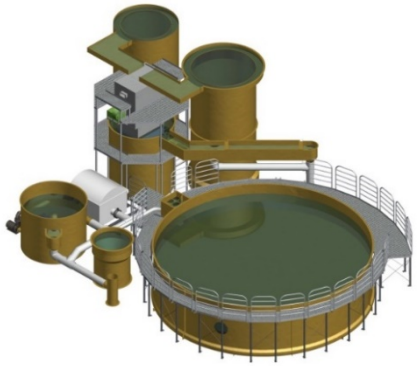
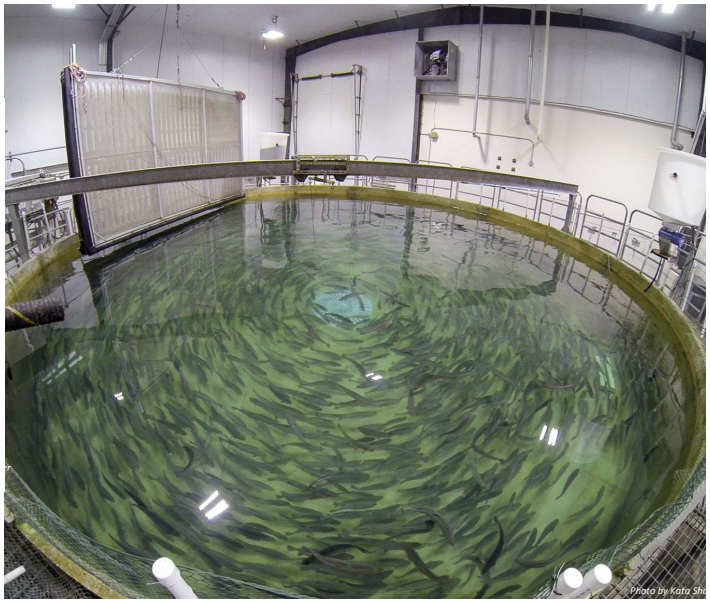
Evaluating the Effects of Ozone on Post-Smolt Atlantic Salmon Growth, Performance, and Maturation in Freshwater Recirculation Aquaculture Systems





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Background – Early Maturation in RAS

- Early maturation is a common problem in land-based RAS
- At the Freshwater Institute we've documented 17 - 50% + Atlantic salmon maturation prior to market size (4-5 kg)
  - Majority of maturing fish are precocious males
- Fillets are generally undesirable due to pale color and reduced quality

Background – Early Maturation

- Atlantic salmon maturation is a highly flexible process
- Many factors involved:
  - Photoperiod
  - Water temperature
  - Growth rate/ condition factor
  - Genetics
  - Water quality
  - Exercise
  - Endocrine disruptors
  - Hormone signaling

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A Review of Factors Influencing Maturation of Atlantic Salmon, *Salmo salar*, with Focus on Water Recirculation Aquaculture System Environments

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Hormone Accumulation in RAS

- Mota et al., 2014 found that sex steroid concentrations accumulate in RAS
- TCFFI followed by evaluating whether ozone could reduce waterborne hormones in RAS water
  - Ozone significantly reduced estradiol levels
  - Testosterone and 11-KT were generally lower in ozonated RAS

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The effects of ozonation on select waterborne steroid hormones in recirculation aquaculture systems containing sexually mature Atlantic salmon *Salmo salar*

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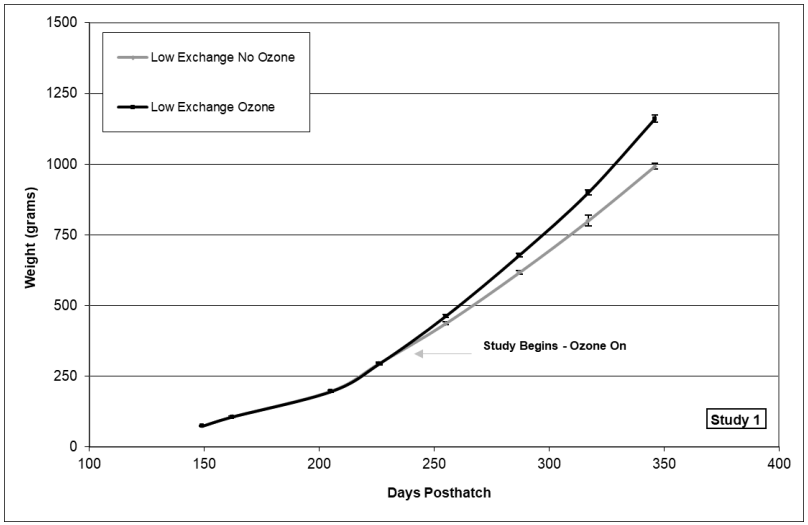
- Ozone can dramatically improve water quality
  - Color
  - UV Transmittance
  - Total Suspended Solids
  - Fine Particles
  - Dissolved Metals
  - Waterborne Hormones

- Ozonation can result in improved growth performance



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- *Can ozone reduce or eliminate early maturation?*

6 Replicate Experimental Scale RAS

Experimental Design

3 RAS Operated with Ozone

3 RAS Operated without Ozone

Fish Stocking

500 post-smolt salmon/RAS (296 ± 4 g to begin)

Photoperiod

12 hr lights on: 12 hr lights dimmed to 5%

Feeding

Around-the-clock, EWOS Dynamic Red (44/29)

Water Exchange

12 day mean retention time

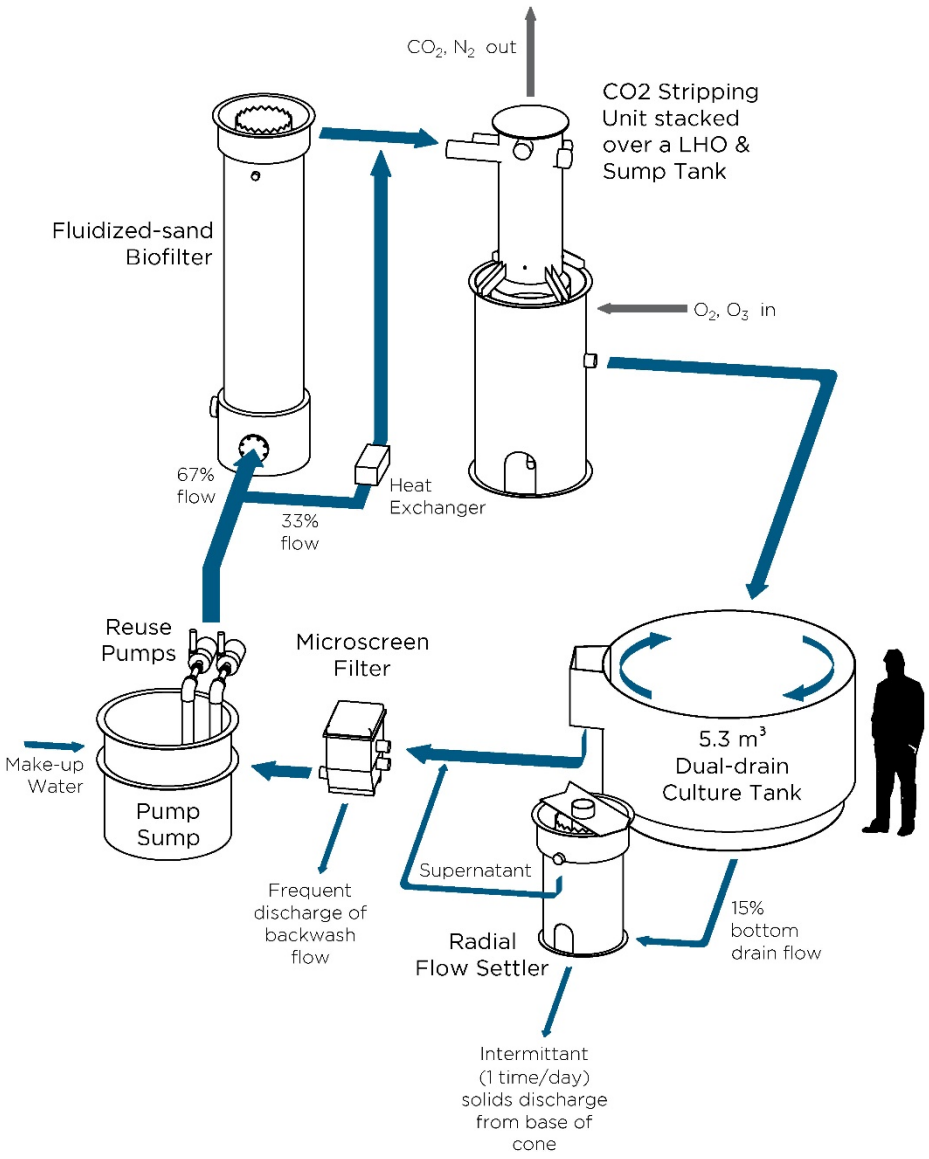
99.8 % water recycle on a flow basis

Hypothesis

Ozone will reduce waterborne hormone accumulation which will lead to reduced early maturation and improved salmon performance

6 RAS Ozone Addition

- Ozone was added at the low head oxygenator (LHO) and monitored via Hach SC100 units with differential ORP sensors



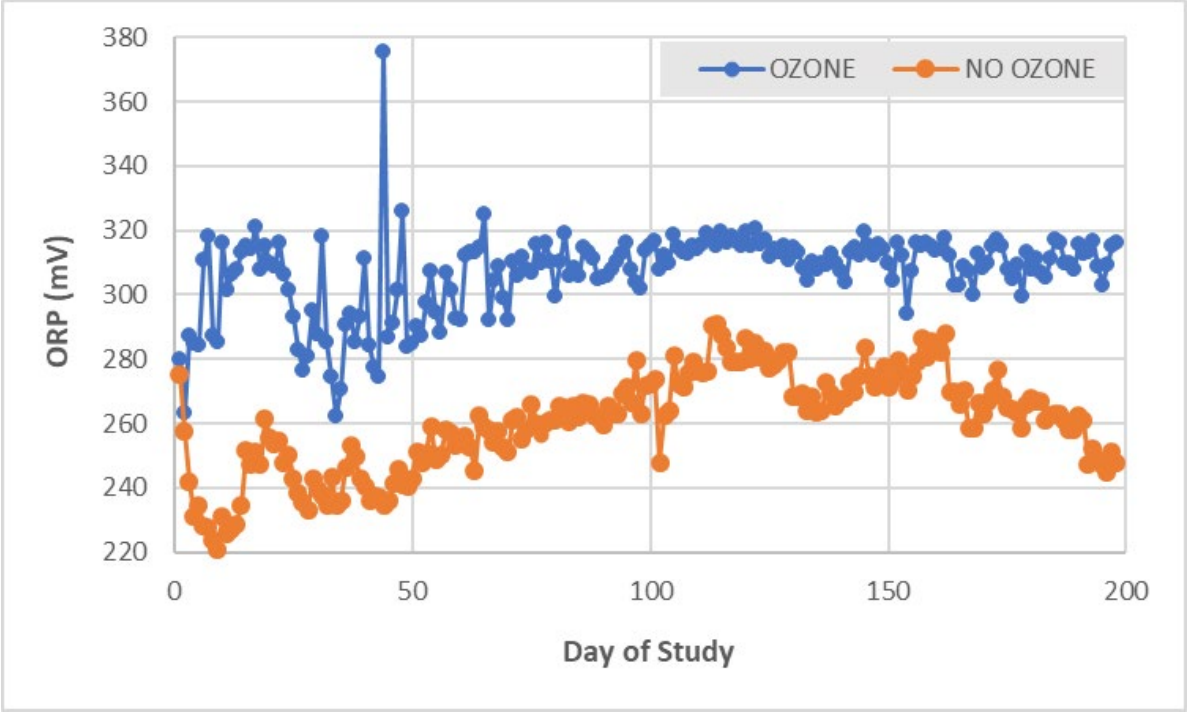
Controlled Environment

- Environmental control within and between treatments is critical !!!

	Ozone	No Ozone
Dissolved Oxygen (mg/L)	10.4 ± 0.1	10.2 ± 0.03
Temperature °C	14.9 ± 0.03	14.9 ± 0.03
pH	7.60 ± 0.03	7.63 ± 0.03
Alkalinity (mg/L)	162 ± 9	176 ± 1
Salinity (ppt)	< 1.0	< 1.0

- Oxidative reduction potential (ORP) was integrated with On/Off ozone addition

- On/Off ozone control at ORP of 315-320 mV

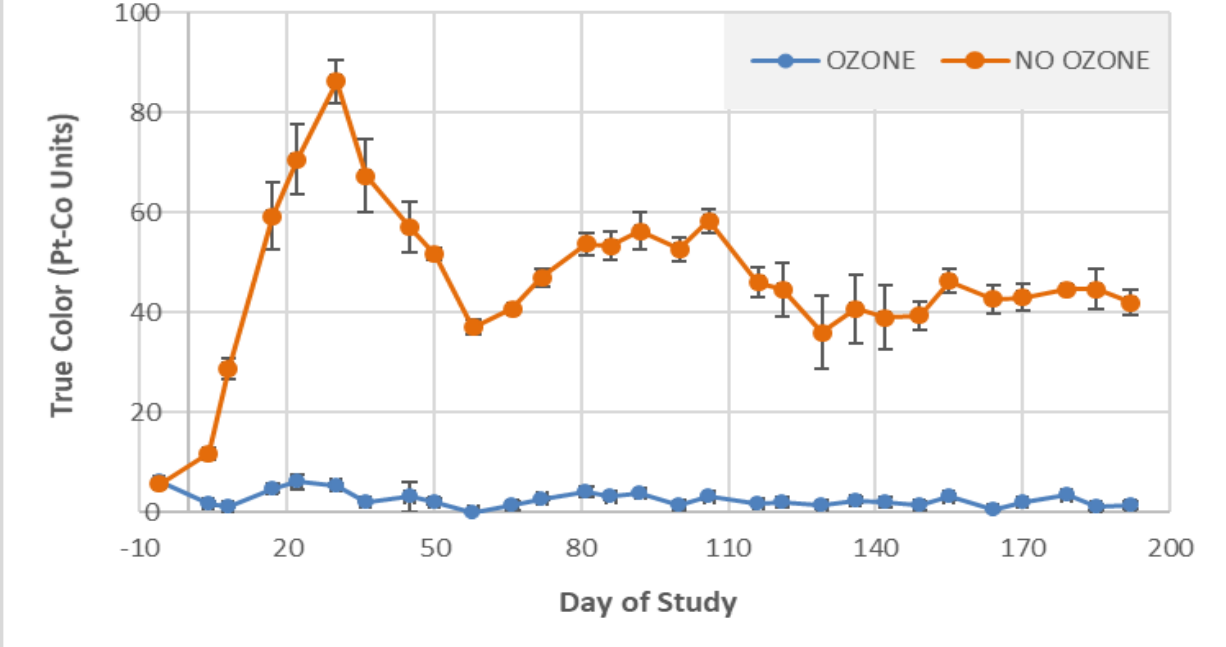


Water Quality Differences

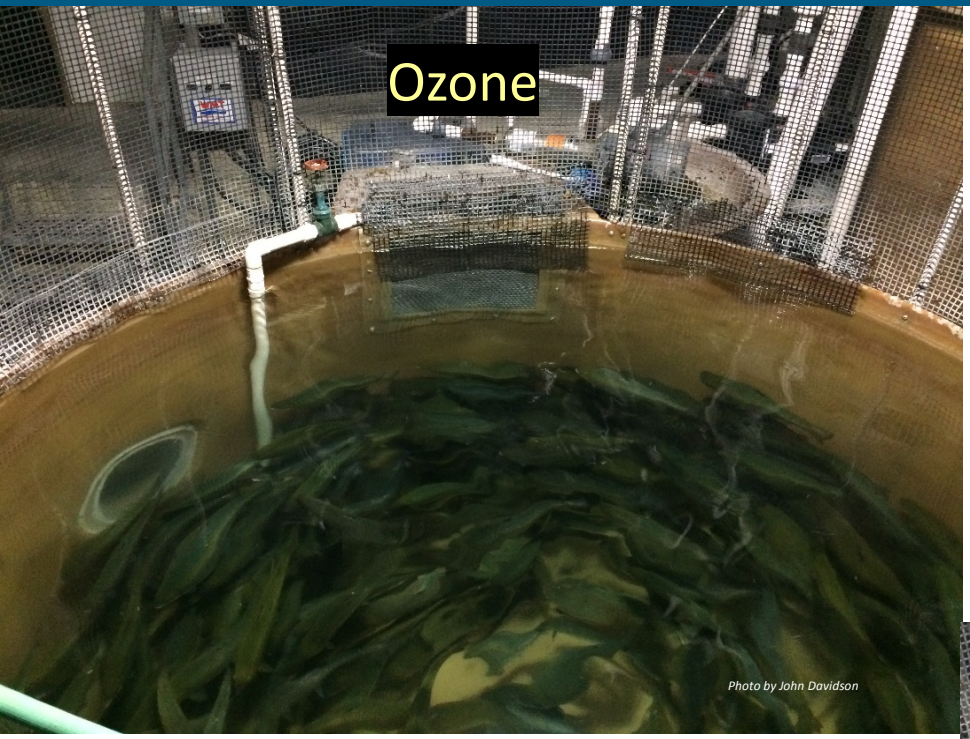
- Ozone significantly improved a range of water quality metrics
- Waterborne hormone data still pending

	Ozone	No Ozone
ORP (mV)	307 ± 1	260 ± 4
True Color (Pt-Co Units)	2 ± 1	47 ± 2
Ultraviolet Transmittance (%)	86 ± 1	63 ± 1
Heterotrophic Bacteria (counts/mL)	36 ± 7	140 ± 22
Dissolved Copper (mg/L)	0.008 ± 0.001	0.025 ± 0.001
Dissolved Iron (mg/L)	0.014 ± 0.002	0.021 ± 0.002
Dissolved Zinc (mg/L)	0.054 ± 0.004	0.065 ± 0.003

- Dramatic increase in color in RAS without ozone when water flushing was reduced
- Approximately 40-fold difference in color of culture water between treatments



Visual Water Quality Differences



- When RAS are operated with low water exchange and long retention times, color of the water increases
- Brown coloration is due to accumulation of naturally occurring tannins/ humic substances

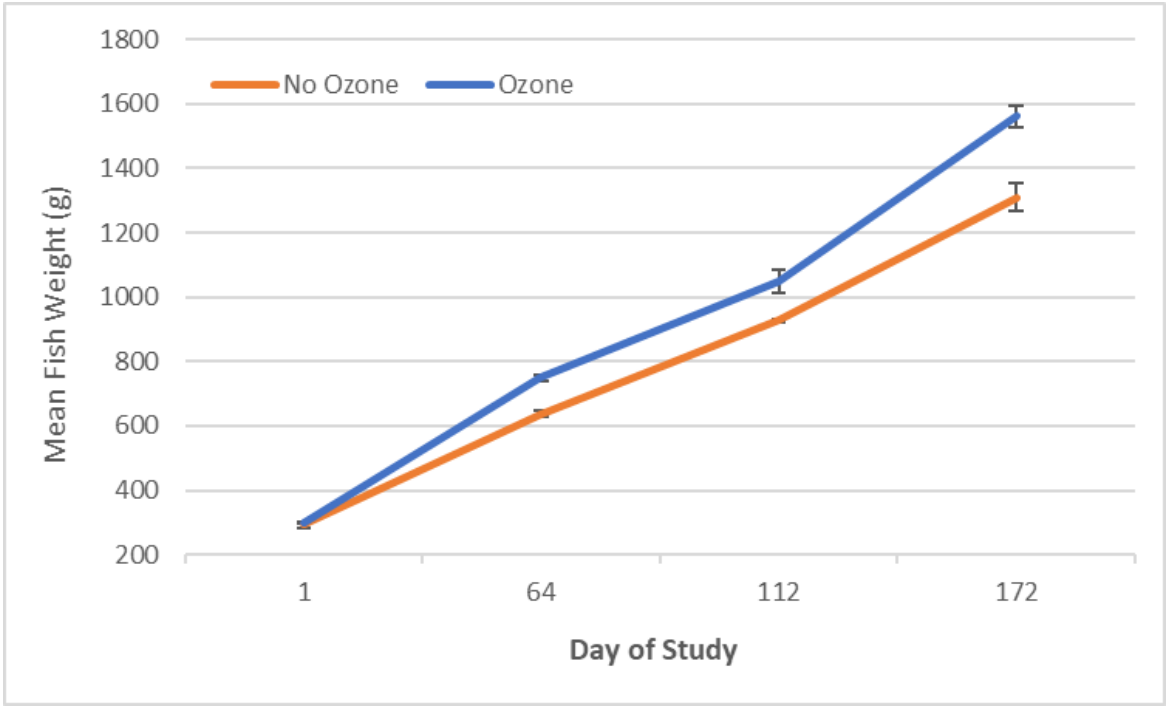
- Tannic Acid (Ozone) – 0.25 ± 0.03 mg/L
  Tannic Acid (No Ozone) – 1.38 ± 0.07 mg/L
- Ozone causes microflocculation of dissolved organics which creates crystal clear water

Salmon Performance

After 6 months of research:

- Salmon cultured in ozonated RAS are growing significantly faster
- Over the first two months, cumulative wasted feed was nearly two times greater in RAS without ozone

	Ozone	No Ozone
Mean Salmon Weight (g) *	1561 ± 35	1309 ± 43
Max Fish Density (kg/m^3) *	96.5 ± 3.2	84.6 ± 0.3
Cumulative FCR	0.96 ± 0.07	0.97 ± 0.05
Fish Survival (%)	97.7 ± 0.6	97.8 ± 0.1
TGC *	1.91 ± 0.04	1.68 ± 0.06



- Reason for growth curve inflection is unknown but could be related to max fish density approaching 100 kg/m^3 or nitrate-N levels
  - (109 ± 2 v. 97 ± 1 mg/L)

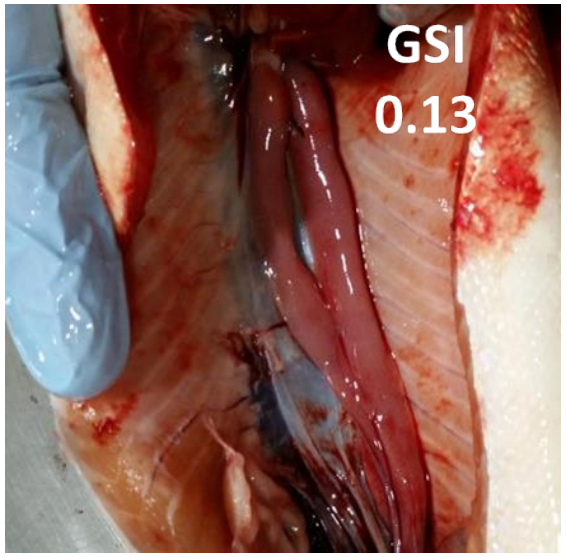
Welfare Scoring

- Utilized welfare scoring system developed by Nofima; 60 fish per replicate RAS
  - 0-4 for cataracts; 0-3 other metrics
- Fin score analyses are pending
- No significant differences between treatments
  - Scale loss bordered significance in favor of ozone

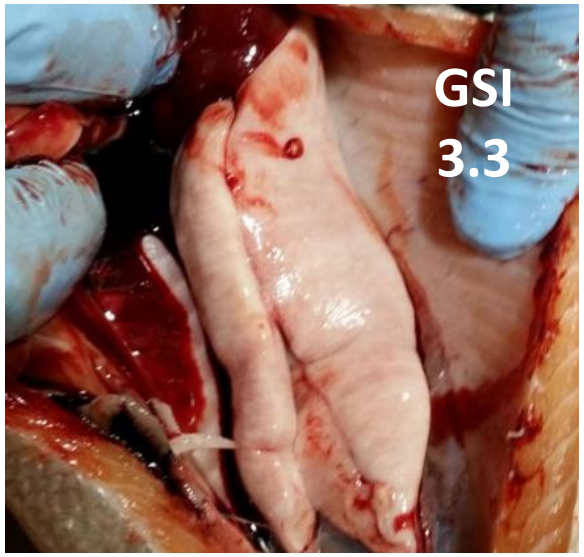
Welfare Metrics (Month 6)	Ozone	No Ozone
Left Eye Cataract	0.3 ± 0.1	0.2 ± 0.03
Right Eye Cataract	0.4 ± 0.1	0.6 ± 0.1
Left Operculum Damage	0.1 ± 0.0	0.1 ± 0.03
Right Operculum Damage	No Damage	No Damage
Snout Damage	0.3 ± 0.1	0.4 ± 0.03
Scale Loss	0.6 ± 0.1	0.9 ± 0.1
Hemorrhages/Sores	0.2 ± 0.1	0.2 ± 0.1

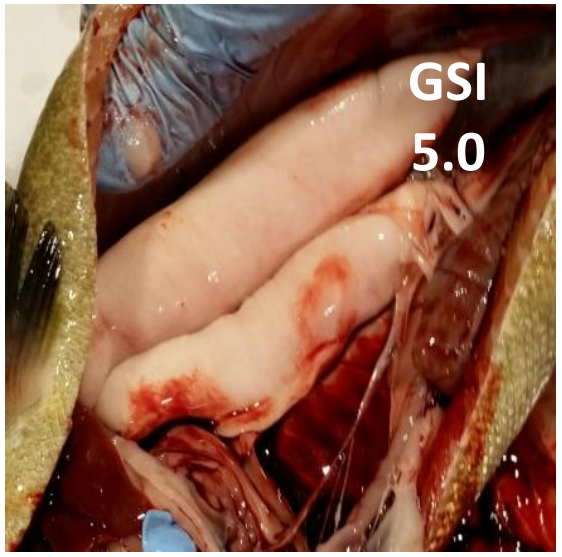
Salmon Maturation – Gonadosomatic Index

Male GSI (%)	2 Months	4 Months	6 Months
Ozone	1.6 ± 1.0	3.7 ± 0.2	4.1 ± 0.5
No Ozone	0.02 ± 0.01	2.8 ± 0.7	3.8 ± 0.2



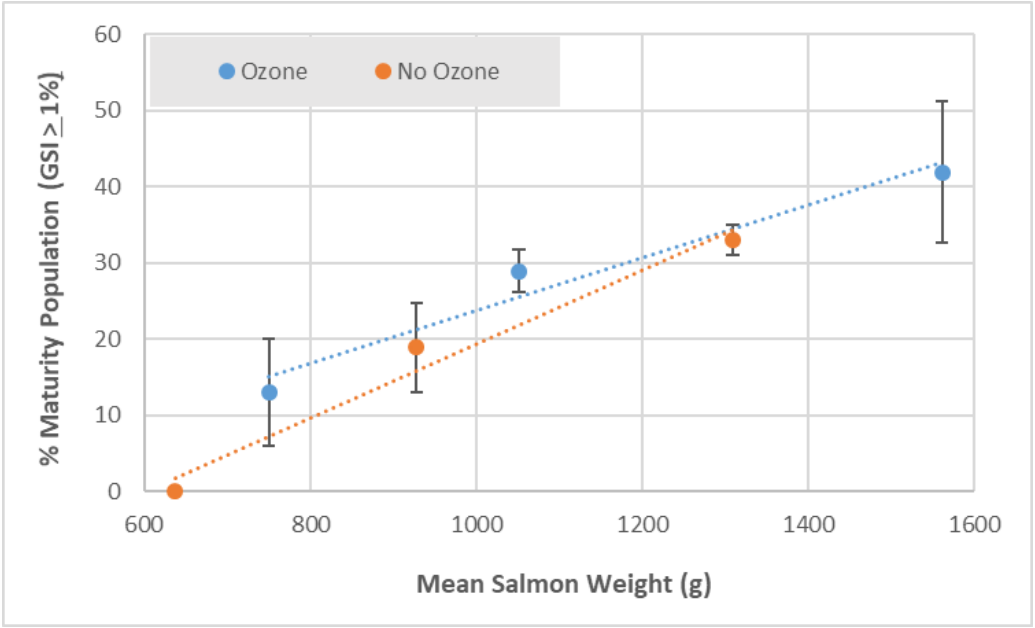
- Male maturation appears to have started sooner in ozonated RAS
- No significant difference in male GSI (%) between treatments at 4 or 6 months
- Male salmon in both treatments exhibited advanced maturation



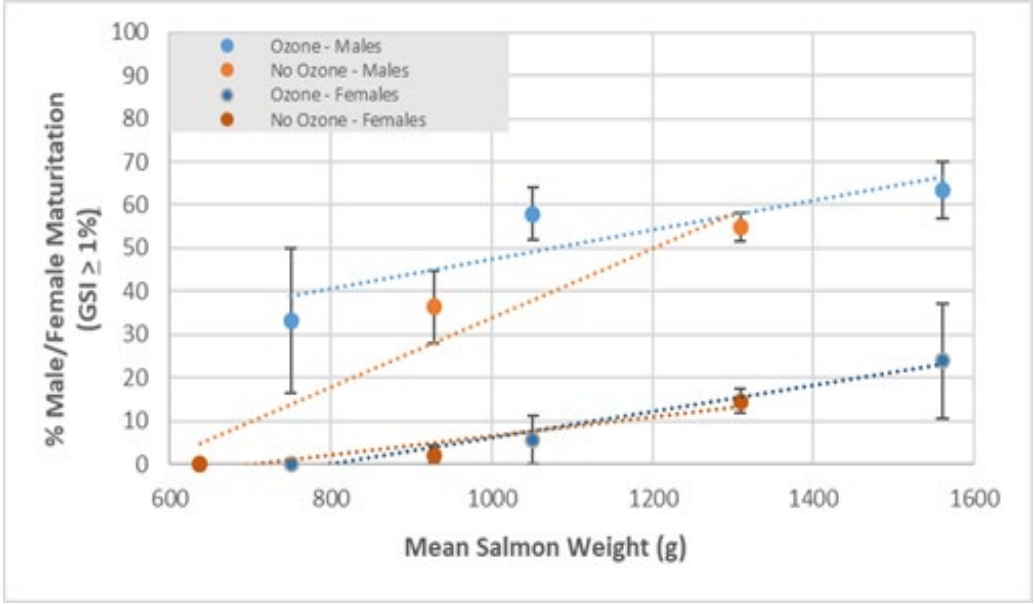


Early Maturation

% Maturity GSI ≥ 1%	2 Months	4 Months	6 Months
Ozone	13 ± 7	29 ± 3	42 ± 9
No Ozone	0 ± 0	19 ± 6	33 ± 2

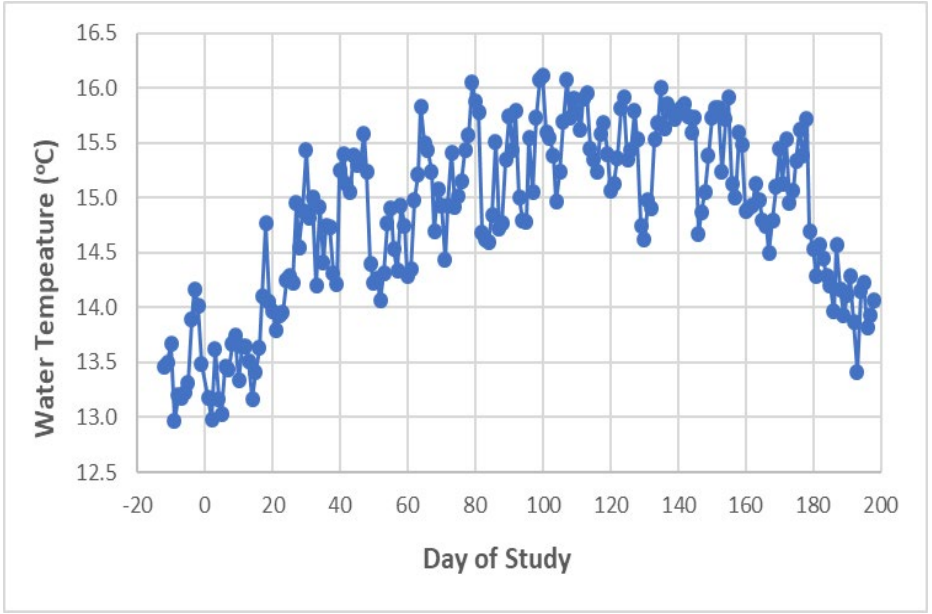


- Ozone did not interrupt early maturation
- Salmon in ozone systems were more mature with time, but trend appears to be growth (weight) dependent



Conclusions

- Ozone is not the holy grail for eliminating early maturation in RAS
  - 40-50% + maturity expected by end of study
  - Appears to be growth dependent

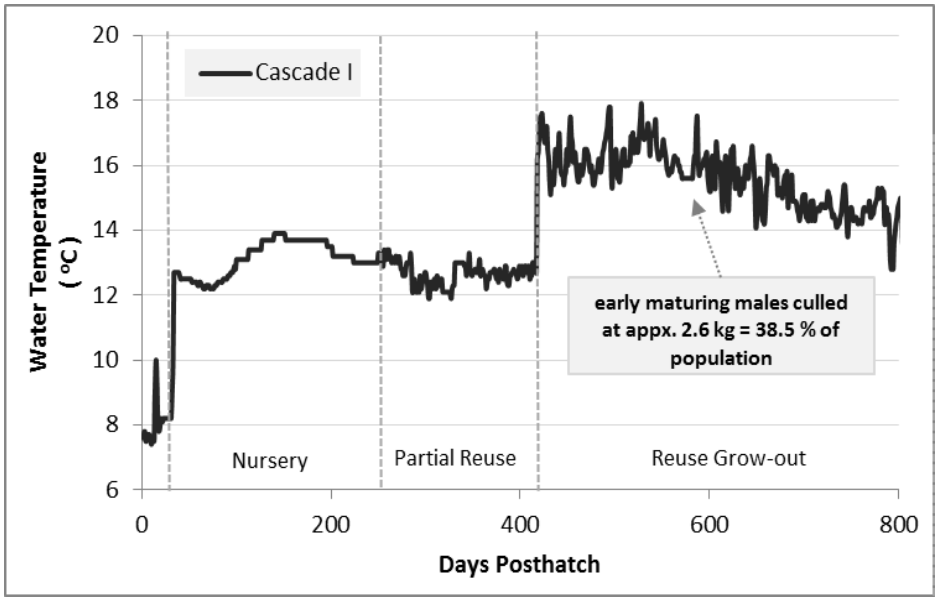


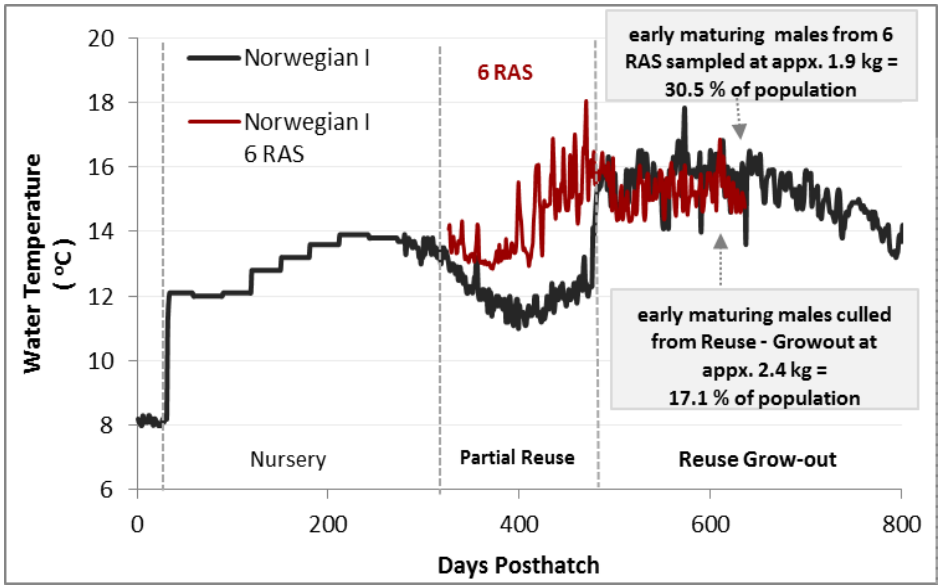
- Other factors are influencing maturation
  - Mean water temperature = 14.9 °C
  - Fish were stocked in the spring
  - Increasing temperature at critical life stage?

- Ozone appears to create a growth advantage
  - Ozone results in many water quality benefits
  - 40-50 fold difference in color of culture water
  - Possible impact to feed capture response?
  - Cumulative effect of other water quality benefits?

Next Steps - *What is the Solution?*

- Process of elimination….
  - Accumulating nitrate
  - Waterborne hormone accumulation
  - Photoperiod alone
- Water temperature
  - Avoid dramatic increases
  - Strategize the timing of onsite temp. increases
- Biology/ Genetics
  - All females?
  - All female triploids?
  - Genetic selection
- Freshwater vs. Seawater





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- All related experimental protocols were in compliance with Animal Welfare Act (9CFR) and approved by the Conservation Fund Freshwater Institute's Animal Care and Use Committee.