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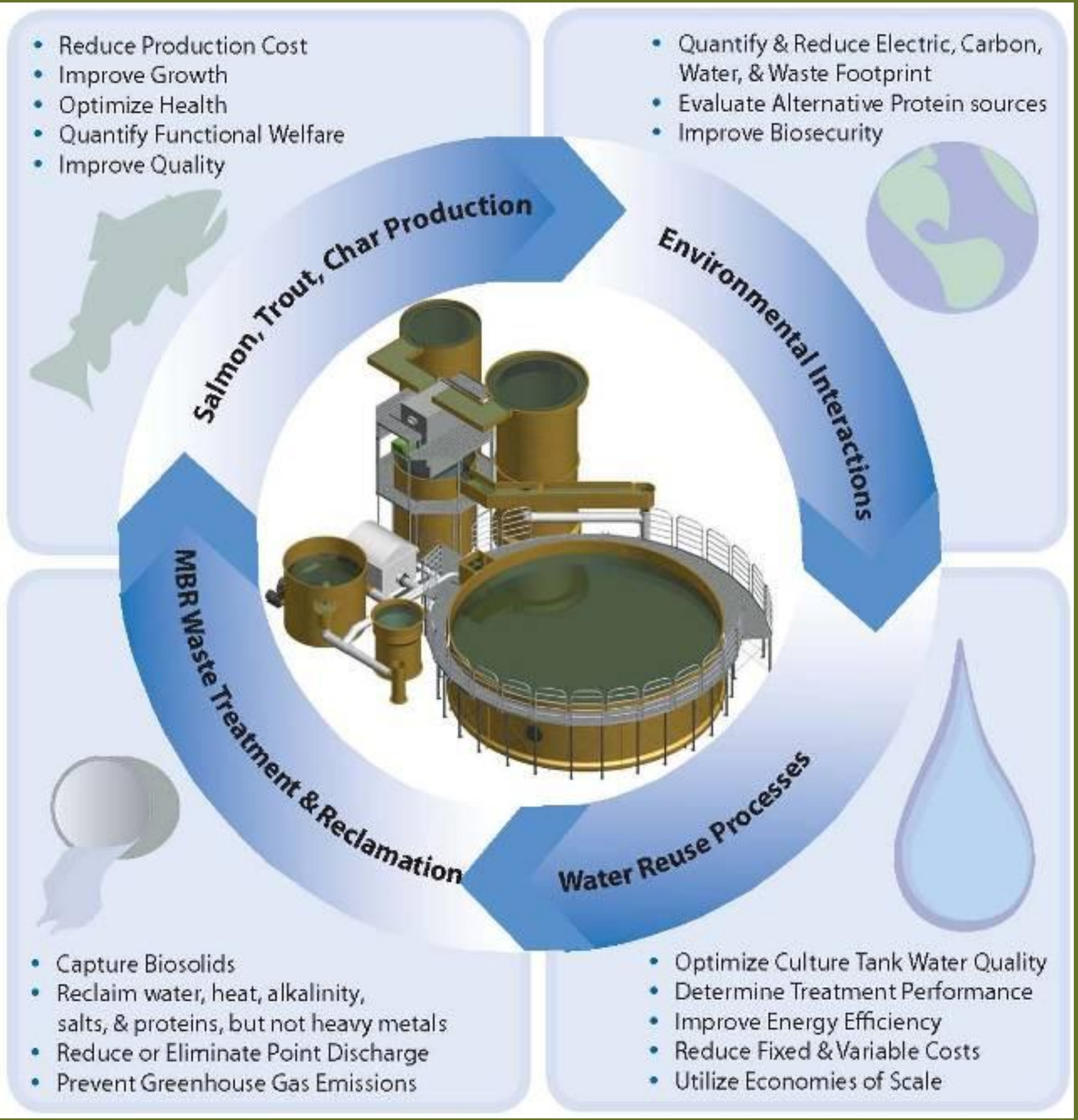
Studies on long-term carbon dioxide exposure during salmonid growout in water recirculation aquaculture systems

Good C, Davidson J, Snekvik K, Takle H, Terjesen B, & Summerfelt S

Aquaculture Innovation Workshop
Comox, BC, Nov 5-6 2012



Research at The Freshwater Institute

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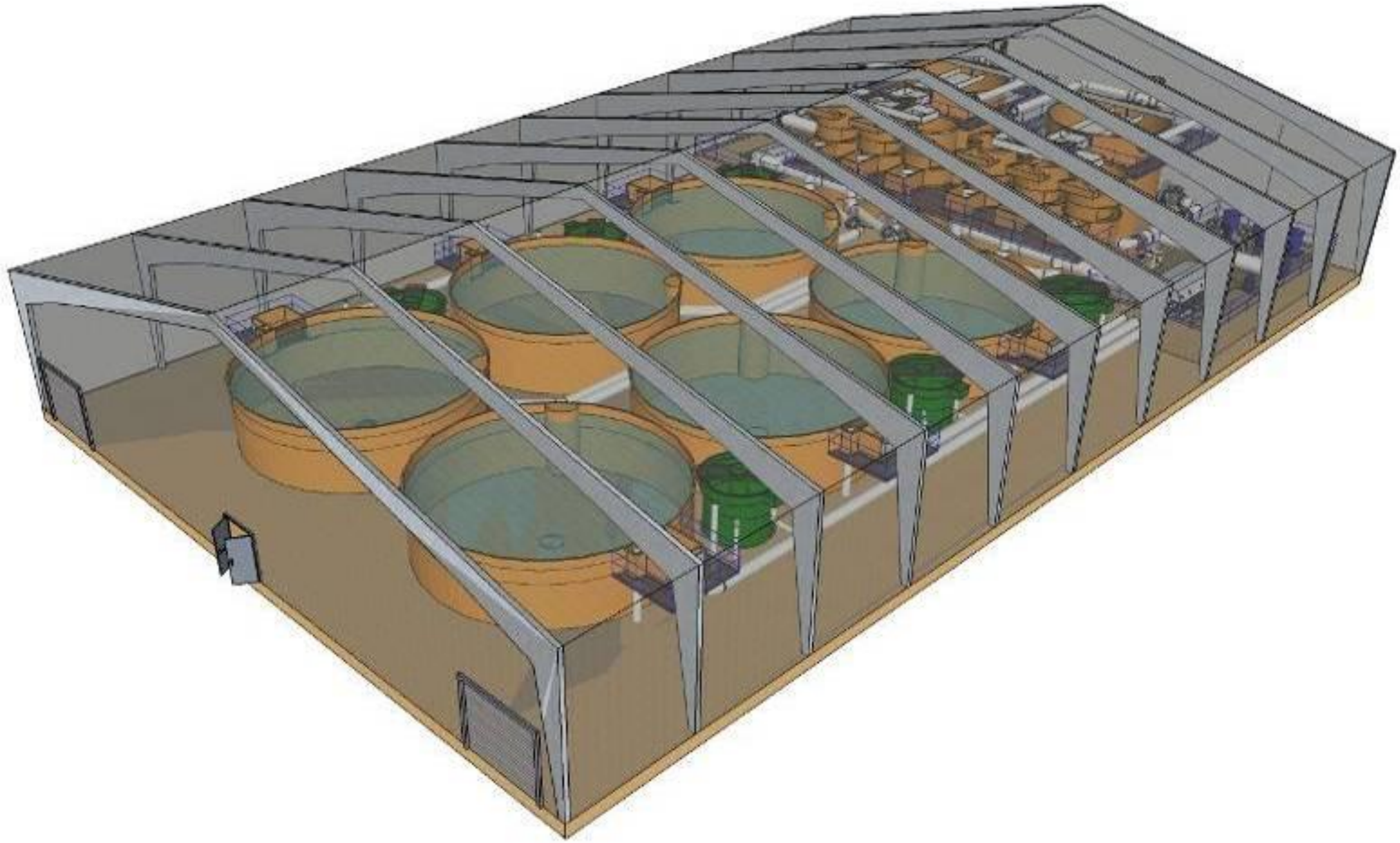
Closed Containment Facilities with Water Recirculation



Background: Dissolved CO_2

- Inverse relationship of CO_2 with pH
- Long-term exposure to elevated CO_2
  - Decreased hemoglobin oxygen binding capacity (Bohr effect)
    - Increased ventilation, elevated blood pressure
  - Reduced growth rate
  - Higher FCR
  - Nephrocalcinosis
- Also, increased solubility of toxic metals at lower water pH

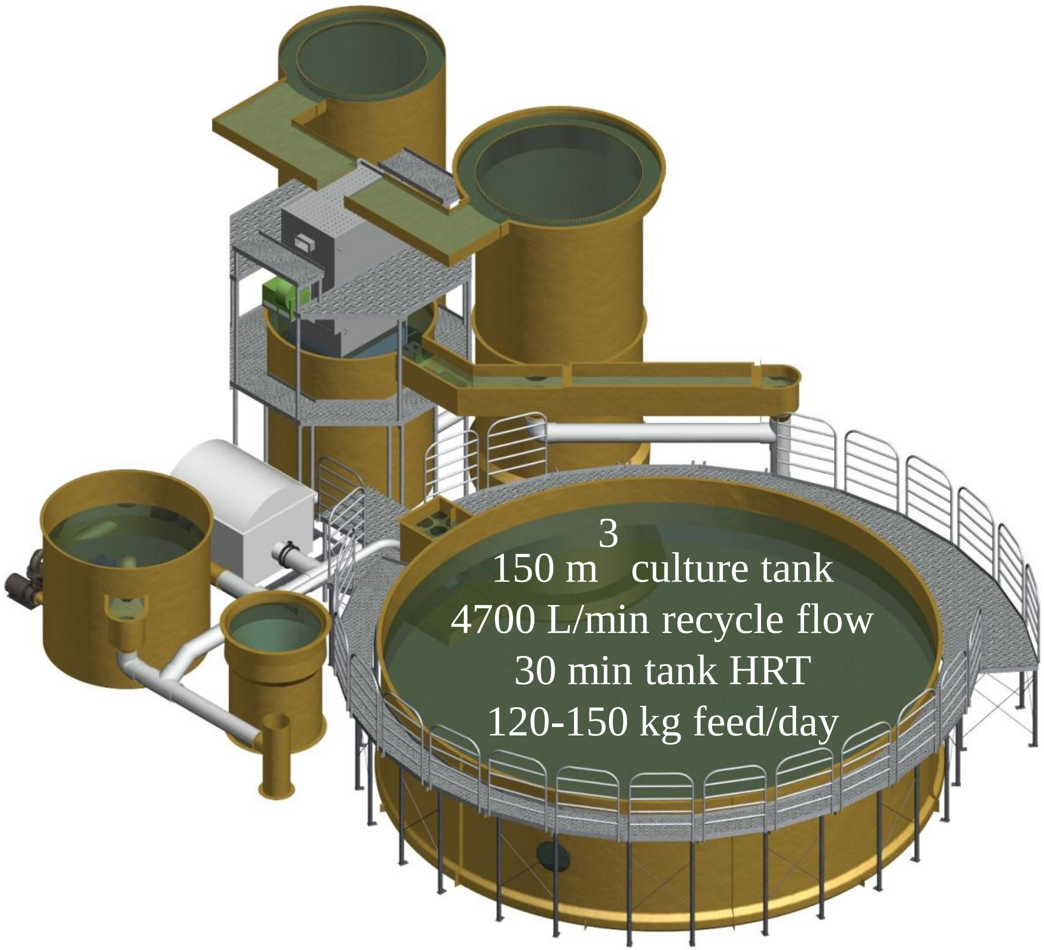
Background: Dissolved CO_2

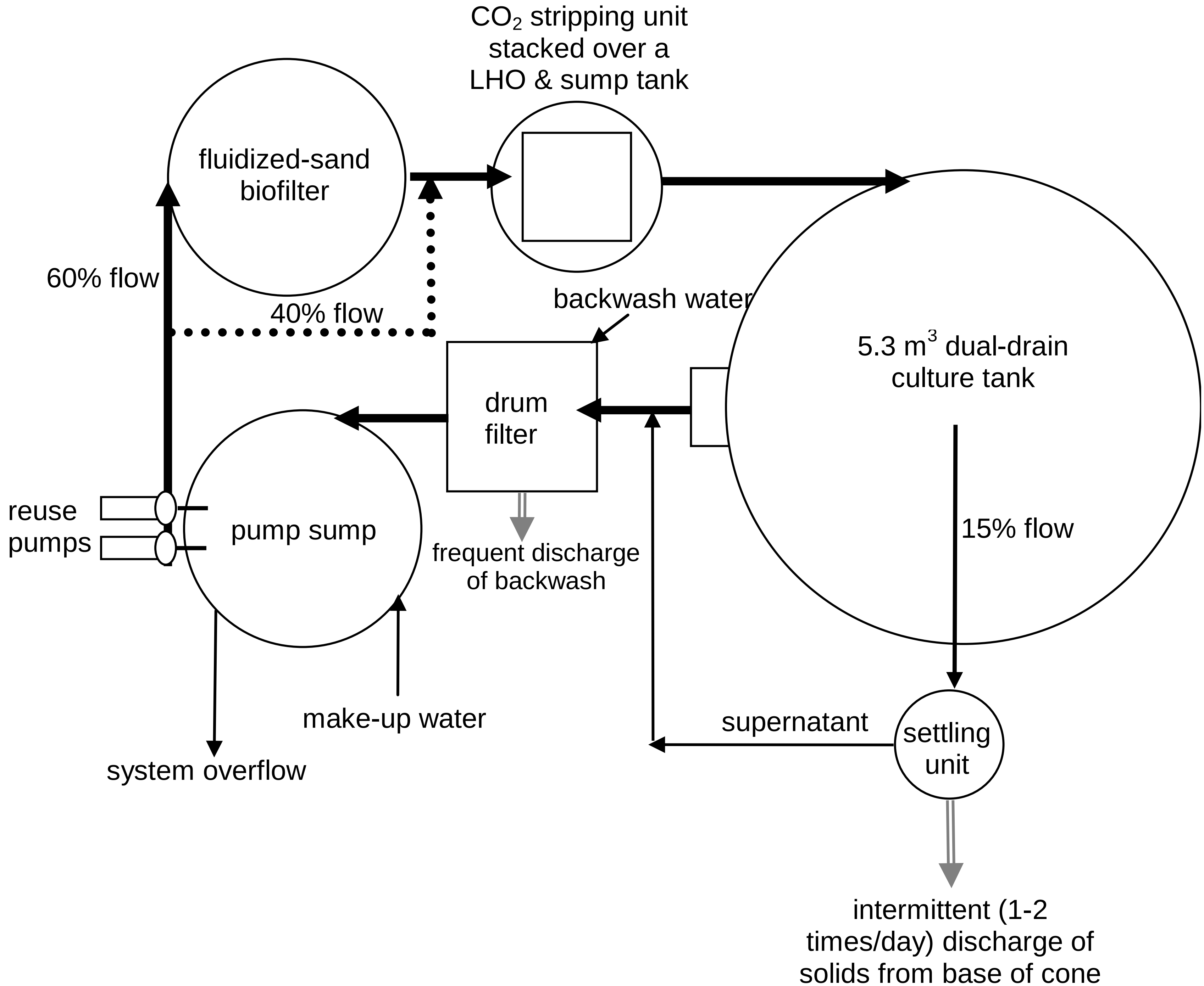
Aside from fish health implications….

Economic Considerations

- Decreasing tank CO_2 concentrations requires pumping more water flow & installing a larger stripping unit (cascade column or aerated basin)
  - increases fixed costs
  - increases variable costs to pump water

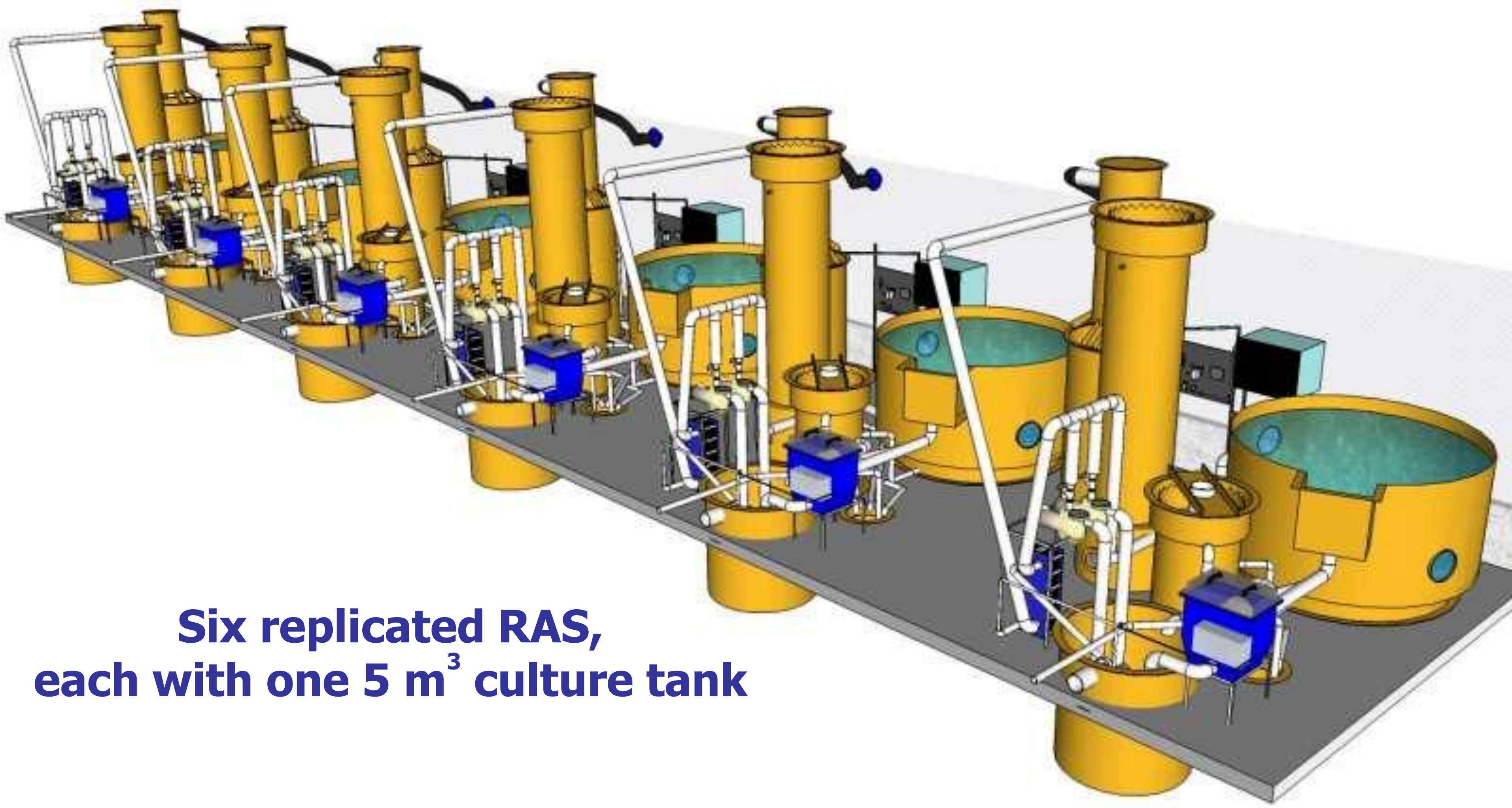
Main System – Freshwater Institute



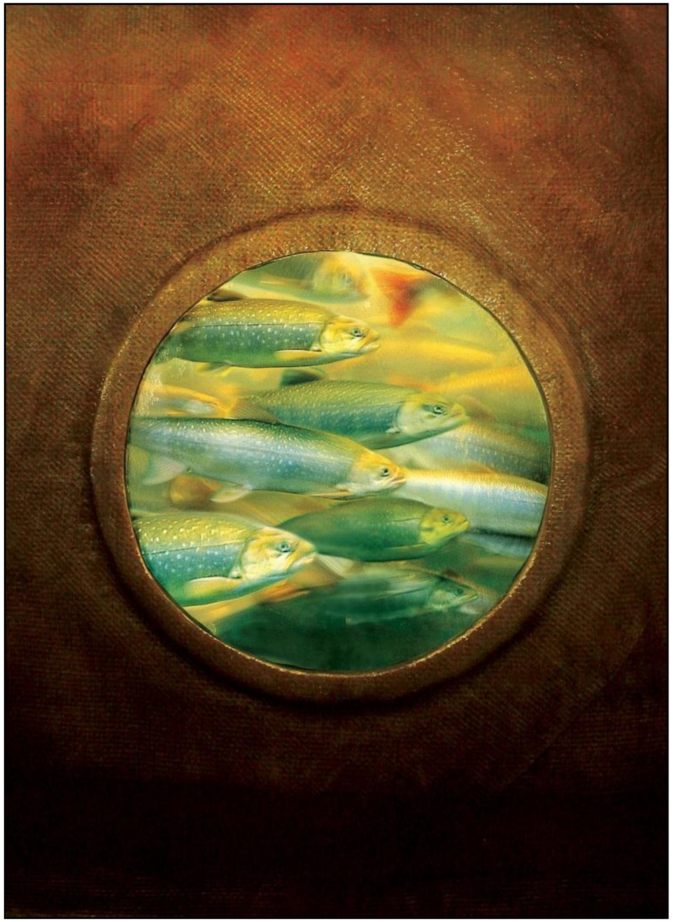


CO2 stripping unit stacked over a LHO & sump tank
fluidized-sand biofilter
60% flow
40% flow
backwash water
5.3 m3 dual-drain culture tank
drum filter
reuse pumps
pump sump
frequent discharge of backwash
15% flow
make-up water
system overflow
supernatant
settling unit
intermittent (1-2 times/day) discharge of solids from base of cone

Replicated RAS



Previous Observations at The Freshwater Institute



- During **low flushing** (1-2% water exchange), and **high feeding** (1.3-2.0 kg/d per m^3/d makeup flow):
  - Elevated morbidity and mortality
  - Unknown causation
  - No infectious causes suspected
  - Water quality parameters within safe limits

First 6-month Study

High Makeup H_2O Exchange (2.6%)

Low Makeup H_2O Exchange (0.26%)

Dissolved Carbon Dioxide?

Greater **tank hydraulic retention time** in main system: approximately **20 mg/L** CO_2, versus approximately **10 mg/L** CO_2 in 6 RAS

Study 1 – Rainbow Trout

- Investigate the effects of high vs. low dissolved carbon dioxide on rainbow trout reared in high feeding, low flushing RAS:
  - Growth
  - Survival
  - Histopathology
- Assess treatment effects on water quality parameters

Materials and Methods

Recirculating Aquaculture Systems – CO_2 added to LHO

Materials and Methods

Small Flow-Through Comparison Tanks (3)

Materials and Methods

- 5100 rainbow trout raised for 6 months
  - Stocked at 62 ± 1 grams in size
  - 800 per RAS plus 100 per flow-through
- 40(min) - 80(max) kg/m^3 density
- 3 RAS with high CO_2 (**25 mg/L**)
- 3 RAS with low CO_2 (**10 mg/L**)
- All 6 RAS with low (0.26%) exchange, high feed loading (4.1 kg/day per m^3/day makeup flow)

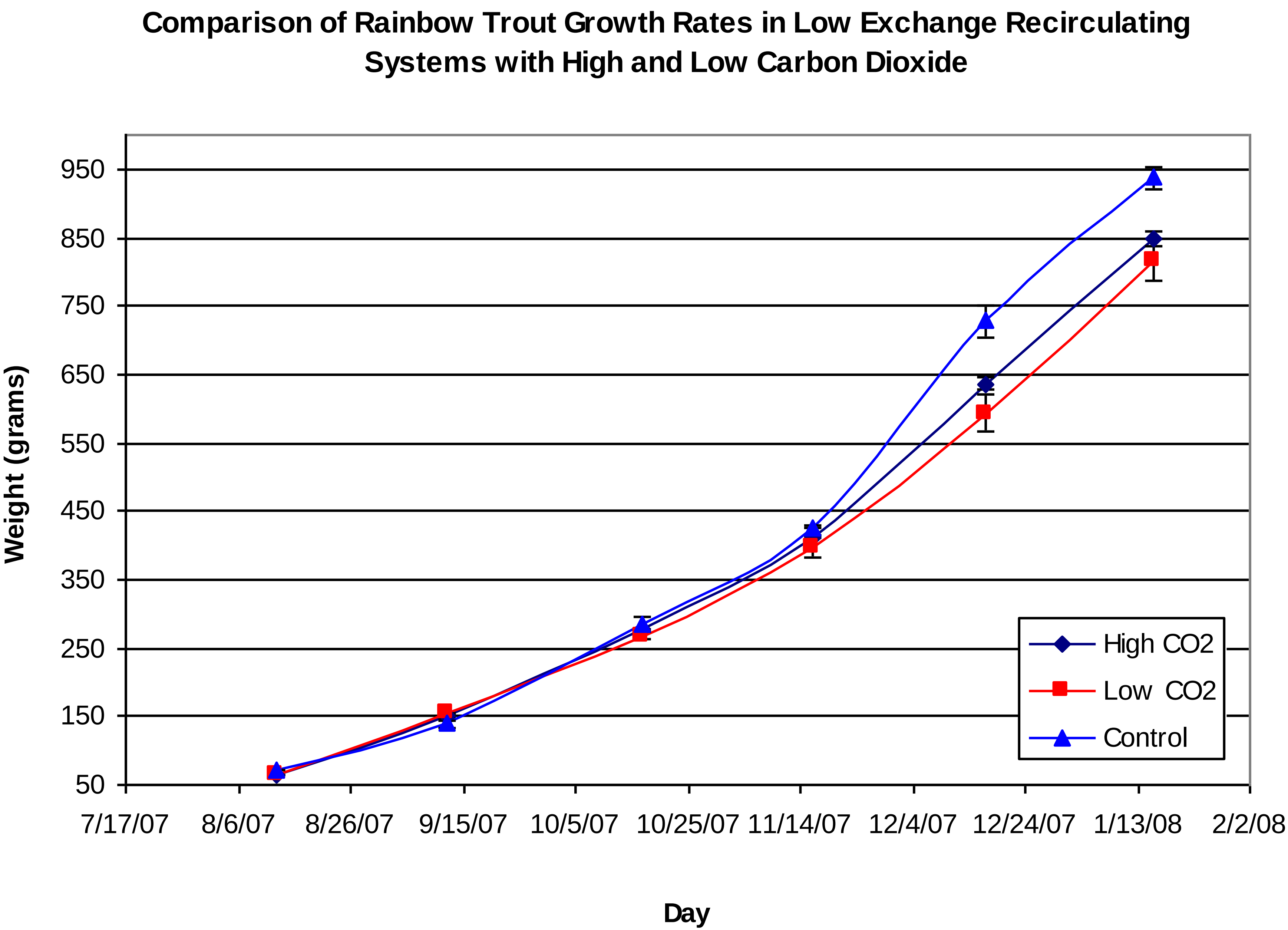
Data Collection

- Monthly length/weight sampling
- Daily mortalities
- Tissue sampling at study's end for histopathological evaluation (5 fish per tank)
  - Skin, gill, heart, liver, spleen, swim bladder, anterior and posterior kidney
- Water quality sampling 2-3 times/week

Water Quality

Parameters	High CO_2	Low CO_2
TAN (mg/L)	0.35 ± 0.01	0.35 ± 0.02
Unionized Ammonia (mg/L) *	0.001 ± 0.000	0.004 ± 0.000
Nitrite Nitrogen (mg/L)	0.054 ± 0.013	0.051 ± 0.008
Nitrate Nitrogen (mg/L)	46 ± 0	50 ± 2
Alkalinity (mg/L)	202 ± 2	202 ± 2
CO_2 (mg/L) *	24 ± 1	8 ± 1
$cBOD_5$ (mg/L)	5 ± 0	6 ± 1
True Color (Pt-Co units)	42 ± 1	48 ± 3
UV Transmittance (%)	70 ± 0	67 ± 1
TSS (mg/L)	7.99 ± 0.37	9.21 ± 1.30
Total Particles (0-60 µm)	1.8×10^4	2.3×10^4
Temperature (^oC)	13.9 ± 0.1	13.7 ± 0.1
pH *	7.17 ± 0.01	7.61 ± 0.03
DO (mg/L)	10.1 ± 0.0	10.1 ± 0.0

* Significant difference $p<0.05$ between high and low CO2 groups


Comparison of Rainbow Trout Growth Rates in Low Exchange Recirculating Systems with High and Low Carbon Dioxide
Weight (grams)
950
850
750
650
550
450
350
250
150
50
7/17/07
8/6/07
8/26/07
9/15/07
10/5/07
10/25/07
11/14/07
12/4/07
12/24/07
1/13/08
2/2/08
Day
High CO2
Low CO2
Control

Rainbow trout

Mean Final Weight:

High CO_2 : **849 ± 7 g**

Low CO_2 : **817 ± 43 g**

Mean TGC:

High CO_2 : **2.56 ± 15**

Low CO_2 : **2.55 ± 12**

Mean FCR:

High CO_2 : **1.25 ± 0.10**

Low CO_2 : **1.25 ± 0.05**

No significant ($p<0.05$) differences between high and low CO_2 groups

Rainbow trout

SURVIVAL

- High CO_2 = **98.3%** ± 0.2
- Low CO_2 = **96.5%** ± 1.8

No nephrocalcinosis diagnosed in either cohort

Study 2 – Atlantic salmon

- Effects of <u>high</u> (**20 mg/L**) & <u>low</u> (**10 mg/L**) dissolved carbon dioxide concentrations on Atlantic salmon performance, health, and welfare during **<u>growout</u>** in <u>freshwater</u> RAS

Atlantic salmon - Study Objectives

- Investigate the effects of high vs. low dissolved carbon dioxide on Atlantic salmon in moderately low flushing RAS:
  - Growth
  - Survival
  - Histopathology
  - Blood gas / chemistry assessments
  - Gill enzyme regulation

Materials and Methods

- 5400 Atlantic salmon smolts raised to market size (~24 months post-hatch)
  - Stocked at 200 grams in size, 900 fish per RAS
- 40(min) - 80(max) kg/m^3 density
- 3 RAS with high CO_2 (20 mg/L)
- 3 RAS with low CO_2 (10 mg/L)
- All 6 RAS with 1.0% exchange
- Feed loading 0.73 kg/d per m^3/d makeup flow

Materials and Methods

- Bi-monthly length/weight sampling
- Daily mortalities
- Gill tissue collected at 1- and 3-weeks post-stocking
- Kidney tissue collected every two months for histopathology; multi-organ final sampling
- Water quality sampling 2-3 times/week

Water Quality

Parameters	High CO_2	Low CO_2
TAN (mg/L)	0.16 ± 0.01	0.16 ± 0.02
Nitrite Nitrogen (mg/L)	0.075 ± 0.013	0.080 ± 0.016
Nitrate Nitrogen (mg/L)	12 ± 0	12 ± 0
Alkalinity (mg/L)	233 ± 2	241 ± 2
CO_2 (mg/L)	20 ± 1	9 ± 1
True Color (Pt-Co units)	11 ± 1	12 ± 3
UV Transmittance (%)	89 ± 0	88 ± 1
TSS (mg/L)	0.85 ± 0.02	1.06 ± 0.07
pH	7.41 ± 0.01	7.86 ± 0.03
DO (mg/L)	10.1 ± 0.0	10.1 ± 0.0

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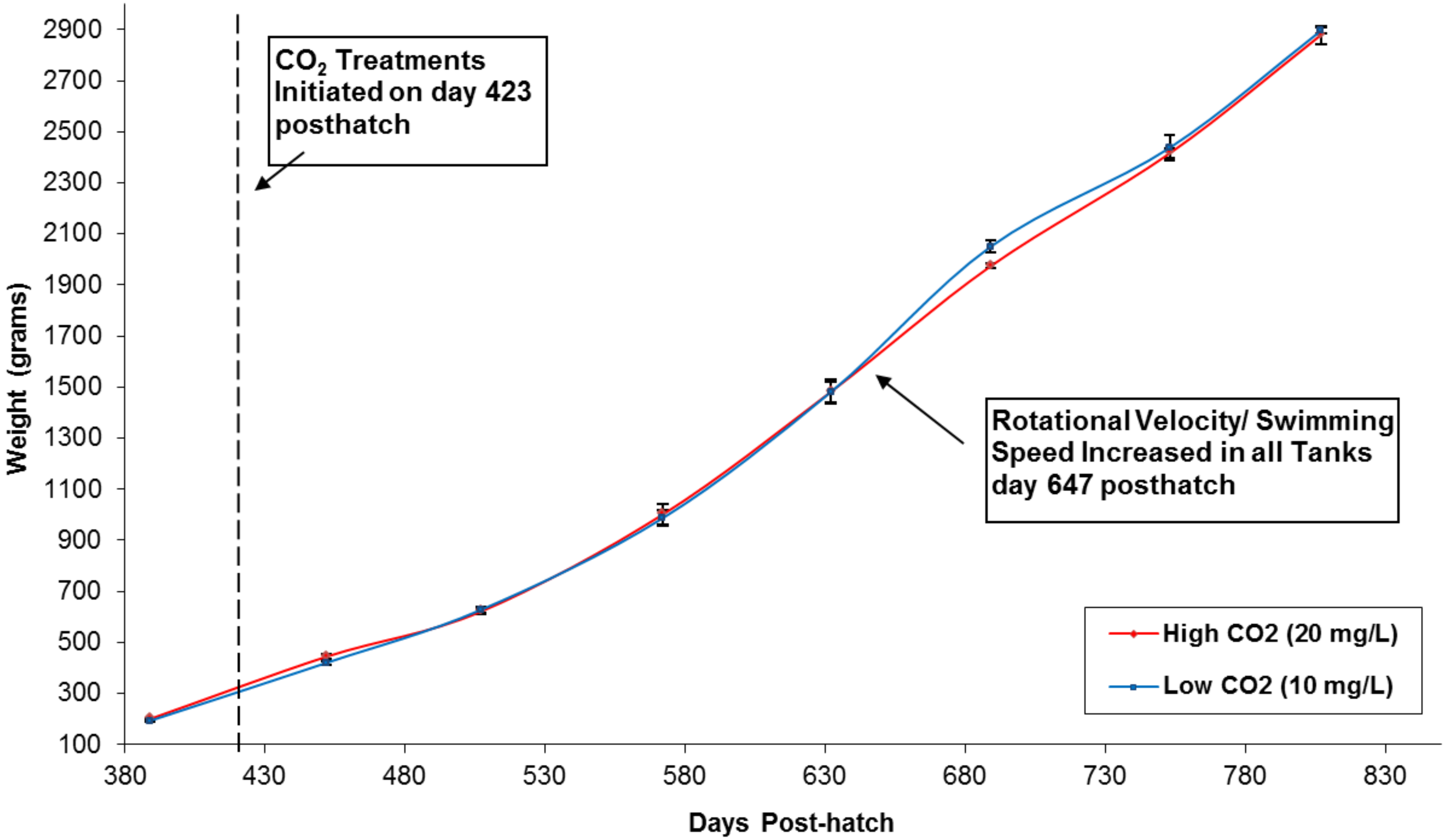
Performance - Growth

Final weight (g) @ 807 days post-hatch

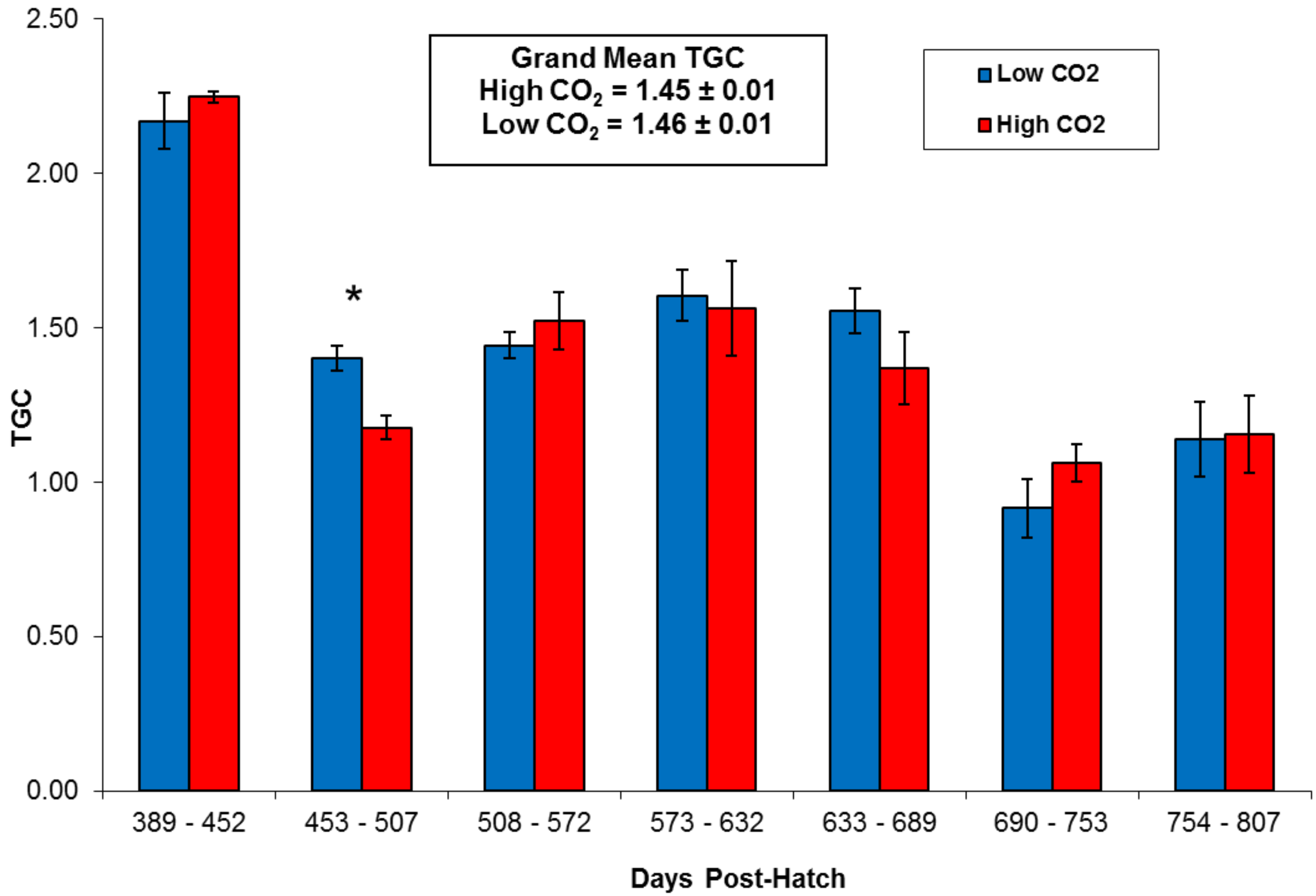
High CO_2: **2879** ± 35

Low CO_2: **2896** ± 12

Equal salmon growth at 10 and 20 mg/L of CO_2



Performance - TGC



Survival

Mean survival (%)

High CO_2: 99.2 ± 0.3

Low CO_2: 99.1 ± 0.3

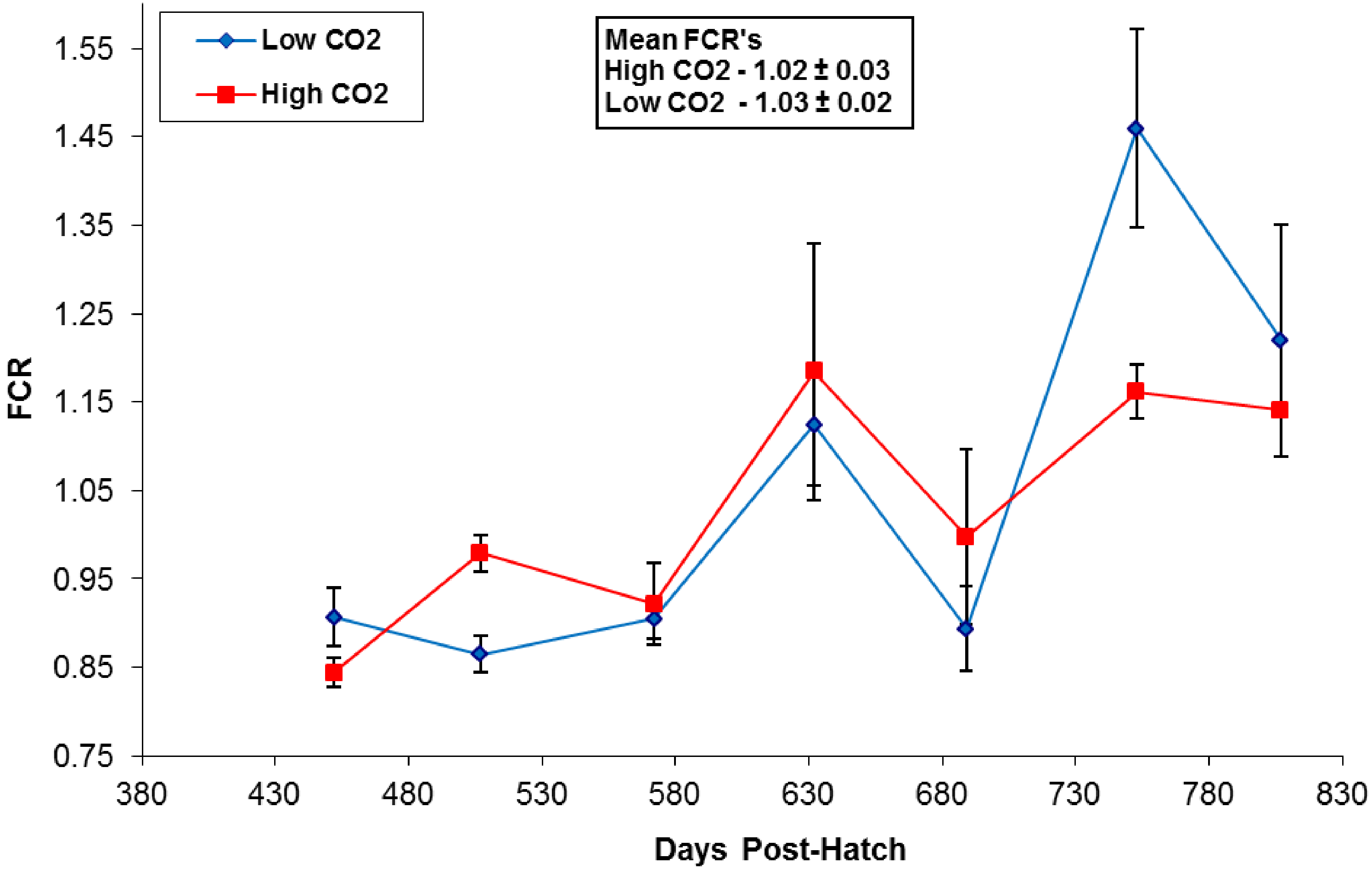
Culls due to fungus (%)

High CO_2: 3.75 ± 1.05

Low CO_2: 3.41 ± 1.27

* approx. 3400 lbs of salt added to each system during study to control fungus

Feed Conversion

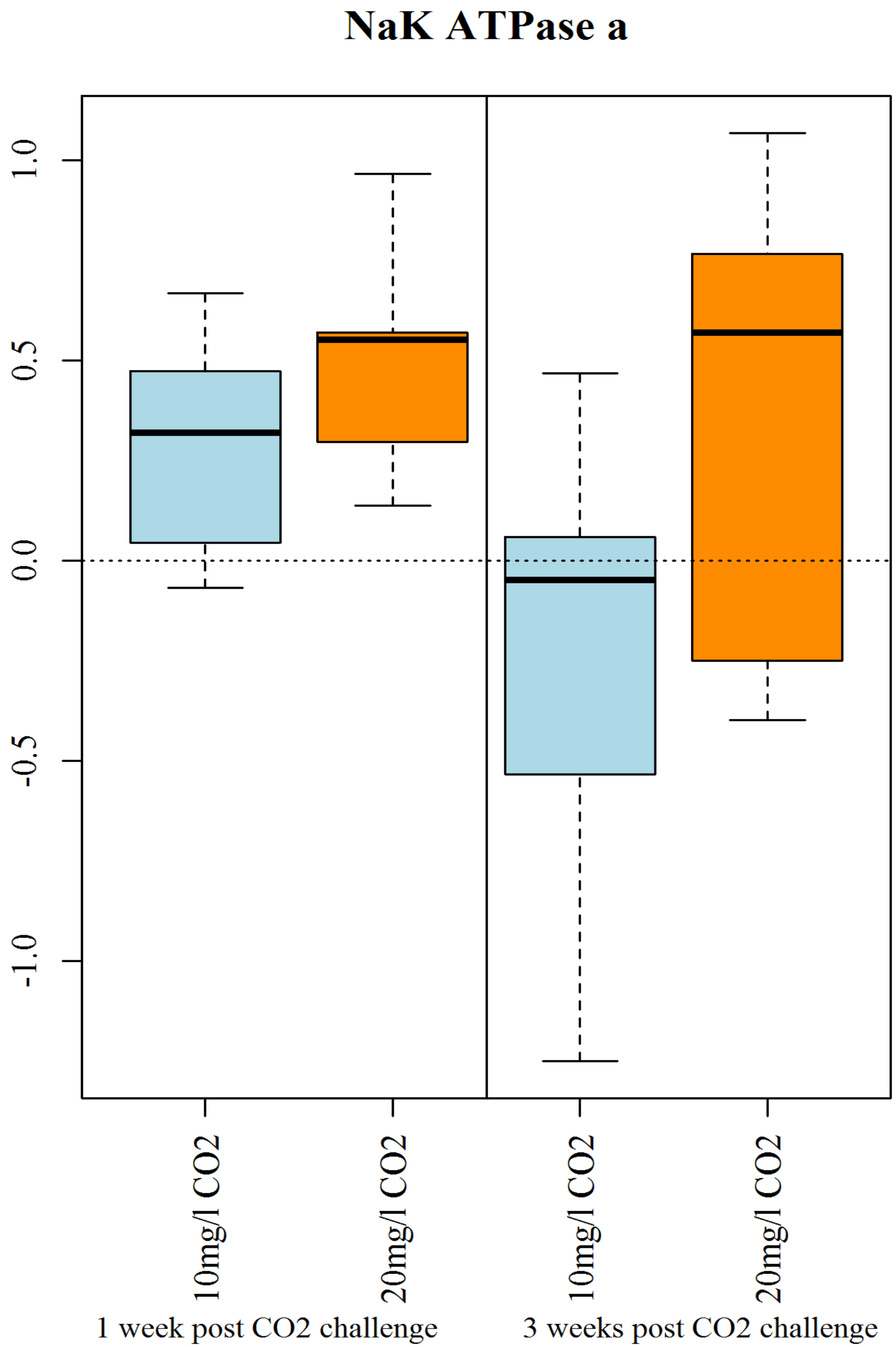


Whole blood analysis

Parameter	CO_2	Mean ± SE	p-value
Chloride (mmol/L)	High	127 ± 0.35	0.002
	Low	132 ± 0.89	
pH	High	7.11 ± 0.01	0.030
	Low	7.06 ± 0.01	
pCO_2 (mmHg)	High	61.8 ± 1.13	0.005
	Low	48.7 ± 1.17	
Bicarbonate (mmol/L)	High	19.6 ± 0.26	<0.001
	Low	13.7 ± 0.26	
Total CO_2 (mmol/L)	High	21.4 ± 0.27	<0.001
	Low	15.2 ± 0.34	

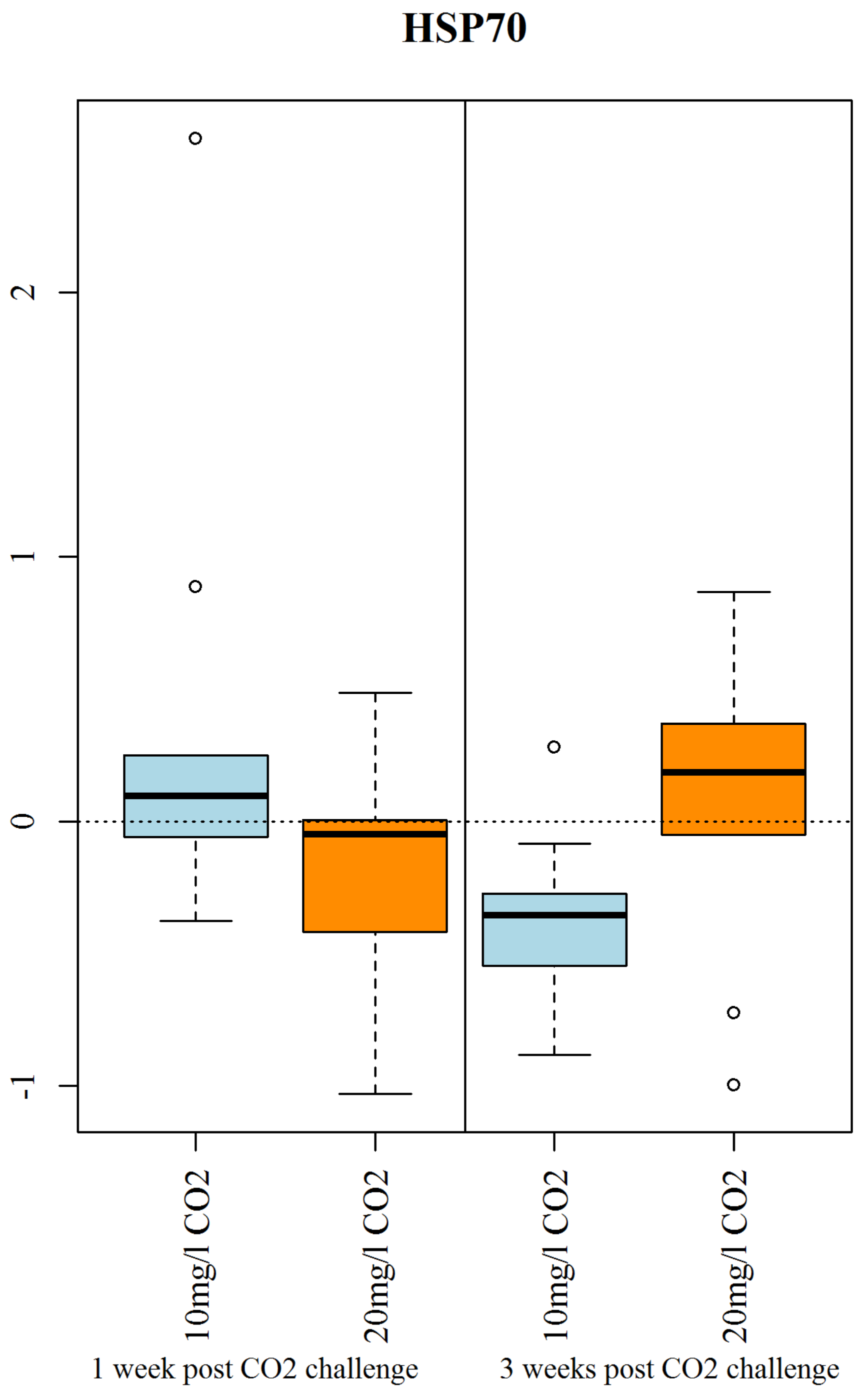
Histopathology

- Very few lesion types observed at any sampling point
- No nephrocalcinosis observed in any kidney samples
- Renal interstitial nephritis more prevalent in the high CO_2 group, particularly at one week post-stocking



ANOVA p-values

	20 mg/L CO_2 1-wk	20 mg/L CO_2 3-wks	10 mg/L CO_2 1-wk
20 mg/L CO_2 3-wks	0.93	-	-
10 mg/L CO_2 1-wk	0.82	0.93	-
10 mg/L CO_2 3-wks	0.00	0.02	0.04



ANOVA p-values

	$20\ CO_2$ 1w	$20\ CO_2$ 3w	$10\ CO_2$ 1w
$20\ CO_2$ 3w	0.99	-	-
$10\ CO_2$ 1w	0.29	0.99	-
$10\ CO_2$ 3w	0.99	0.60	0.12

Conclusions

- Salmon performance unaffected by 20 mg/L carbon dioxide, with oxygen at saturation and alkalinity >200 mg/L
- Compensatory physiological and haematological differences noted between groups
- No nephrocalcinosis observed

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Acknowledgements

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- Opinions, conclusions, and recommendations are of the authors and do not necessarily reflect the view of the USDA.

- All experimental protocols were in compliance with Animal Welfare Act (9CFR) and have been approved by the Freshwater Institute Animal Care and Use Committee.

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