



**Effects of water
exchange rate and
biofiltration on
circulating hormones in
water recirculation
aquaculture systems
containing sexually
maturing Atlantic salmon**

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Aquaculture Innovation
Workshop #5
Shepherdstown WV - Sept 2013

- Reduce Production Cost
- Improve Growth
- Optimize Health
- Quantify Functional Welfare
- Improve Quality



Salmon, Trout, Char Production

- Quantify & Reduce Electric, Carbon, Water, & Waste Footprint
- Evaluate Alternative Protein sources
- Improve Biosecurity



Environmental Interactions



MBR Waste Treatment & Reclamation



- Capture Biosolids
- Reclaim water, heat, alkalinity, salts, & proteins, but not heavy metals
- Reduce or Eliminate Point Discharge
- Prevent Greenhouse Gas Emissions

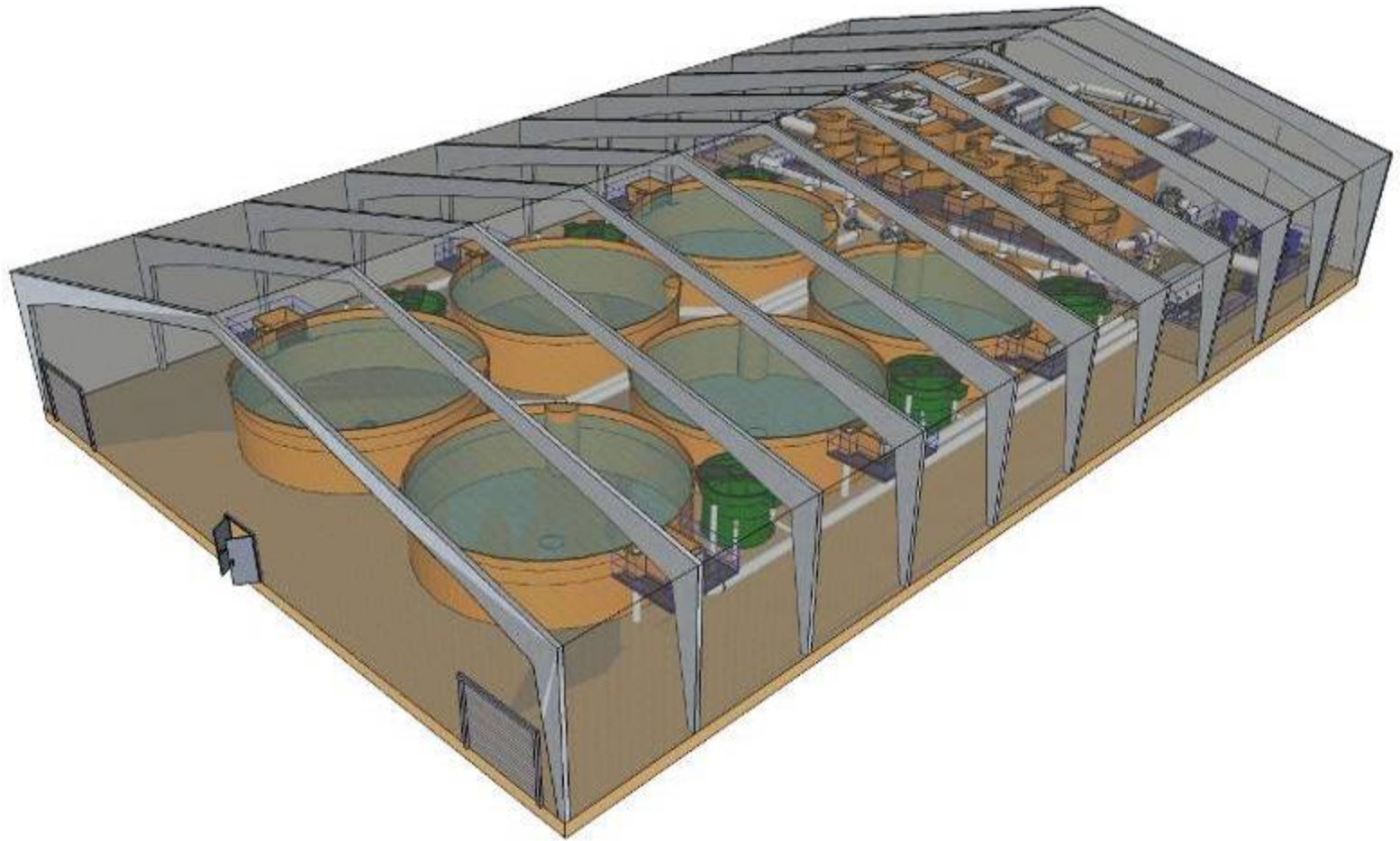
Water Reuse Processes



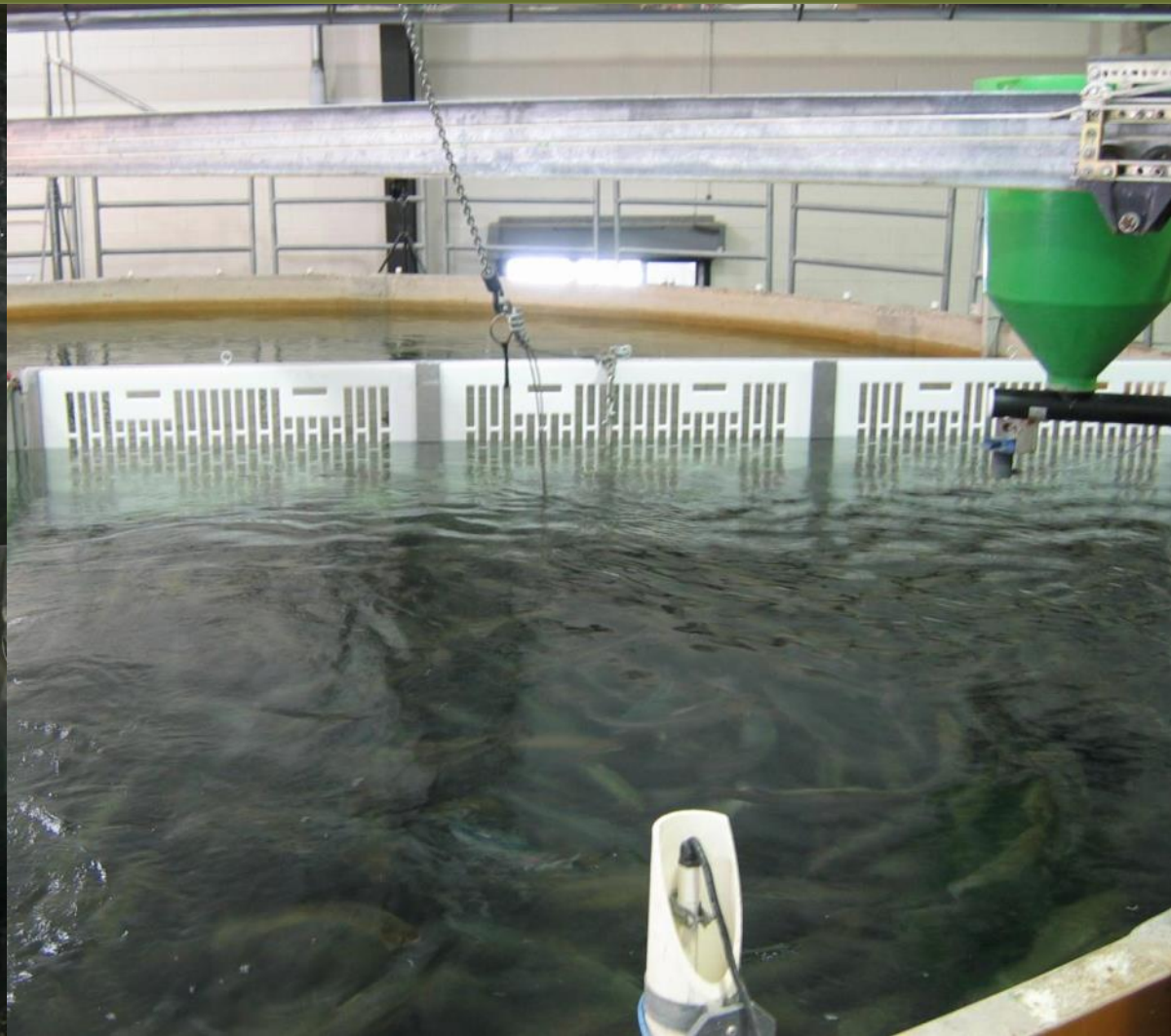
- Optimize Culture Tank Water Quality
- Determine Treatment Performance
- Improve Energy Efficiency
- Reduce Fixed & Variable Costs
- Utilize Economies of Scale

Research at The Freshwater Institute

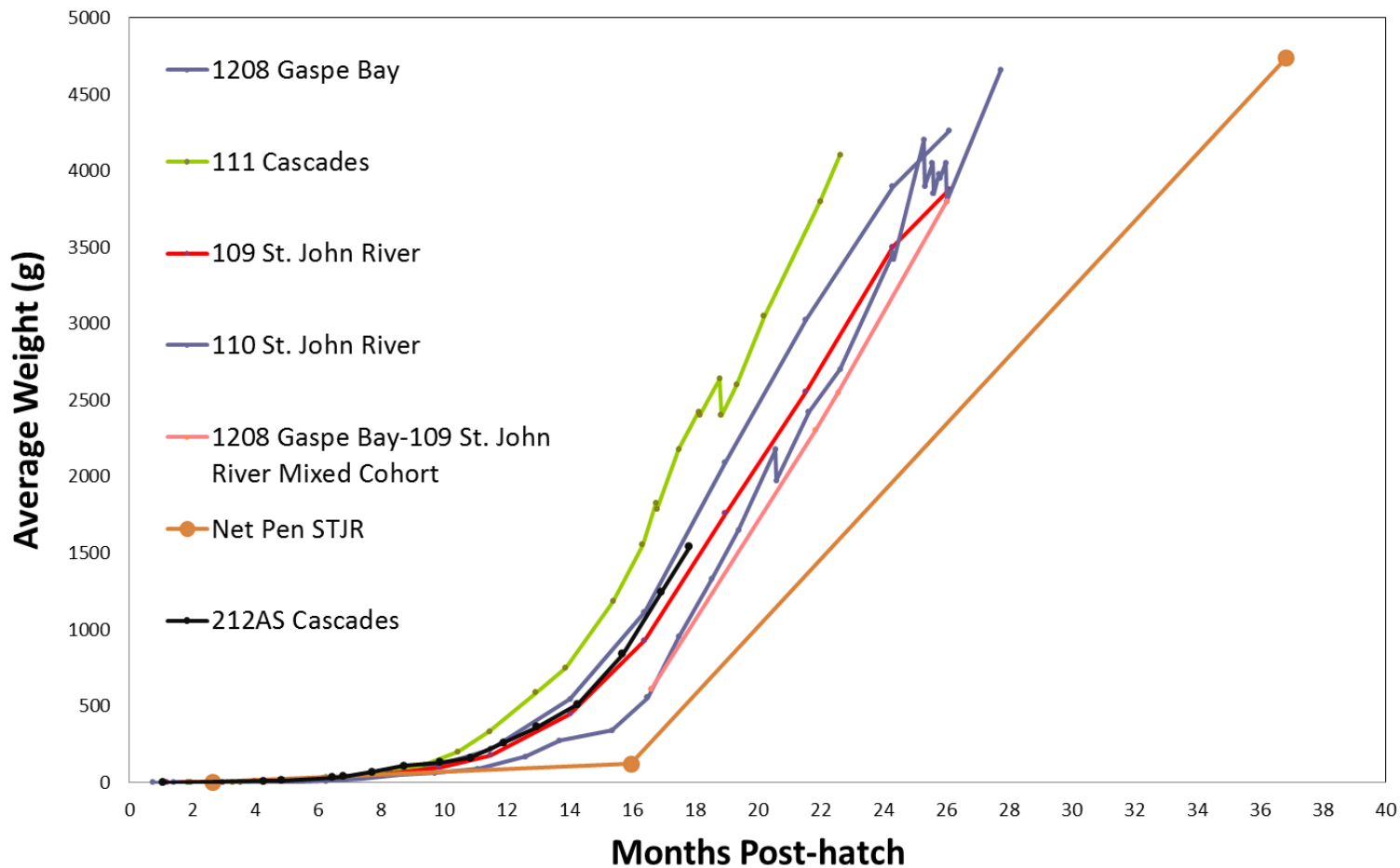
Closed Containment Facilities with Water Recirculation



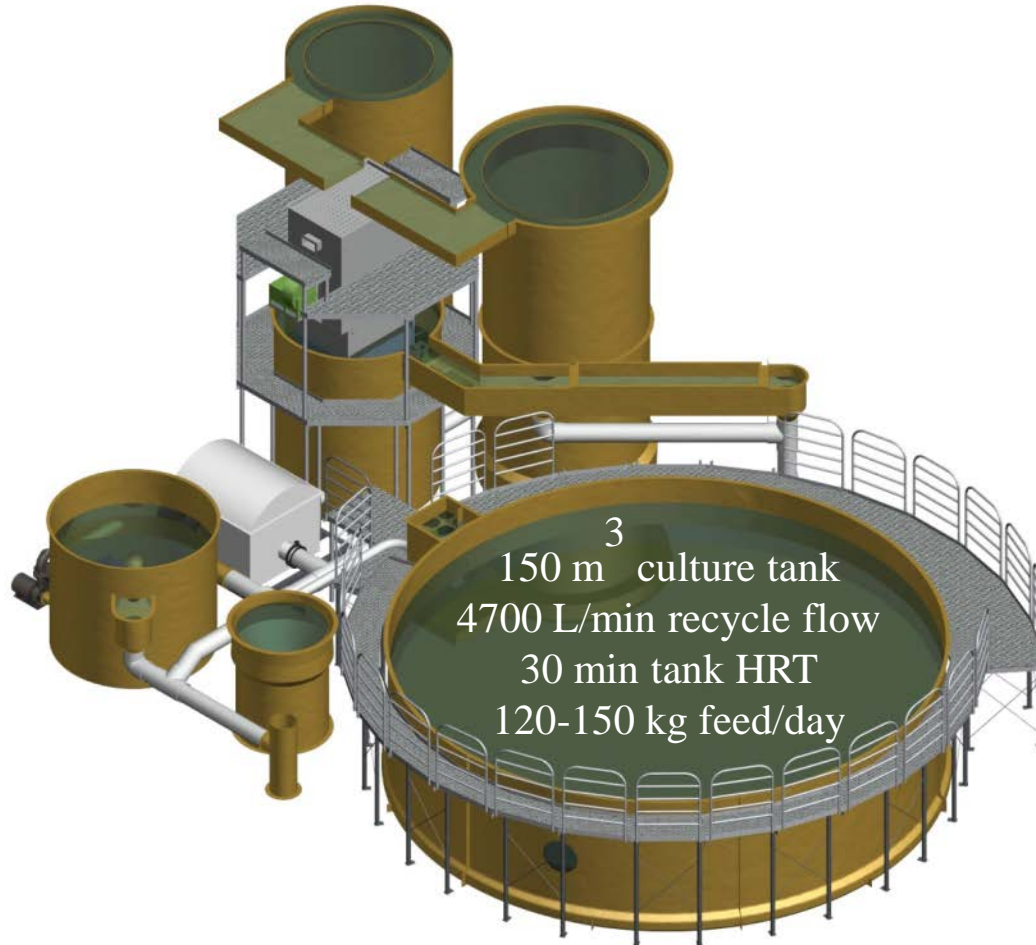
Background: Atlantic salmon growout trials



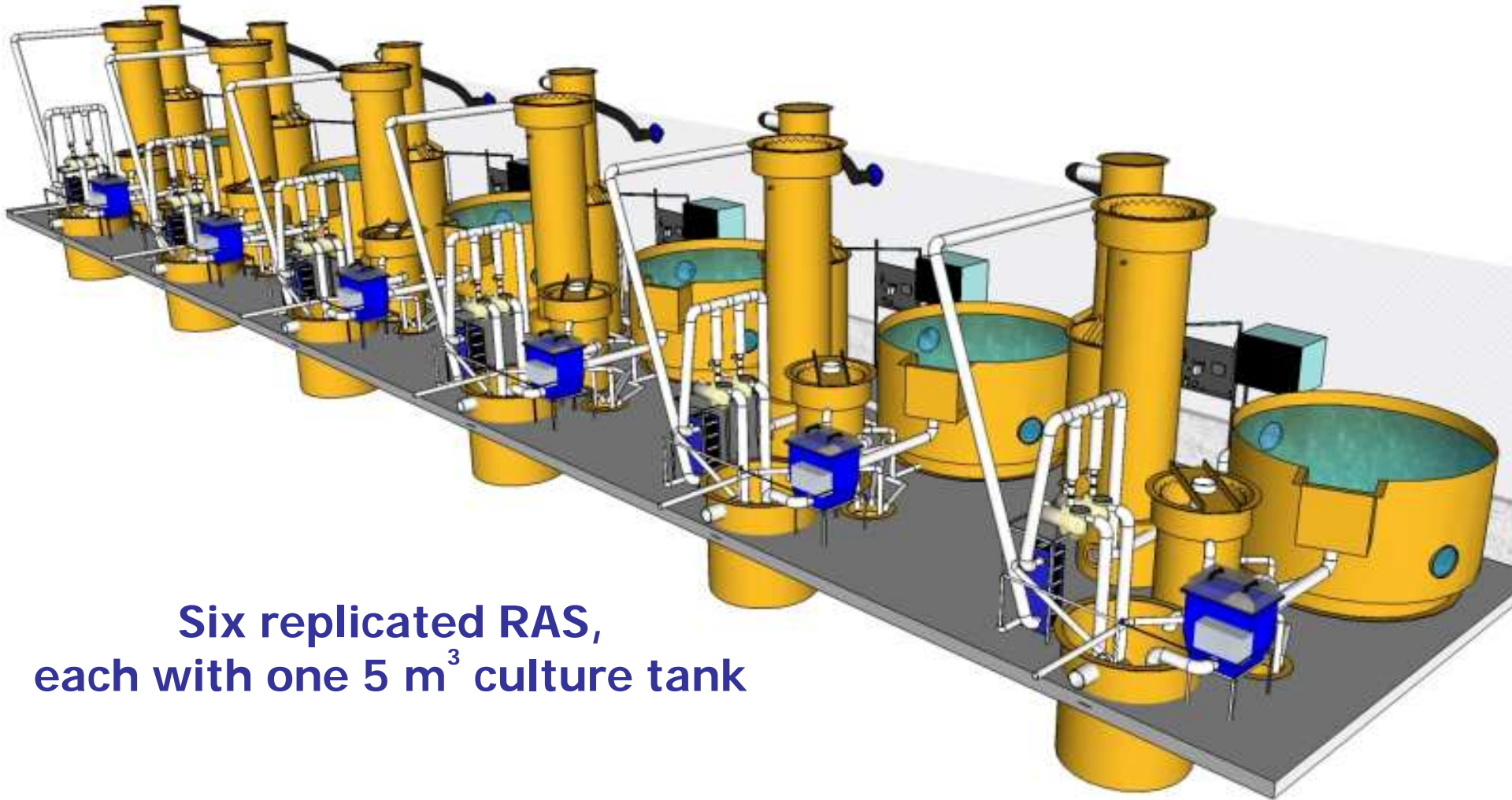
Background: Atlantic salmon growout trials



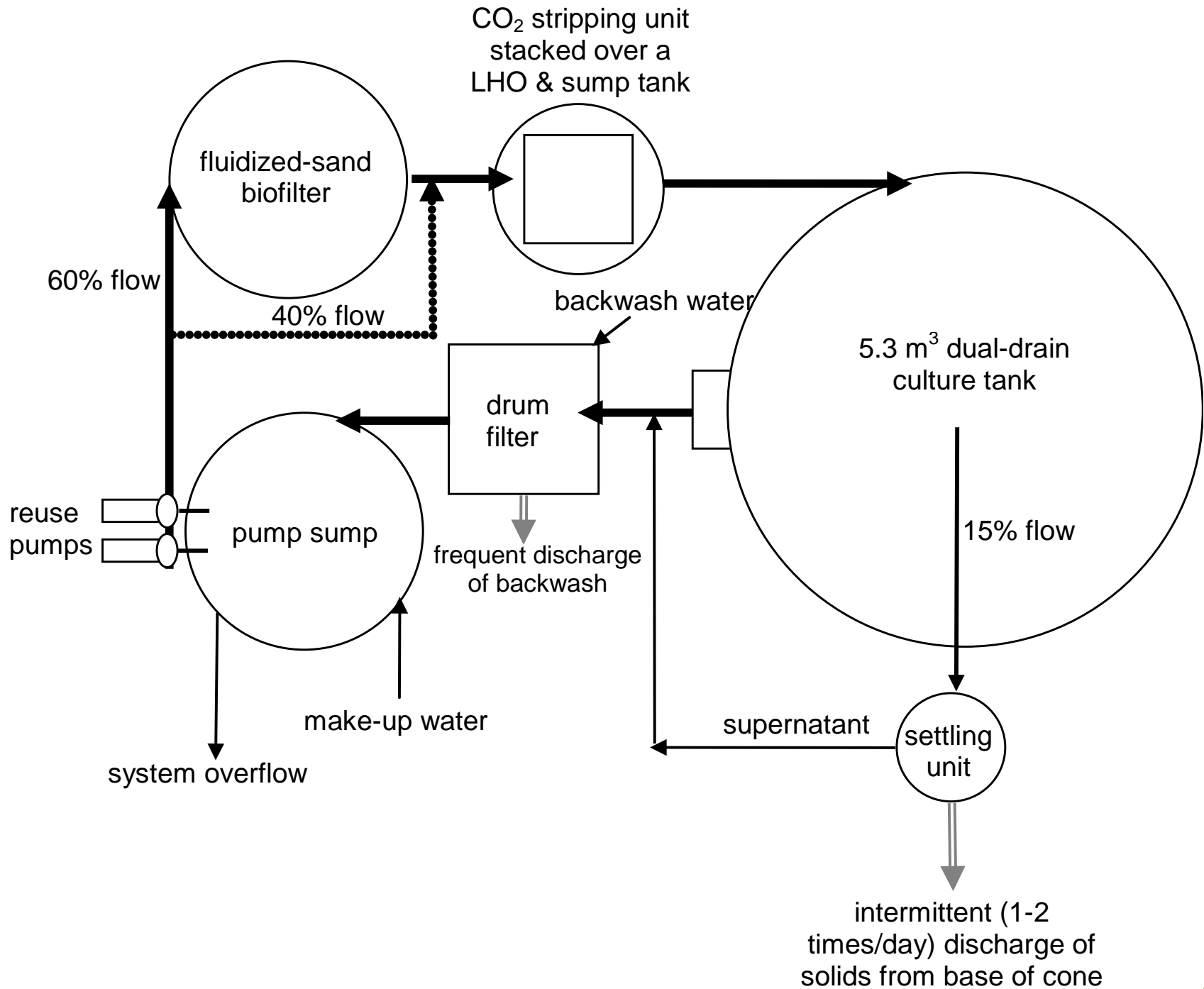
Main Reuse System Freshwater Institute



Replicated RAS



**Six replicated RAS,
each with one 5 m³ culture tank**



Background: Atlantic salmon growout trials

Precocious maturation

- 80% of male salmon matured early
- 40% of all fish removed as early maturing males
- approximately half at 2 kg and half at 3.5 kg



Sexual maturation in *S. salar*:

A highly flexible process, influenced by

- Photoperiod
- Water temperature
- Feed intake
- Nutrition
- Lipid reserves
- Growth rate
- Stock genetics
- Etc.



Accumulation of steroid hormones?



High Makeup H₂O Exchange (2.6%)



Low Makeup H₂O Exchange (0.26%)

Atlantic salmon growout – High vs. Low Exchange RAS

- Recirculating System (9.5 m³)
- 5.3 m³ Dual-drain tank
- Radial flow settler
- Drum filter (60 µm screens)
- Pump sump
- 1-HP centrifugal pump
- Heat Exchanger
- Fluidized Sand Biofilter
- Low Head Oxygenator (LHO)
- CO₂ Stripping Column



Atlantic salmon growout – High vs. Low Exchange RAS

➤ High vs. Low Water Exchange Rates – 3 RAS per treatment

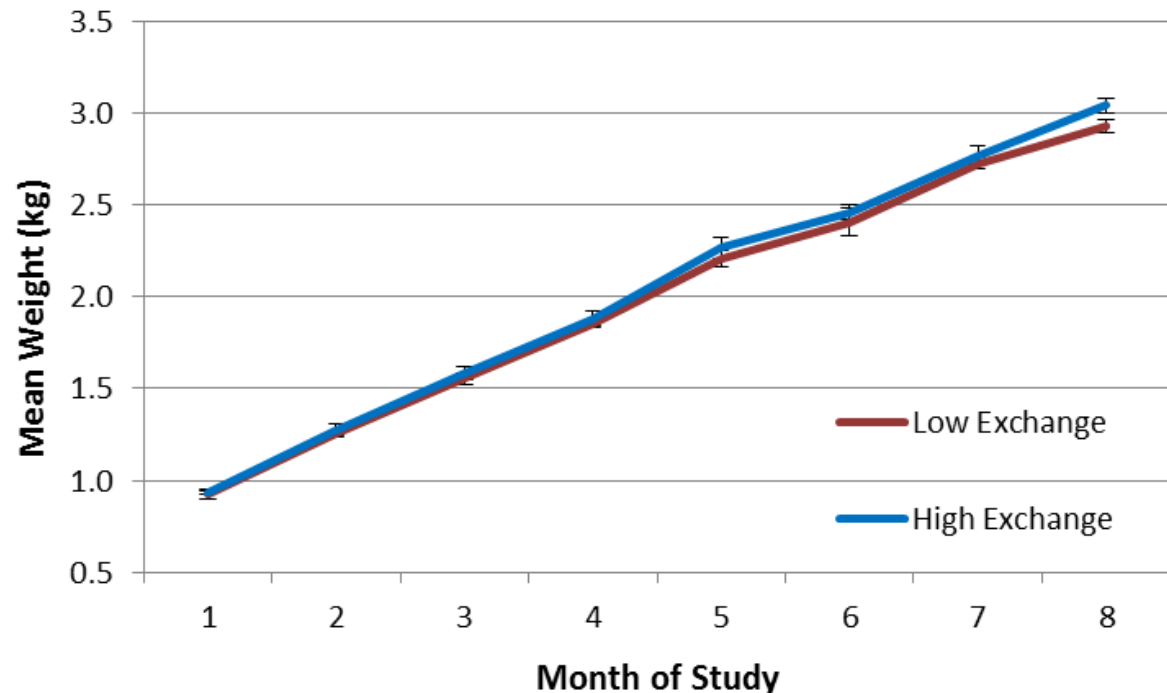
	Low Water Exchange	High Water Exchange
Flushing Rates (% of Recycled Flow)	0.25	2.60
Feed Loading Rate (kg feed/m ³ makeup water/ day)	1.3	0.13
Hydraulic Retention Time (days)	7.0	0.7

Atlantic salmon growout – High vs. Low Exchange RAS

Parameter (mg/L)	High Exchange	Low Exchange
Temperature °C	15.0 ± 0.0	14.9 ± 0.0
Dissolved Oxygen	10.3 ± 0.1	10.3 ± 0.0
Carbon Dioxide	9 ± 1	9 ± 1

Atlantic salmon growout – High vs. Low Exchange RAS

- Atlantic salmon were stocked at 0.93 ± 0.01 kg to begin
- No significant difference in mean weight throughout the study
- Slight separation in growth curves but difference not statistical
- End Mean weight
 - High Exchange
 3.04 ± 0.04 kg
 - Low Exchange
 2.93 ± 0.04 kg



Atlantic salmon growout – High vs. Low Exchange RAS

Parameter (mg/L)	High Exchange	Low Exchange
Thermal Growth Coefficient	1.45 ± 0.02	1.40 ± 0.02
FCR	1.03 ± 0.02	1.16 ± 0.13
Survival	> 99%	>99%

Atlantic salmon growout – High vs. Low Exchange RAS



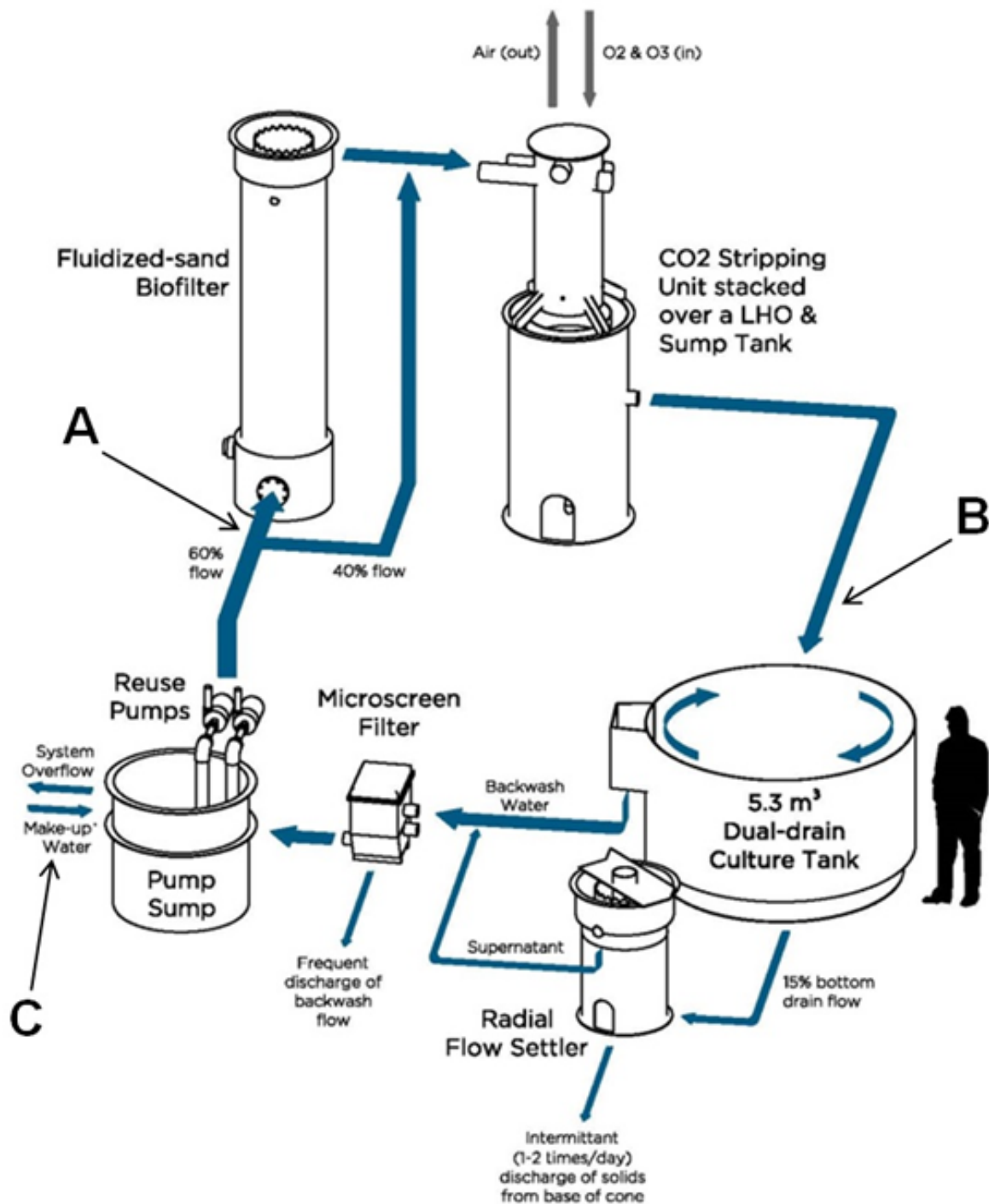
Very high prevalence of apparently mature males and females by study's end (24-months post-hatch)

Atlantic salmon growout – High vs. Low Exchange RAS



- Photoperiod?
- Rapid growth?
- Water temperature?
- Freshwater environment?
- Accumulating steroid hormones? - testable

- **Objectives:**
 - Determine whether important hormones accumulate in RAS relative to exchange rate, and whether this is associated with increased early maturation
 - Determine the effects of treatment processes on hormone concentrations
- Target hormones: **testosterone, 11-KT, estradiol, progesterone, cortisol**



Triplicate water samples collected from each RAS:

A – pre-water treatment processes

B – post-water treatment processes

C – makeup water influent

EIA quantification

Hormone	Exchange rate	<u>Water sample location</u>		
		Pre-Treatment (A)	Post-Treatment (B)	Makeup influent (C)
Testosterone	High	518.7 ± 118.0 ^{ab}	443.7 ± 86.32 ^a	123.7 ± 7.313 ^c
	Low	768.4 ± 88.88 ^d	758.5 ± 155.5 ^{bd}	124.0 ± 45.24 ^c
11-KT	High	194.5 ± 21.19 ^a	127.9 ± 11.08 ^b	4.783 ± 0.390 ^c
	Low	183.0 ± 17.73 ^a	124.7 ± 11.90 ^b	4.526 ± 1.008 ^c
Estradiol	High	168.7 ± 61.80 ^a	168.8 ± 66.25 ^a	39.55 ± 6.341 ^b
	Low	223.5 ± 28.53 ^a	239.8 ± 20.69 ^a	38.92 ± 25.06 ^b

No differences in measured concentration for cortisol or progesterone

		<u>RAS</u>	
		High exchange	Low exchange
Visual signs of maturity (%) (n=357)	M	75.6 ± 13.7 ^a	67.8 ± 8.07 ^a
	F	11.3 ± 3.27 ^a	3.23 ± 1.47 ^b
Gonadosomatic index (n=24)	M	6.79 ± 0.30 ^a	5.94 ± 0.79 ^a
	F	3.06 ± 1.38 ^a	5.24 ± 4.97 ^a

- **Testosterone** the only measured hormone significantly accumulating in RAS relative to exchange rate
- **11-KT** the only measured hormone to be significantly reduced across the water treatment processes
- **Testosterone, 11-KT, and estradiol** sig. higher in RAS compared to makeup water
- Mature male % unrelated to exchange rate

- Controlled studies incorporating:
 - Water sampling at multiple time points
 - More sampling locations throughout the RAS
 - Plasma hormones assessments for water sampling validation
 - Increased GSI sampling
 - Other physiological assessments in parallel, e.g. vitellogenin, MIH, etc.

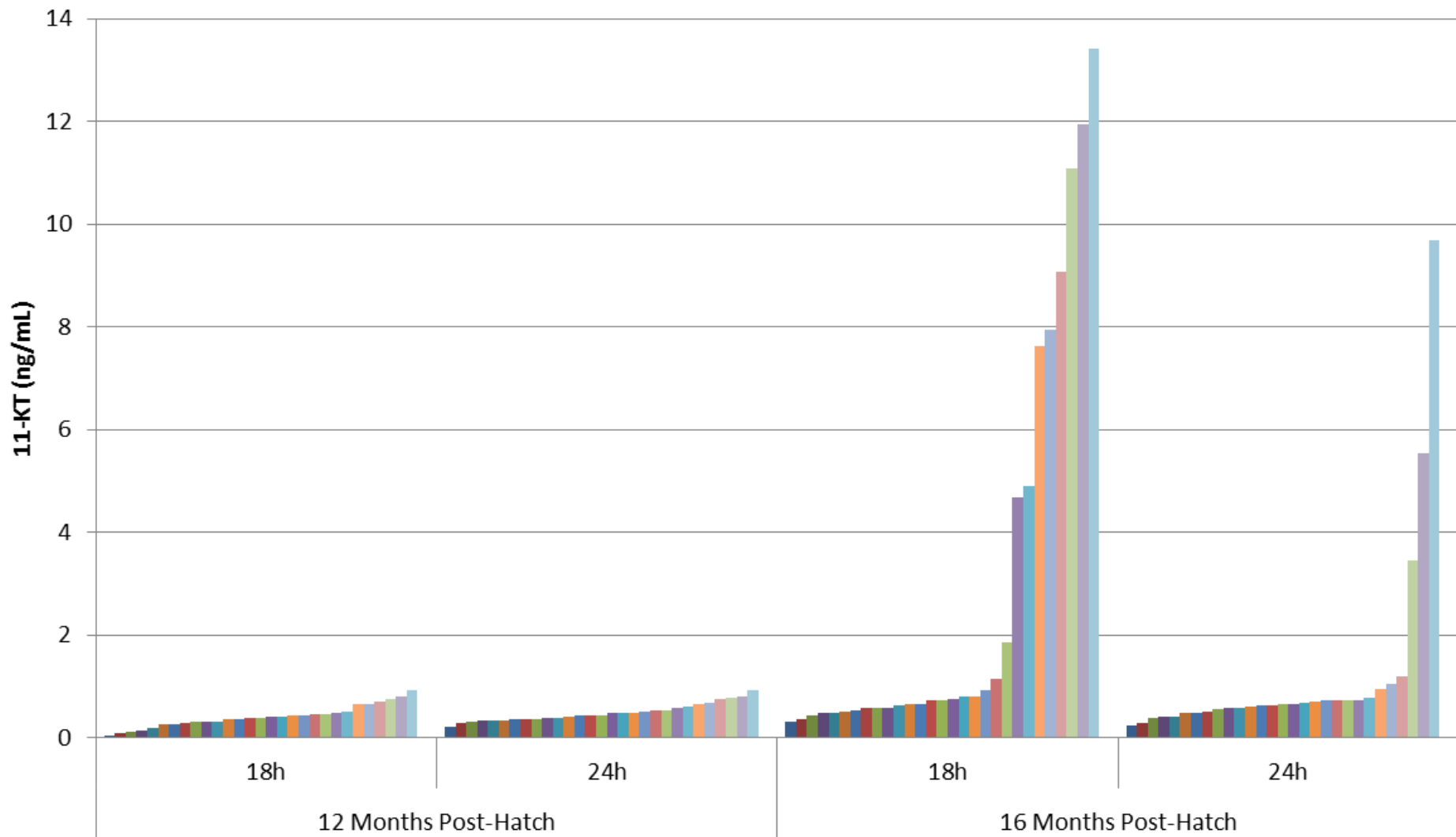
Photoperiod effect?

Two treatment groups:

- 24-hour photoperiod
- 18h:6h photoperiod



2013 Maturation Assessments



- Mature males at 16 months:
 - 18h:6h = 23%
 - 24h = 10%
- GSI vs 11-KT:
 - 18h:6h correlation coefficient = 0.1808
($p=0.3538$)
 - 24h correlation coefficient = 0.4613
($p=0.0103$)

- No evidence that 18h:6h photoperiod reduces early male maturation
- Further sampling at ~2.5 kg and final harvest (4-6 kg)

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