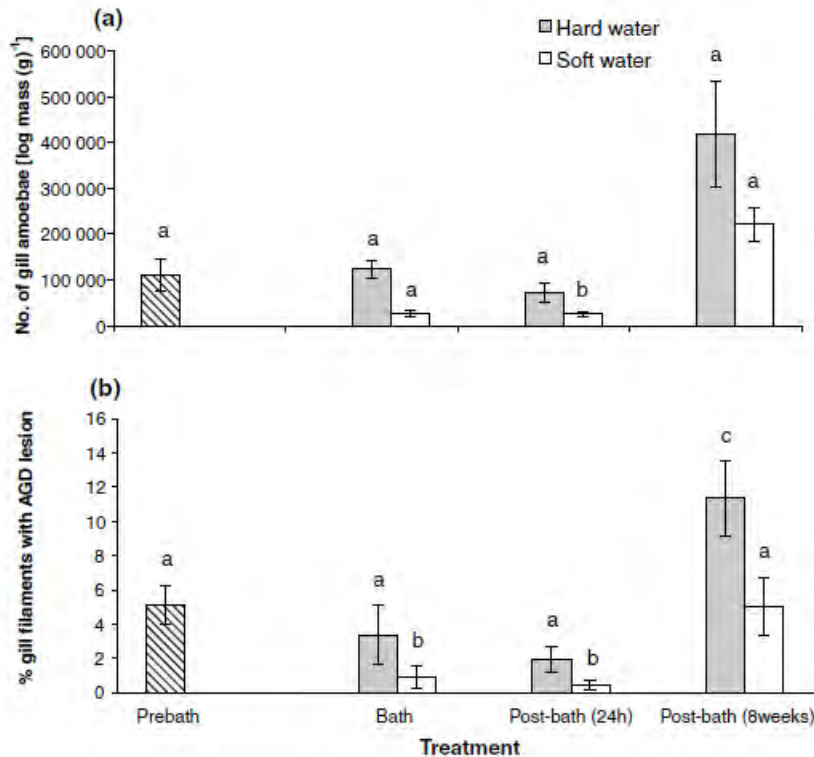
Figure 3.2. Water availability for instantaneous AGD treatment at twelve flow gauging locations on the West coast of Scotland. \* = key AGD treatment season. Note: Ewe at Poolewe, Lochy at Camisky, and Shiel at Shielfoot all overlap the X-axis throughout the year (i.e. none have insufficient water for instantaneous abstraction at any time).

Source: APEM (2014) Availability and use of freshwater resources for the treatment of amoebic gill disease at Scottish salmon farms. APEM report 413028 to the Scottish Aquaculture Research Forum.

# Gill amoeba survival in freshwater: implications for freshwater bathing



# Effects of water hardness on efficacy of freshwater bathing

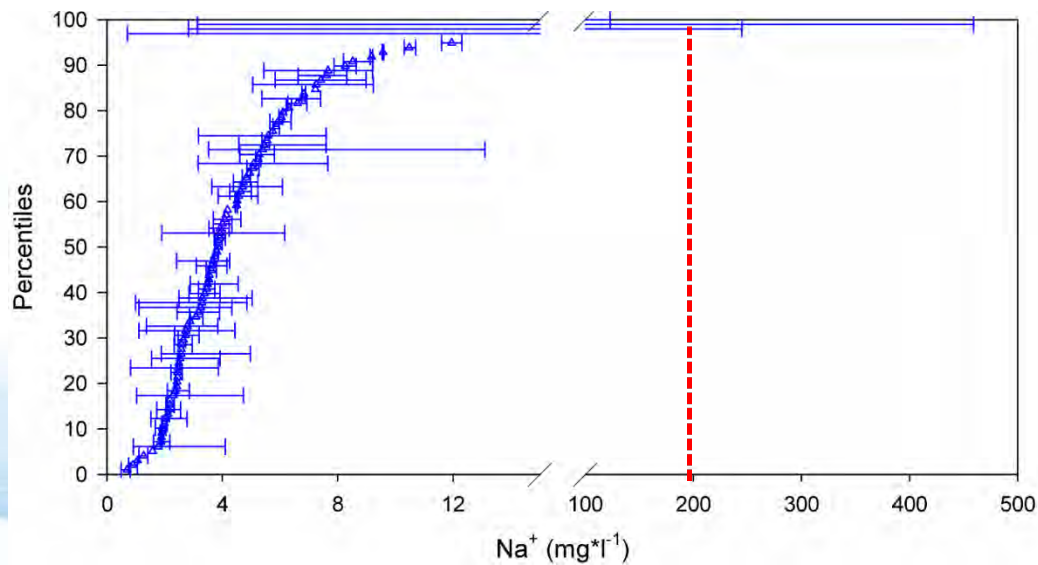
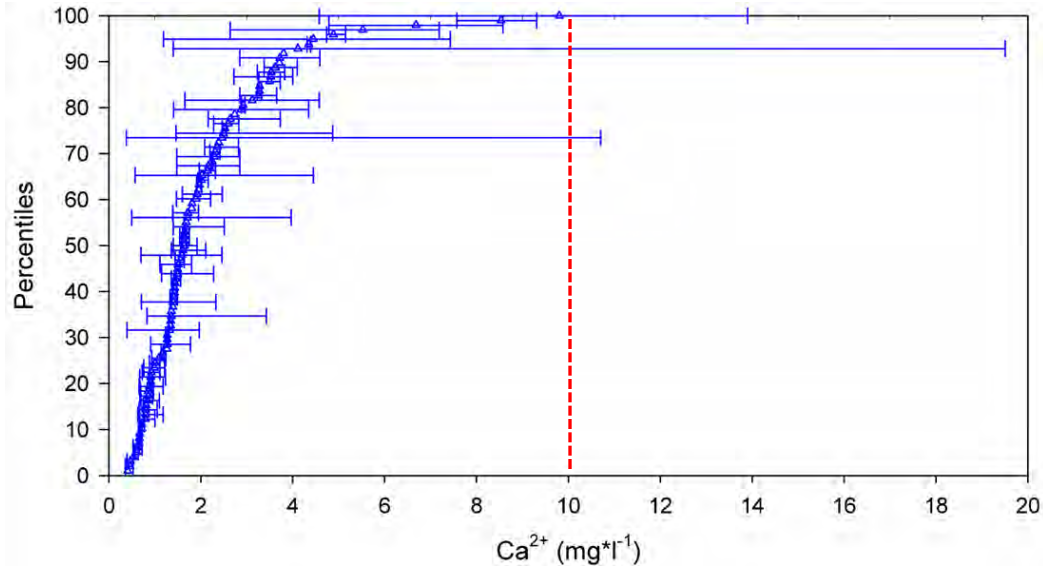


Roberts and Powell 2003 Journal of Fish Diseases 26: 591-599

Powell et al. 2005 FRDC project report 2001/205

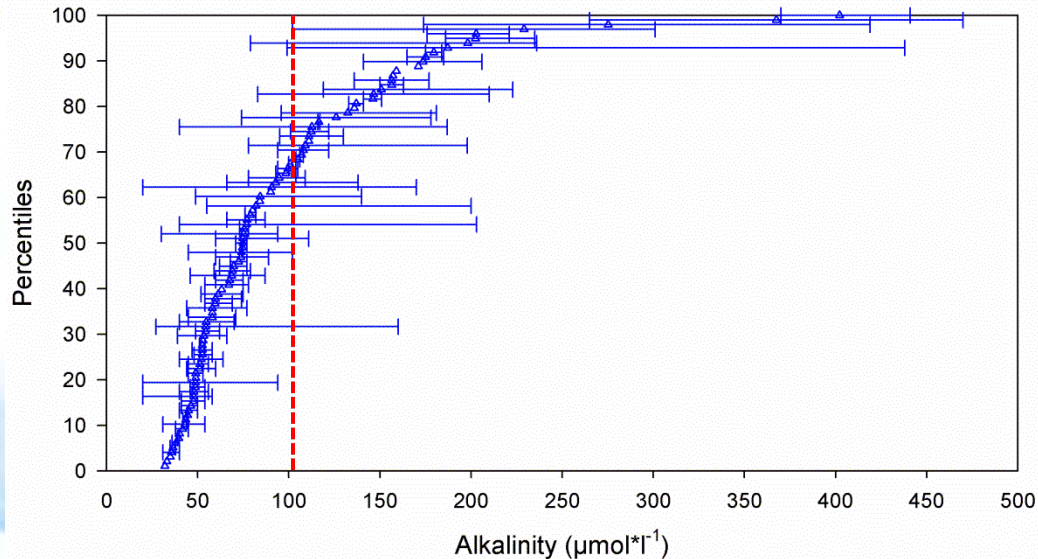
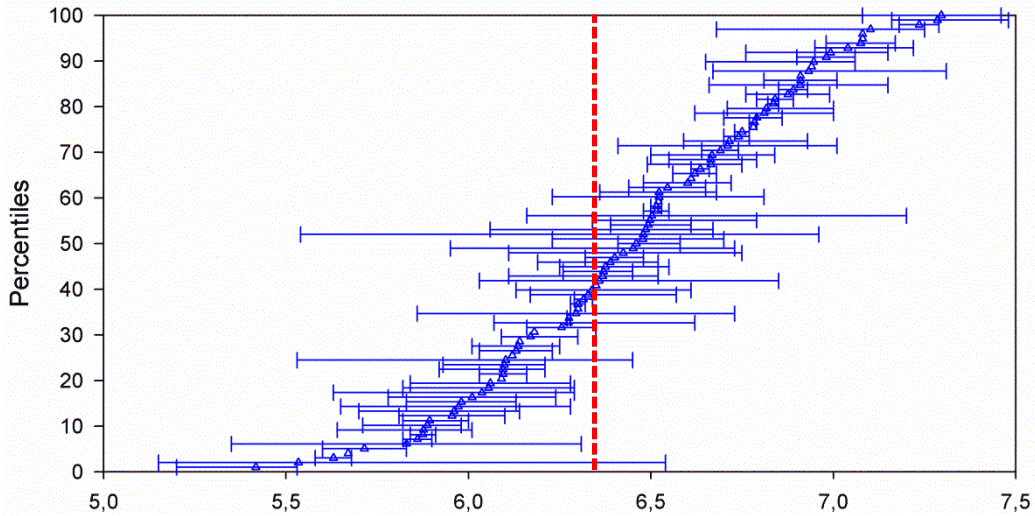


# Norwegian freshwater (WQ 99-06)

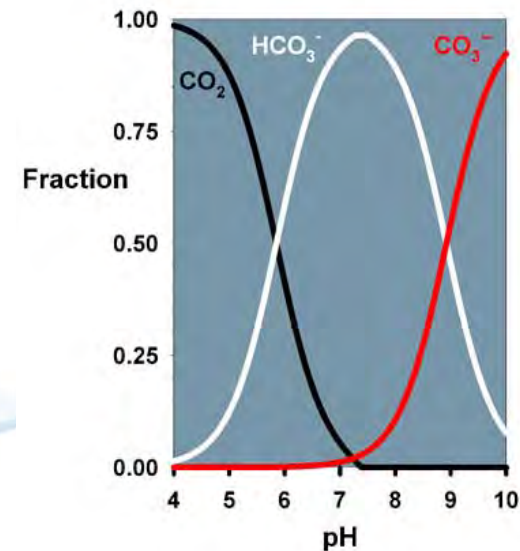


- Low major ion concentrations
- Norwegian WQ is expected to give good treatment efficacy (AGD)

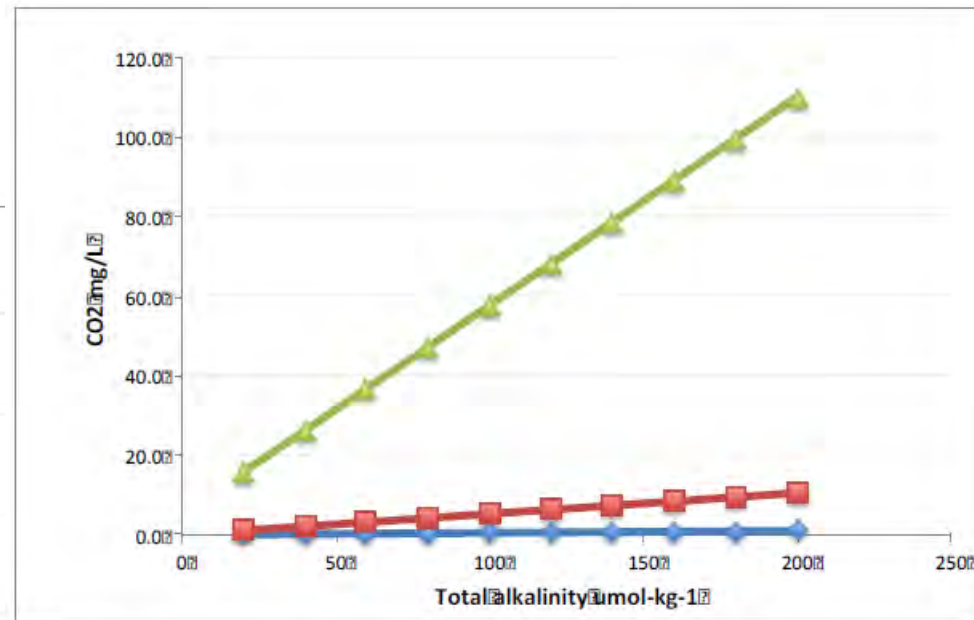
# Norwegian freshwater (WQ 99-06)



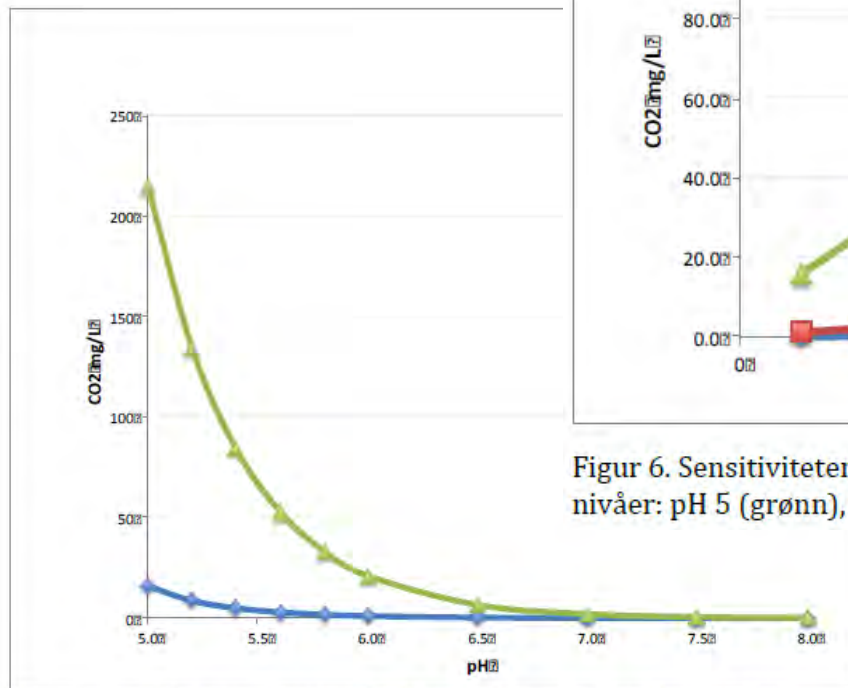
- Generally low pH and buffering capacity
- Closed FW treatment systems with high biomass will generate high degree of  $\text{CO}_2$  (g) accumulation and pH drop
- Aeration and oxygenation may result in steady-state conditions



# Sensitivity of CO<sub>2</sub> concentration to alkalinity and pH



Figur 6. Sensitiviteten av beregnede CO<sub>2</sub> verdier i forhold til endret total alkalitet ved tre ulike pH-nivåer: pH 5 (grønn), pH 6 (rød) og pH 7 (blå).



Figur 7. Sensitivitet av beregnede CO<sub>2</sub> verdier ved ulike pH og to alkalitetsnivåer (blå – 20 µmol/kg, grønn – 400 µmol/kg). Sensitiviteten øker ved lavere pH og ved høyere alkalitet.

Source: NIVA report 2015  
Behandling av amøbegjellesykdom i brønnbåt

# ...but remember

CO<sub>2</sub> is a weak acid in solution



NH<sub>3</sub> is a weak acid in solution





# Modeling the accumulation of CO<sub>2</sub> during high density, re-circulating transport of adult Atlantic salmon, *Salmo salar*, from observations aboard a sea-going commercial live-haul vessel

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Henderson–Hasselbalch equation:

$$P_{CO_2} = \frac{TCO_2}{\alpha 10^{pH-pK^1} + 1} \tag{1}$$

$P_{CO_2}$  partial pressure of CO<sub>2</sub> (mmHg)  
 $TCO_2$  total CO<sub>2</sub> in solution (mmol L<sup>-1</sup>)  
 $\alpha$  solubility constant of CO<sub>2</sub> (mmol L<sup>-1</sup> mmHg<sup>-1</sup>)  
 pH pH of solution  
 $pK^1$  first dissociation constant of CO<sub>2</sub>

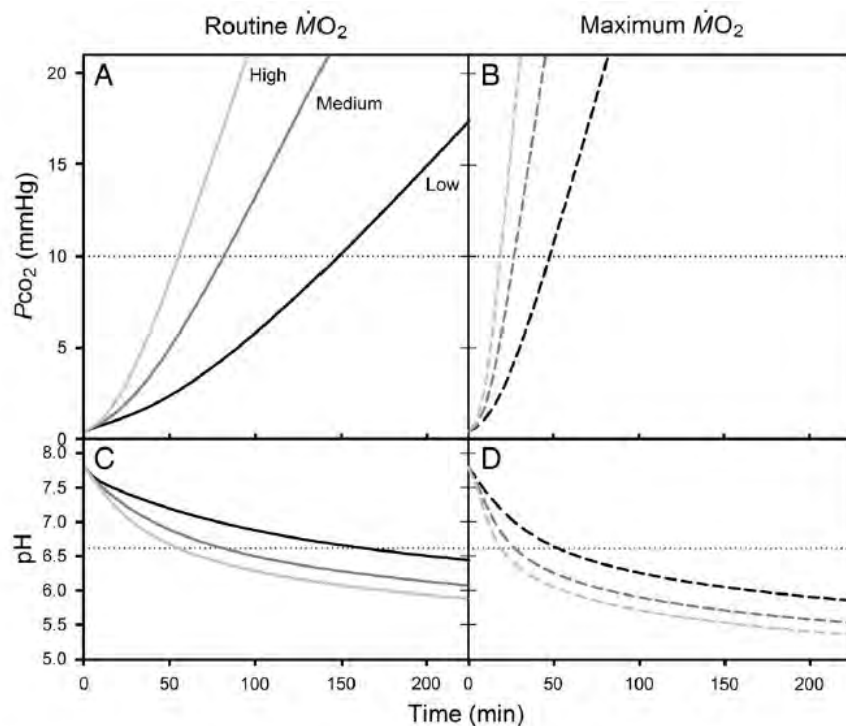
$$P_{CO_2} = \frac{TCO_{2ac} + TCO_{2bg}}{\alpha 10^{pH-pK^1} + 1} \tag{2}$$

$TCO_{2ac}$  moles of accumulated total CO<sub>2</sub> from respiration (mmol L<sup>-1</sup>)  
 $TCO_{2bg}$  moles of background total CO<sub>2</sub> (mmol L<sup>-1</sup>)

The amount of CO<sub>2</sub> created through respiration is calculated from the equation:

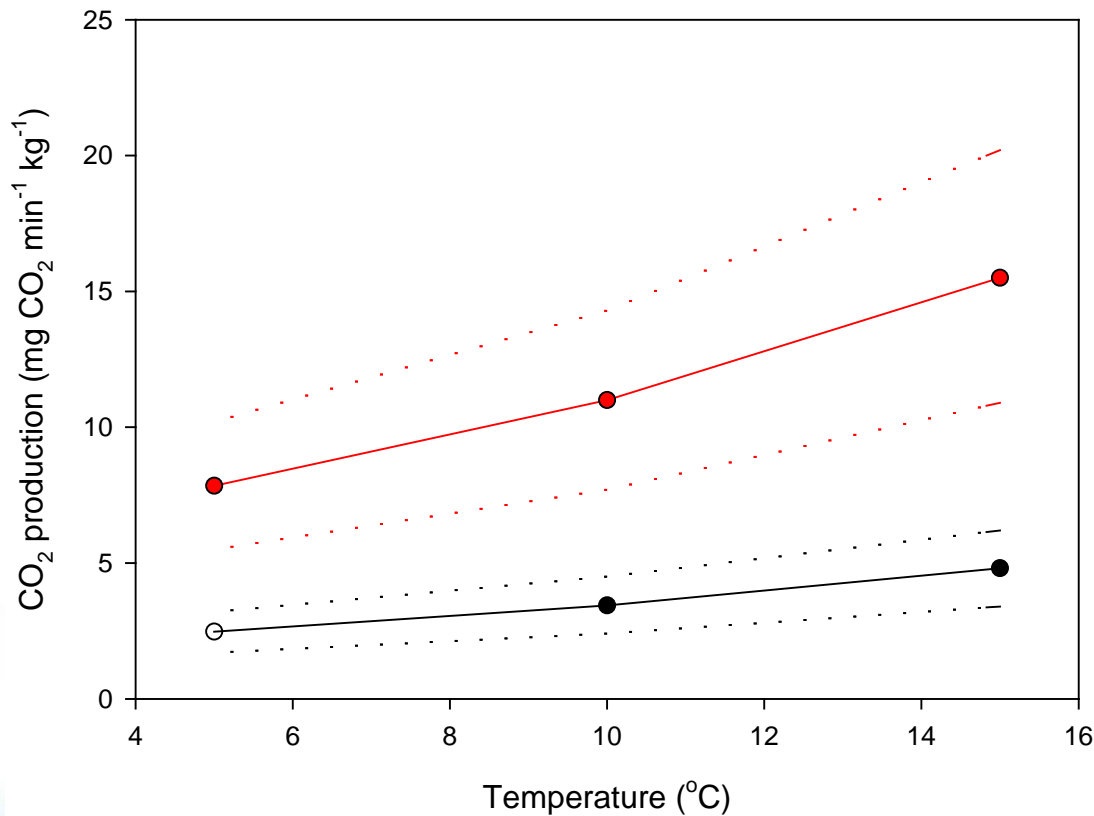
$$TCO_{2ac} = \frac{(\dot{M}O_2 \times RER \times mass) \times t}{V_r} \tag{3}$$

$\dot{M}O_2$  O<sub>2</sub> consumption rate (input as mg O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>, converted to mmol O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>)  
 RER respiratory exchange ratio (moles CO<sub>2</sub> excreted per moles O<sub>2</sub> consumed), RER can vary between 0.7 and 1.3, increasing with activity level and in relation to the tissue level O<sub>2</sub> consumption and CO<sub>2</sub> production ratio, or respiratory quotient (RQ). An RER of 1.0 was used in the present modeling, but this can be varied.  
 mass total fish mass per hold (kg)

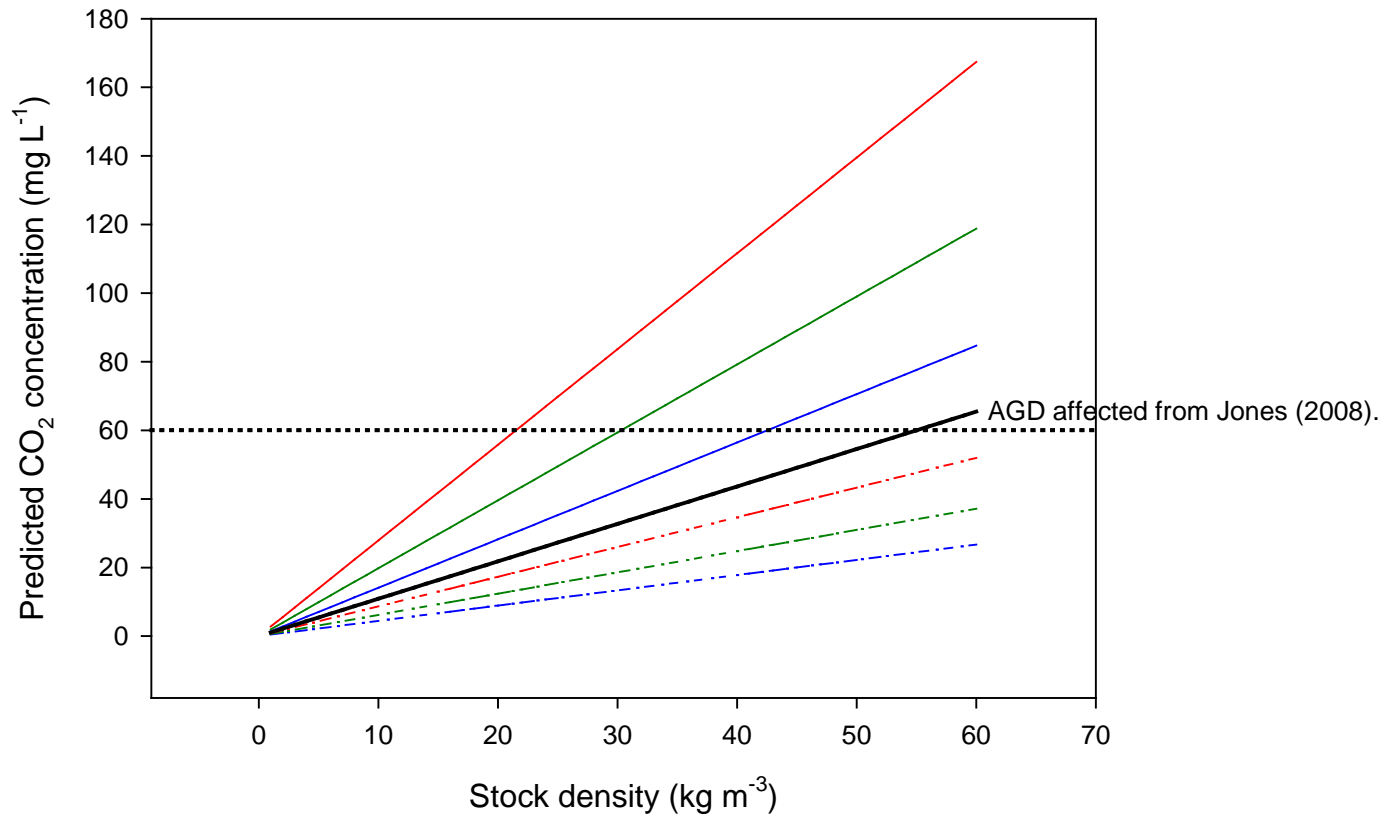


**Fig. 3.** Modeled changes in water quality during various closed-hold transport scenarios. Simulated  $P_{CO_2}$  accumulation at routine  $\dot{M}O_2$  (2.5 mg O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>, solid lines) and maximum  $\dot{M}O_2$  (8.0 mg O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>, dashed lines) across a range of loading densities; low density (70 kg m<sup>-3</sup>), medium density (120 kg m<sup>-3</sup>) and high density (170 kg m<sup>-3</sup>). (A) Under low stress conditions, low loading density allows a re-circulation time of up to 150 min while at a high density this time is reduced to 56 min. (B) Under high stress conditions, low loading density levels allow re-circulation time of 48 min while high loading densities further reduce the time to reach the 10 mmHg CO<sub>2</sub> threshold to 19 min. The associated water pH changes at routine  $\dot{M}O_2$  (C) and maximum  $\dot{M}O_2$  (D) at low, medium and high loading densities show that water pH decreases with increasing water  $P_{CO_2}$ . The arbitrary threshold  $P_{CO_2}$  of 10 mmHg is reached at a water pH of 6.6 (dotted lines) under all scenarios. All trips were modeled using the same water conditions: temperature = 10 °C, salinity = 30 ppt, starting water pH = 7.8.

# CO<sub>2</sub> prediction of salmon at different temperatures

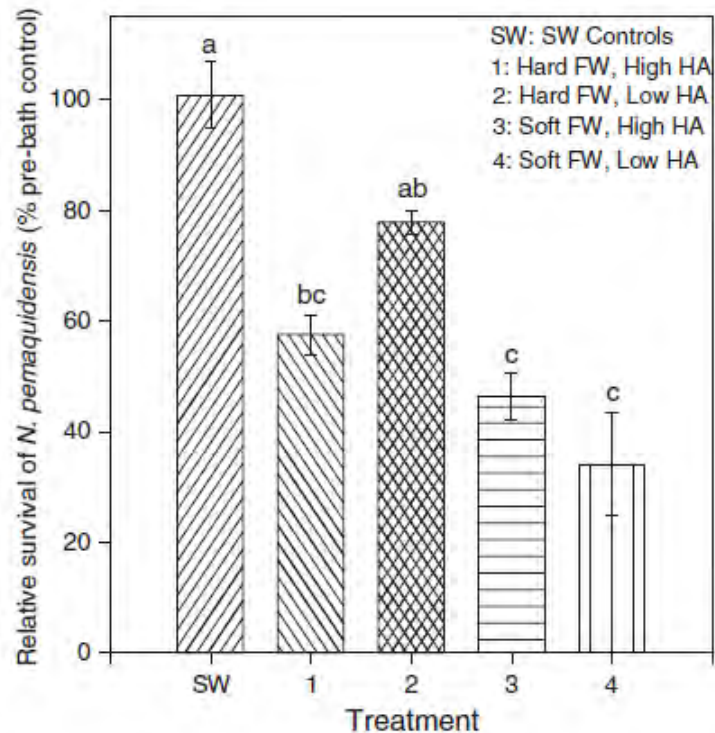


# CO<sub>2</sub> predictions for salmon at different stocking densities (12C)

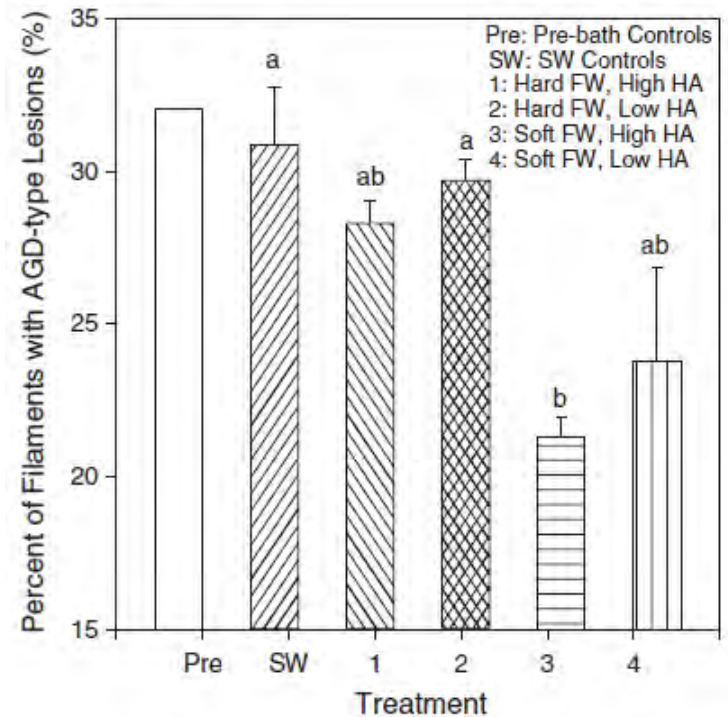


Source: NIVA report 2015  
Behandling av amøbegjellesykdom i brønnbåt

# Effects of DOC and water hardness on AGD bathing efficacy



**Figure 3** The percentage of viable amoebae present on the gills of Atlantic salmon bathed in freshwater for 2.5 h with varying levels of dissolved organic carbon and total water hardness when compared with pre-bath numbers (mean  $\pm$  SE). Different letters indicate significant differences between treatments ( $P > 0.05$ ).

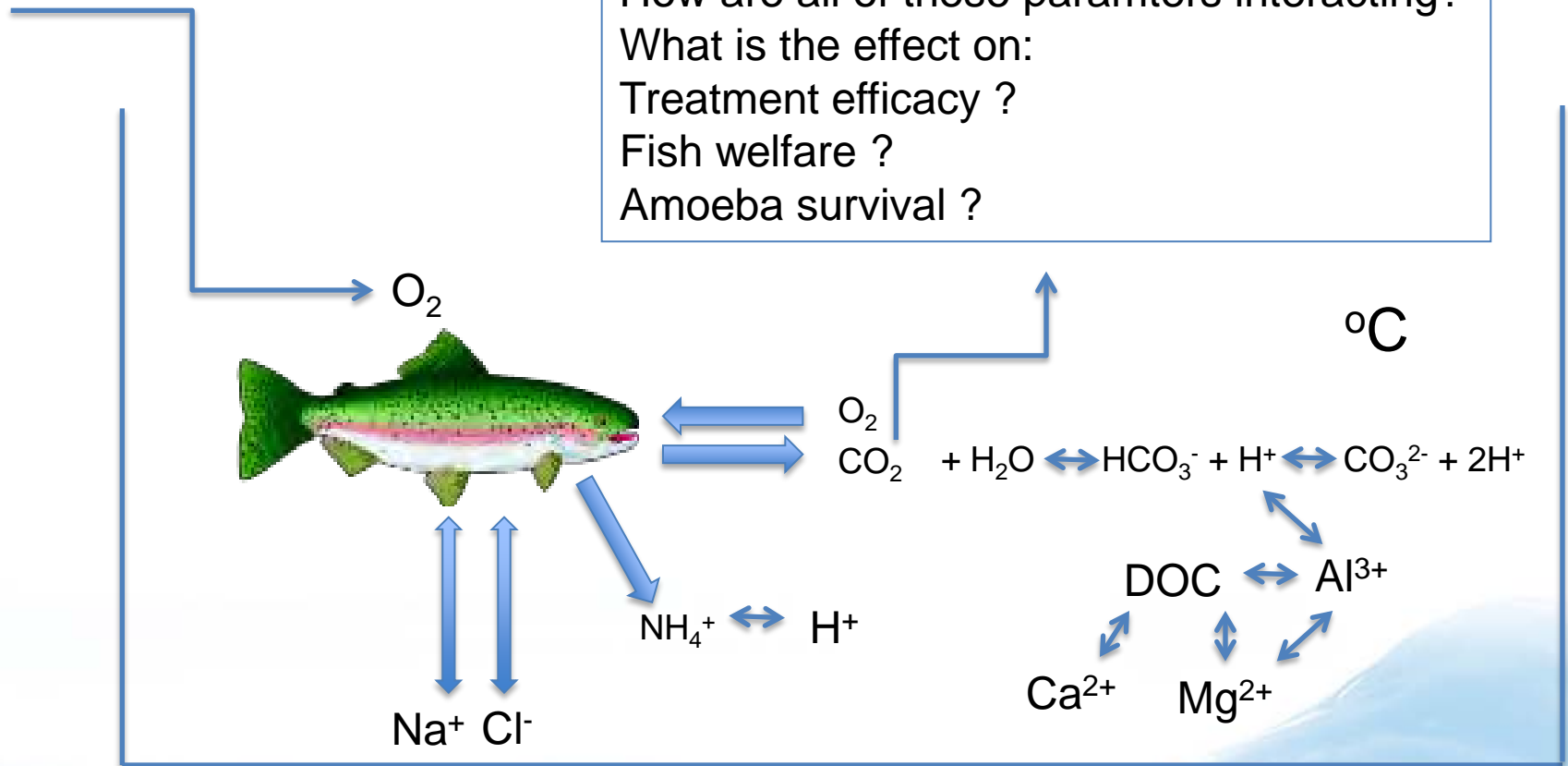


**Figure 4** The percent of gill filaments with ameobic gill disease (AGD)-type lesions post bathe (mean  $\pm$  SE). Different letters indicate significant differences between treatments ( $P > 0.05$ ).



# Simplified illustration of water chemistry interactions during freshwater bathing

How are all of these parameters interacting?  
What is the effect on:  
Treatment efficacy ?  
Fish welfare ?  
Amoeba survival ?



# Efficacy of chemical additives to freshwater baths



Chlorine dioxide and chloramine-T

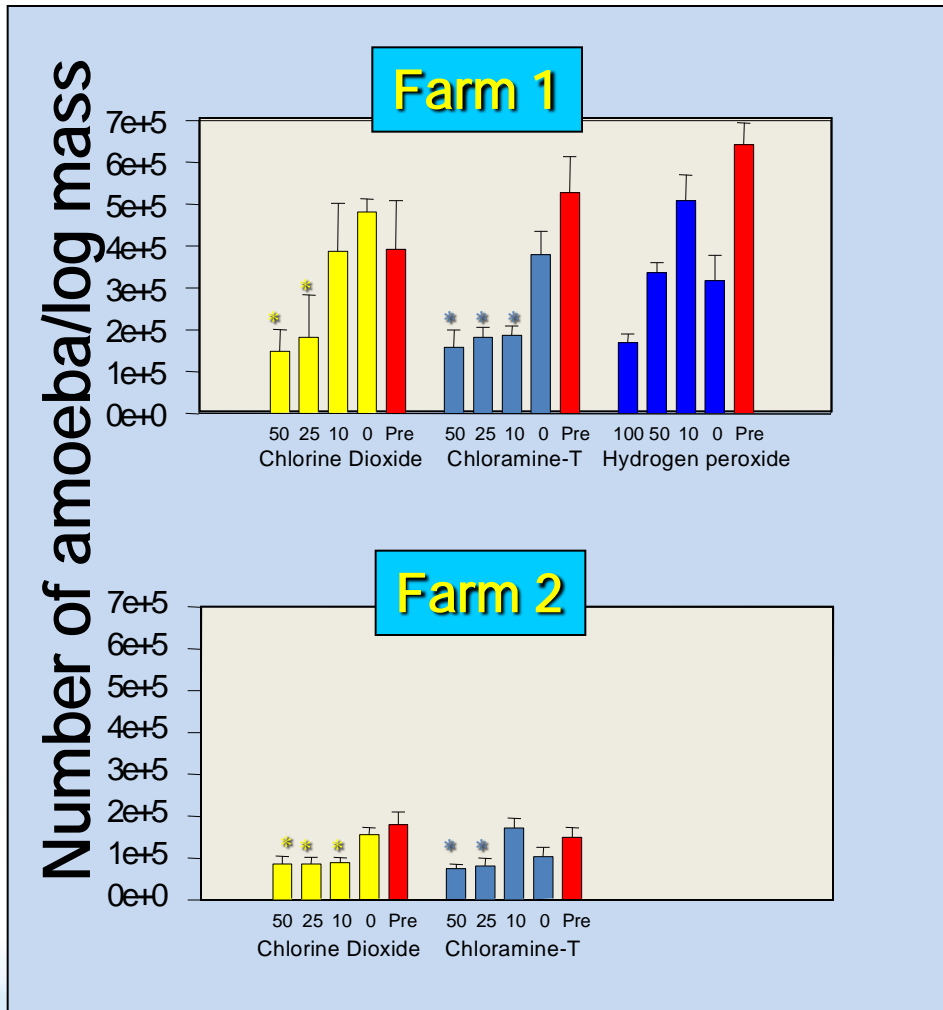
- Significantly reduce gill amoeba numbers

Hydrogen peroxide < 100 mg l<sup>-1</sup>

- Results were equivocal

Interactive effects:

- DOC, water chemistry?
- Reactive oxygen based agents are active through the generation of toxic oxy radicals
- The generation of oxy radicals depends in reactive metal concentrations and organic compounds to enhance breakdown



# Key points for consideration

- Water availability
- Water composition and chemistry
- Effects of fish metabolism
- Additives to freshwater
- Alternatives to freshwater
- Alternatives to bathing

# Thank you for your attention

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