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A Review of Factors Influencing Maturation of Atlantic Salmon in RAS Environments

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A Project of Tides Canada's Salmon Aquaculture Innovation Fund

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Background: Atlantic salmon growout trials

Grilsing

Precocious male maturation

Up to 80% of male salmon mature early

Negative consequences of maturation

- Decreased growth and feed conversion
- Reduced product quality
- Increased susceptibility to opportunistic infections

Historical background

- Long history of maturation affecting Atlantic salmon production:
  - Major source of economic loss for farmers
    - Johnston et a., 2006; McClure et al., 2007
  - $11M – 24M in annual lost revenue ($250M industry)
    - McClure et al., 2007
  - In-cage grilsing estimated at 20-30% (1998-2002)
    - Peterson et al., 2003

Maturation in Atlantic salmon

Sexual maturation in *S. salar*:

A highly flexible process, influenced by

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

Salmo salar: A highly flexible life history

- **Many variations in life history traits among and within populations**
  - Freshwater occupancy/age at smoltification (Randall et al., 1987; Økland et al., 1993)
  - Time of ocean residency and age at reproductive maturity (Scarnecchia, 1983; Saunders, 1986; Thorpe, 1986)
  - Adult size at maturity (Hutchings and Jones, 1985; Saunders, 1986)
  - Non-anadromous versus anadromous forms (Berg, 1985).
- **Evolutionary strategy designed to maintain biodiversity and genetic contribution of a cohort** (Saunders and Schom, 1985).
- **Evolutionary adaptation to optimize reproductive success and to perpetuate the species** (Fleming, 1996; Thorpe et al., 1998).
- **The Atlantic salmon life cycle is motivated by procreation and recruitment of successive generations.**

Salmo salar: A highly flexible life history

- **The path to reproductive maturity is likely triggered by a combination of heritable, physiological/biochemical, and environmental factors and their interactions.**
- Saunders (1986) **proposed that genetic influence provides a basis for maturation but with "rather wide latitude," when the appropriate environmental and physiological/biochemical conditions are met.**
- Mangel and Satterwaite (2008) **described optimization of environmental conditions as creating an opportunity for maturation along with traits that typically parallel optimal growth performance, such as the accumulation of adipose tissue.**

MAJOR FACTORS INFLUENCING MATURATION OF ATLANTIC SALMON

Aquaculture Innovation Workshop
Shepherdstown WV Oct 14-15, 2015

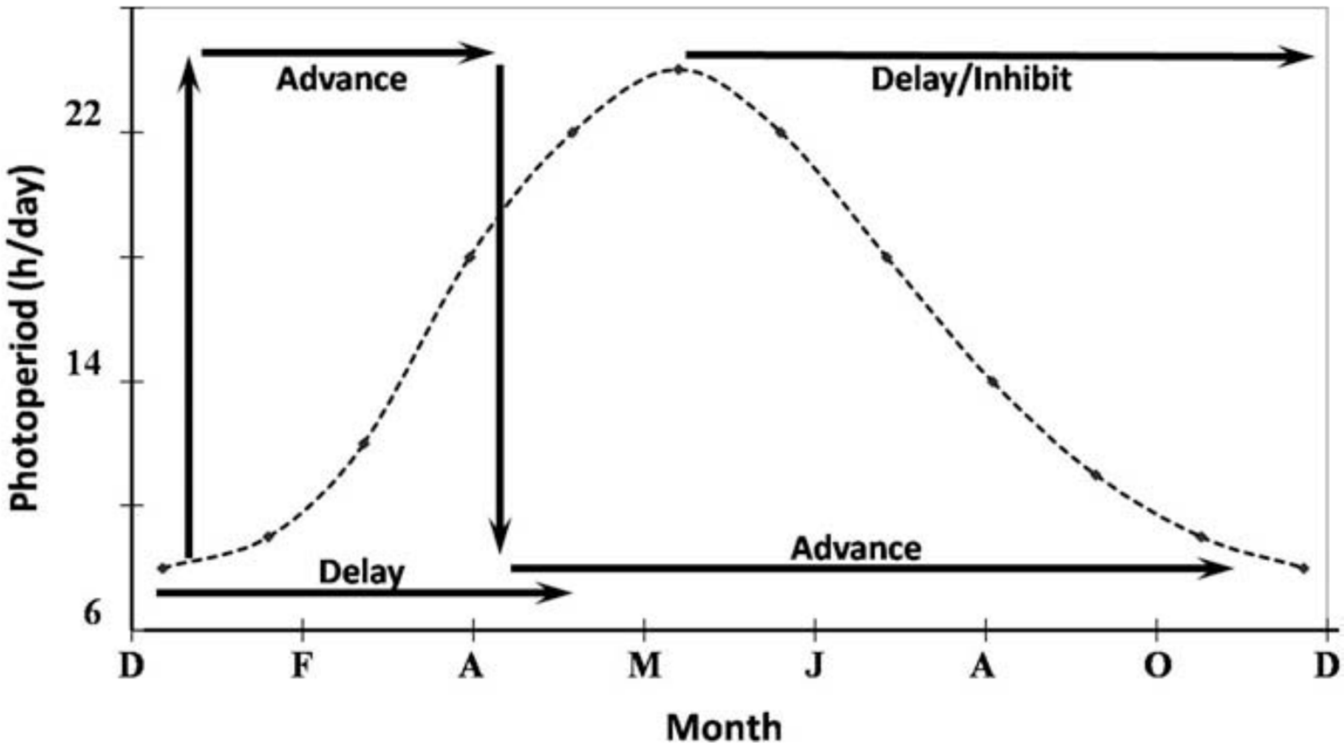
Photoperiod

- An essential determinant for initiating sexual maturation in teleosts Taranger et al., 2010
- Evolutionary strategy to ensure juveniles hatch during advantageous environmental conditions

  Bromage et al., 2001
- Direction of daylength change more important than specific hours of daylight Bromage and Duston, 1986

Photoperiod

- "Decision" to mature is made during late autumn (declining photoperiod) but not fixed before early spring



- Source: Taranger et al., 2010

Photoperiod in Closed Containment

- *Very little research specific to CCS*

<u>First-year photoperiod study at FWI</u>

Two treatment groups:

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

Reared under different photoperiods for 12 months, then comingled for growout

18h:6h vs 24h Photoperiod

- Assessments (18-30 males per treatment):
  - – Gonadosomatic Indices
  - – Plasma 11-KT
- Samplings:
  - – 12 months post-hatch
  - – 16 months post-hatch
  - – 19 months post-hatch (prior to grilse harvest)
  - – 24 months post-hatch

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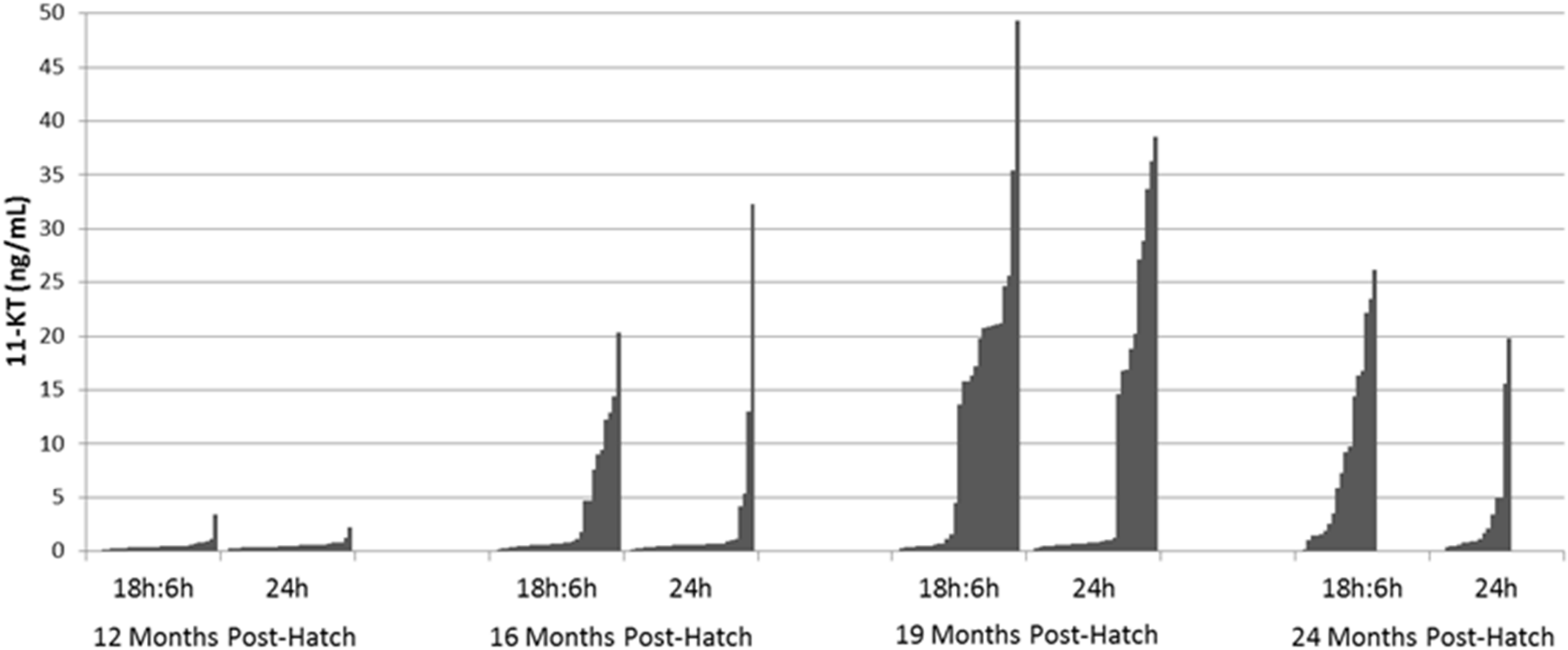
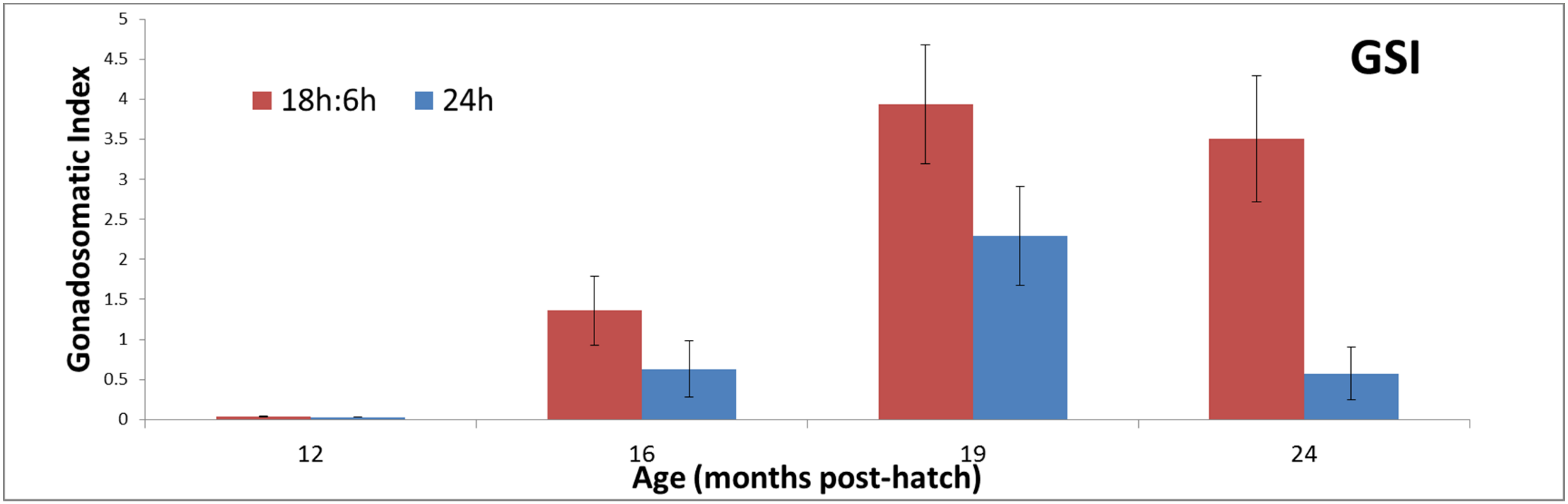
18h:6h vs 24h Photoperiod



$p<0.05$ $p<0.05$



Sampling	Treatment	Mean	SE	P-value
12 months	18h:6h	0.037	0.004	0.165
	24h	0.029	0.002	
16 months	18h:6h	1.359	0.429	0.175
	24h	0.632	0.355	
19 months	18h:6h	3.939	0.741	0.029
	24h	2.296	0.618	
24 months	18h:6h	3.510	0.787	0.002
	24h	0.573	0.329	

Photoperiod

- Taranger et al. 1998
  - Sea cage final year salmon exposed to either i) LDN, ii) LD24:0 beginning January, or iii) LD24:0 beginning in March
  - Transferred at midsummer to brackish land-based systems with either i) LDN, ii) LD24:0, or iii) LD8:16
- Very high grilse in LDN & LD24:0 (March)
- Lowest grilse in LD24:0 followed by LD24:0

Light Quantity & Quality

- Melatonin studies have determined that light intensity threshold for perception by Atlantic salmon is around 0.016 W/m^2
- Salmon are also sensitive to spectral composition; suppress melatonin more efficiently with blue & green light (450nm & 550nm)
- Data strongly suggest light intensity the most important factor

Water Temperature

- Water temperature is one of, if not the most important environmental parameter that affects fish physiology

  Metabolic rate, growth rate, migratory behavior, time of spawning, egg hatching, yolk absorption, etc.

- Atlantic Salmon Metabolism/ Growth Rate

  Austreng et al. (1987) measured fastest Atlantic salmon growth (0.15 - 75 g) in freshwater at 16 ^{o} C

  Austreng et al. (1987) measured fastest Atlantic salmon growth (30 - 2000 g) in marine net cages at 14 ^{o} C

  Handleland et al. (2008) also reported fastest Atlantic salmon growth (70-300 g) in seawater at 14 ^{o} C

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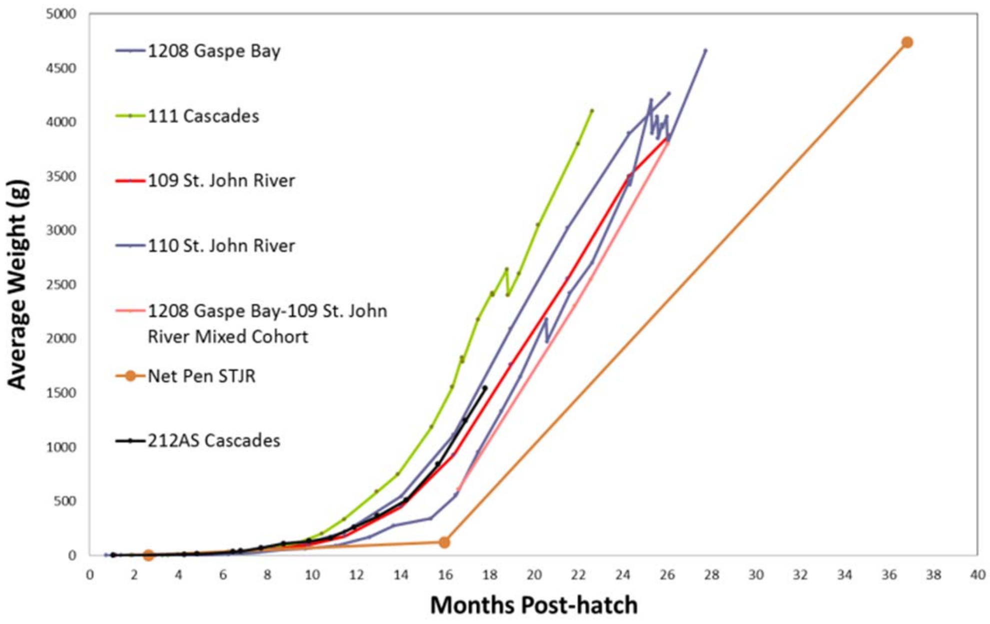
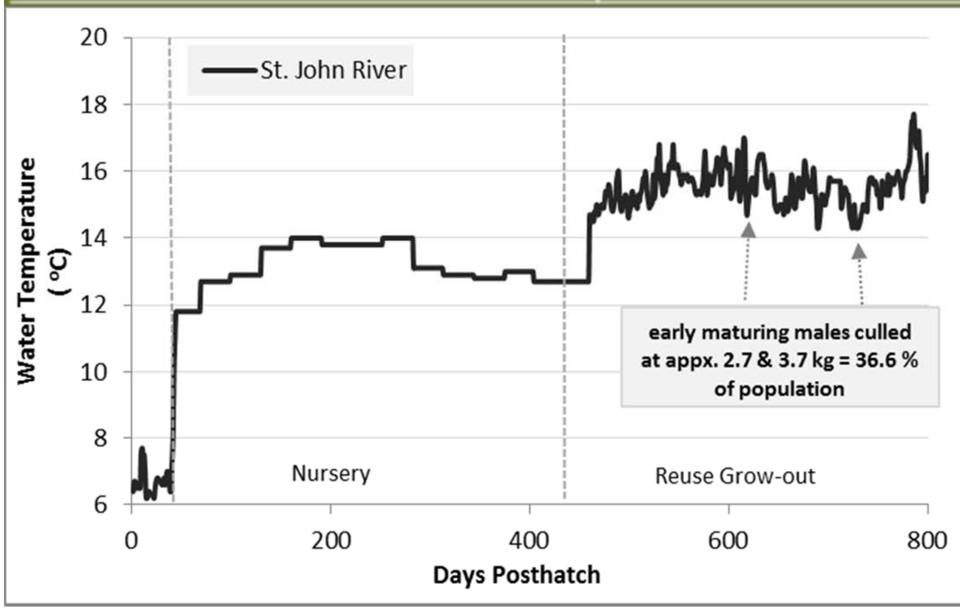
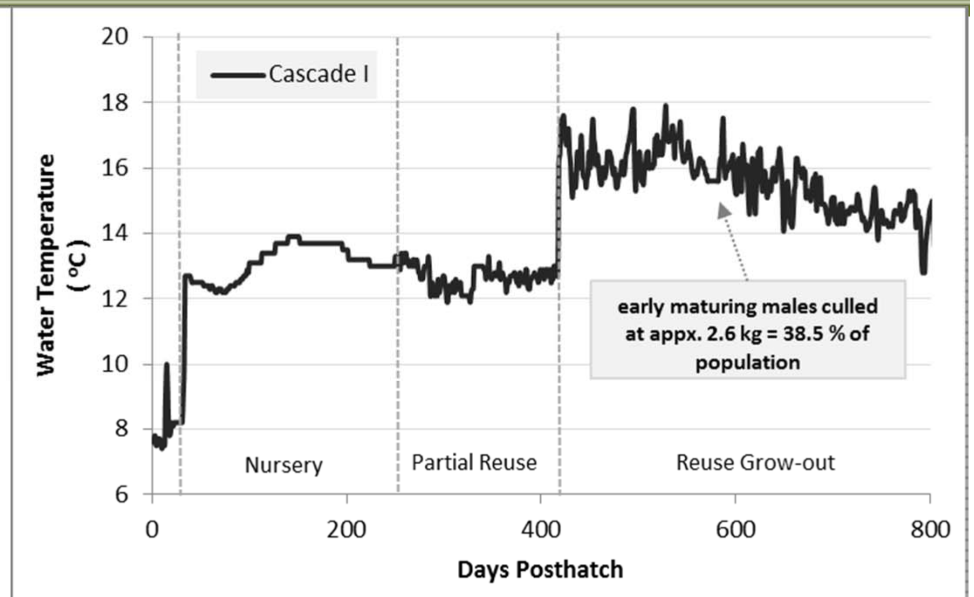
Water Temperature



We've demonstrated that Atlantic salmon can be cultured to market size months faster than salmon produced in the average Atlantic Coast net pen.

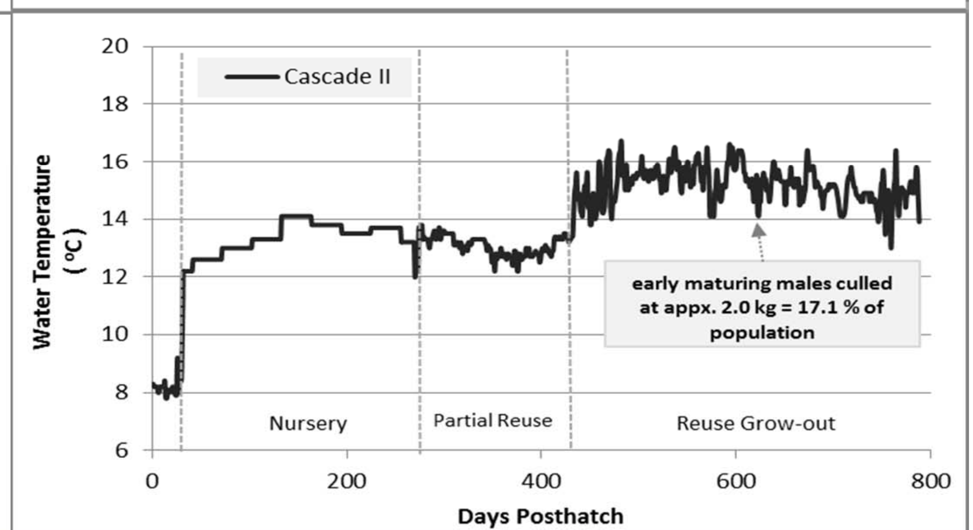
Evidence of Increased Maturation with Increasing Water Temperature





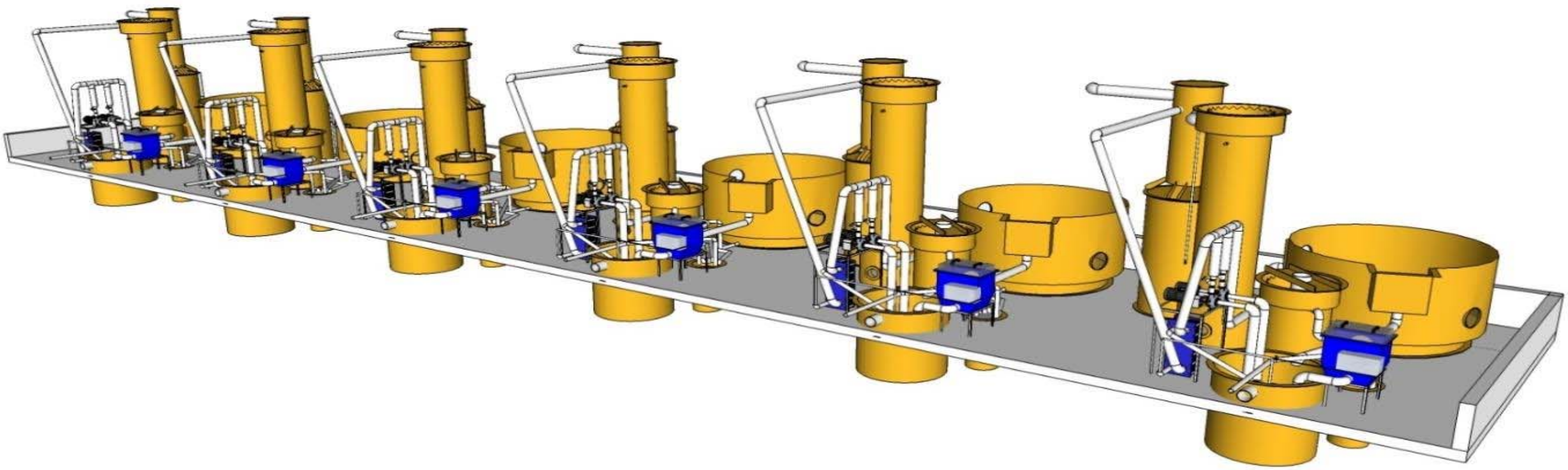
Maintaining temperatures that accelerate salmon growth could be at odds with inhibiting maturation

A sudden increase in temperature could exacerbate maturation



Increased Maturation in Replicated RAS

- 6 Identical Recirculating Aquaculture Systems
  - Located in greenhouse-style building
  - 9.5 m^3 total volume
  - 5.3 m^3 culture tank volume
  - Hydraulic retention time varies from 1- $\geq$ 20 days depending on study



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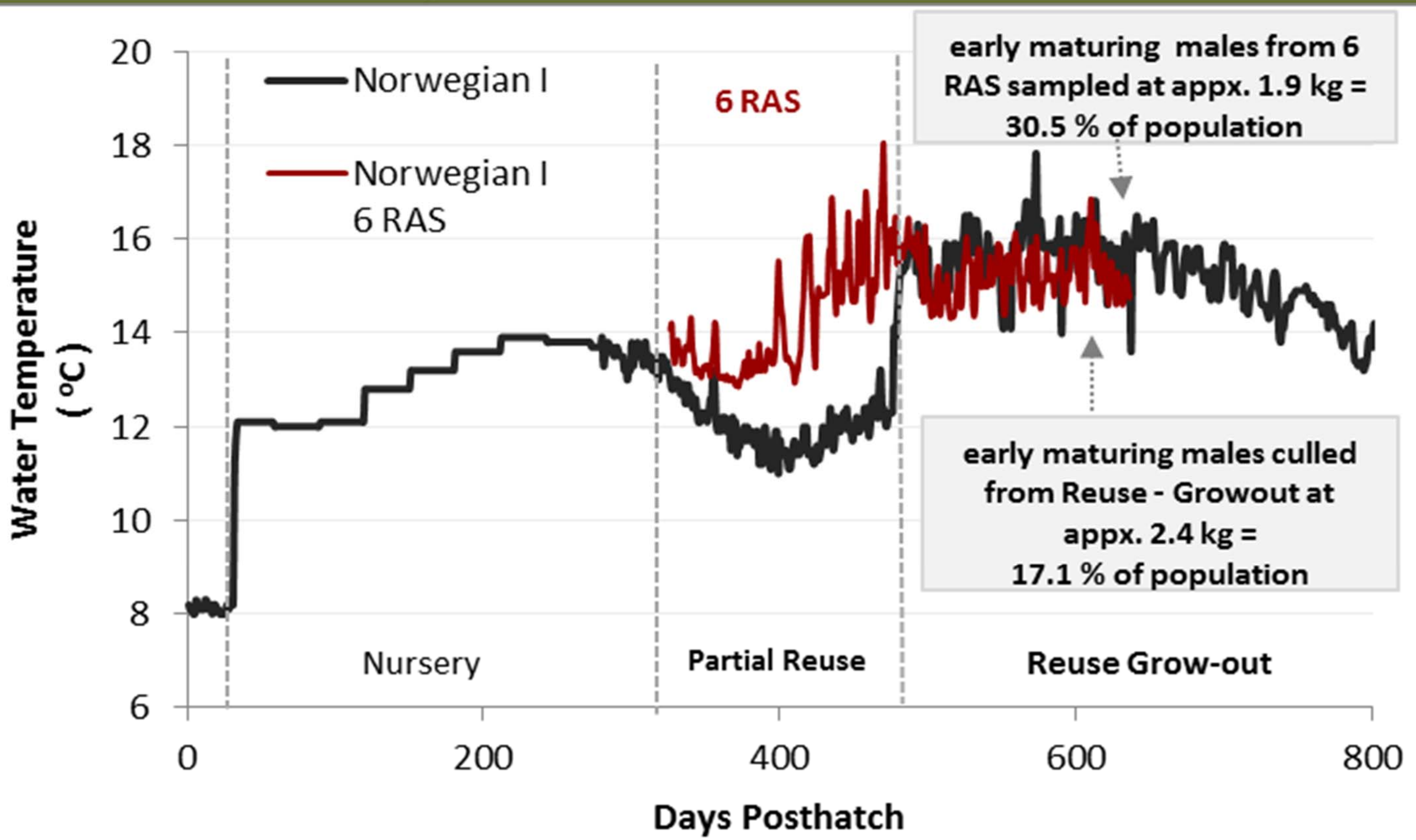
Water Temperature



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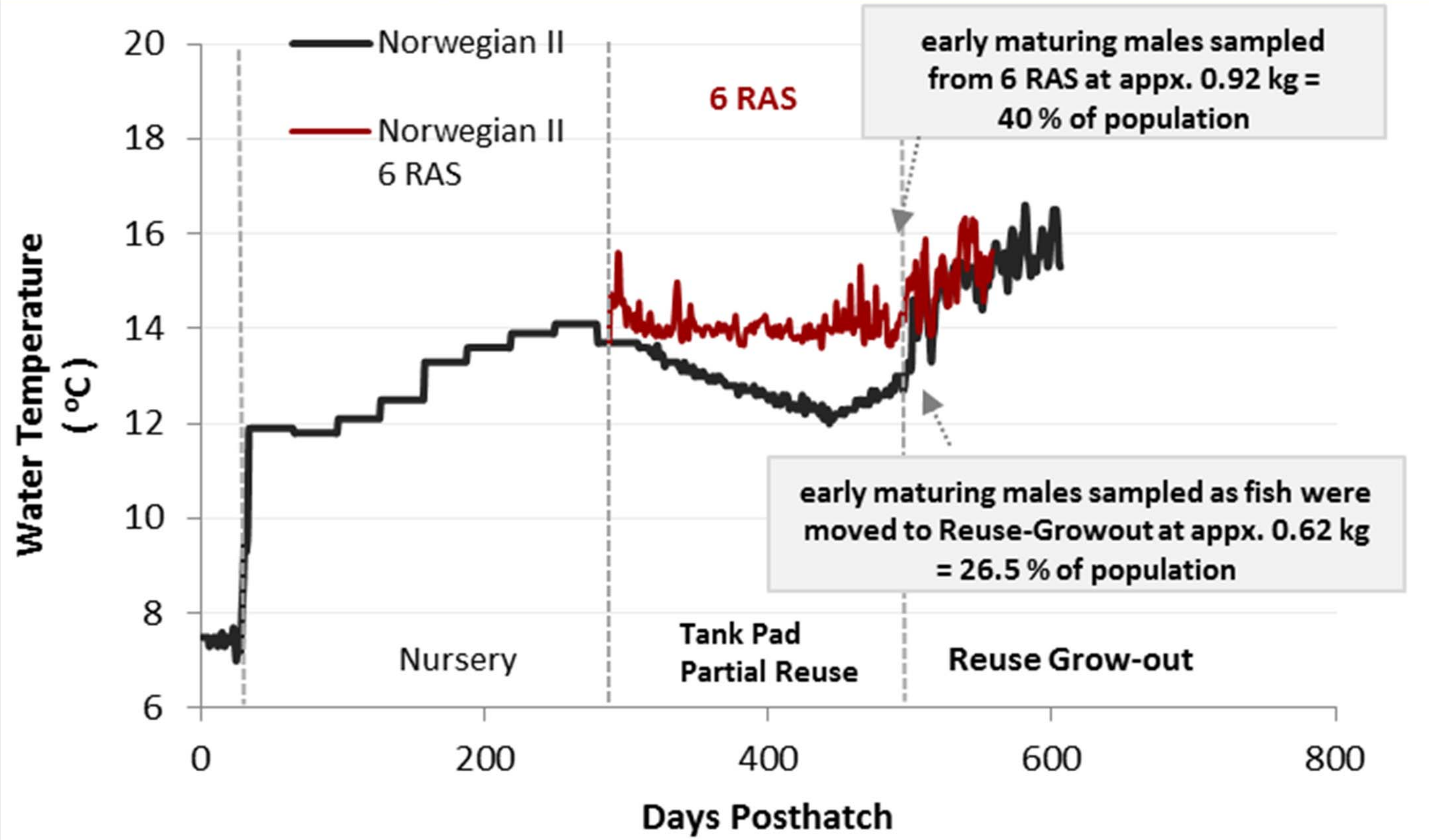
Water Temperature



Literature Review - Temperature

- **Fjelldal et al. (2011)**

  Atlantic salmon parr cultured at 16 ^{o}C with continuous light began to mature (47%) while salmon cultured at 5 and 10 ^{o}C did not mature.

- **Imsland et al. (2014)**

  Pre-smolt A. salmon cultured at 12.8 ^{o}C grew much faster but matured at a much greater rate (66 % vs. 11%) than salmon cultured at 8.3 ^{o}C.

  Concluded that photoperiod was the primary directive for maturation onset but temperature controlled the magnitude .

- **Melo et al. (2014)**

  Described a sudden change from 12 ^{o}C to 16 ^{o}C at the onset of post-smolt stage as a "maturation regime" for Atlantic salmon.

Future Research/ Early Recommendations

- What is the optimal temperature for post-smolt Atlantic salmon culture to market-size in RAS that balances growth and reduced maturation?

  Anecdotal evidence and literature review suggest 16 ^{o}C or lower could be upper limit

- How important is it to avoid sudden increases in water temperature within a RAS or during transfer between systems?

  Anecdotal evidence and literature review suggest that sudden increases in water temperature should be avoided

Physiological Triggers for Maturation

- Rapid growth rates/ fish size

  Taranger (2010) "…the reproductive system [of fish] is usually silenced until an individual's somatic development has proceeded sufficiently to permit investment in pubertal development."

  Policansky (1983) "Under stable conditions with abundant food, fish should grow rapidly and mature as soon as they are developmentally able to do so."

- Adipose tissue/ energy reserve threshold

  Herbinger and Friars (1991) linked grilsing to specific levels of lipids that are stored up in the spring

- Condition factor threshold

  Peterson and Harmon (2005) found that increasing condition factor and GSI were correlated in post-smolt Atlantic salmon

Maturation Control via Diet/ Feeding Regime ?

- Restricted ration at critical life stage

  No difference in maturation during restricted ration study at FI

- Lower lipid/ energy diets at critical life stage

  Increased lipid to reduce FCR – Is this counterproductive relative to early maturation??

  Jobling et al. (2002) found that reducing lipid content of Atlantic salmon diets resulted in reduced body lipid

  Should the closed containment industry consider life-stage specific diets with reduced lipids at certain life stages??

Exercise

- Sustained swimming exercise can prevent precocious maturation Palstra and Planas, 2011
- Adiposity?
- Whole body lipid content has been shown to influence early maturation Shearer et al., 2006
- No consensus regarding effects of exercise on overall energy deposition and whole body composition Rasmussen et al., 2011
- Exercise therefore likely to be exerting influence through other means

Research at The Freshwater Institute

Two swimming speeds:

<0.5 (BL/s)

1.5-2 (BL/s)

Two dissolved oxygen levels:

High: 100% saturation

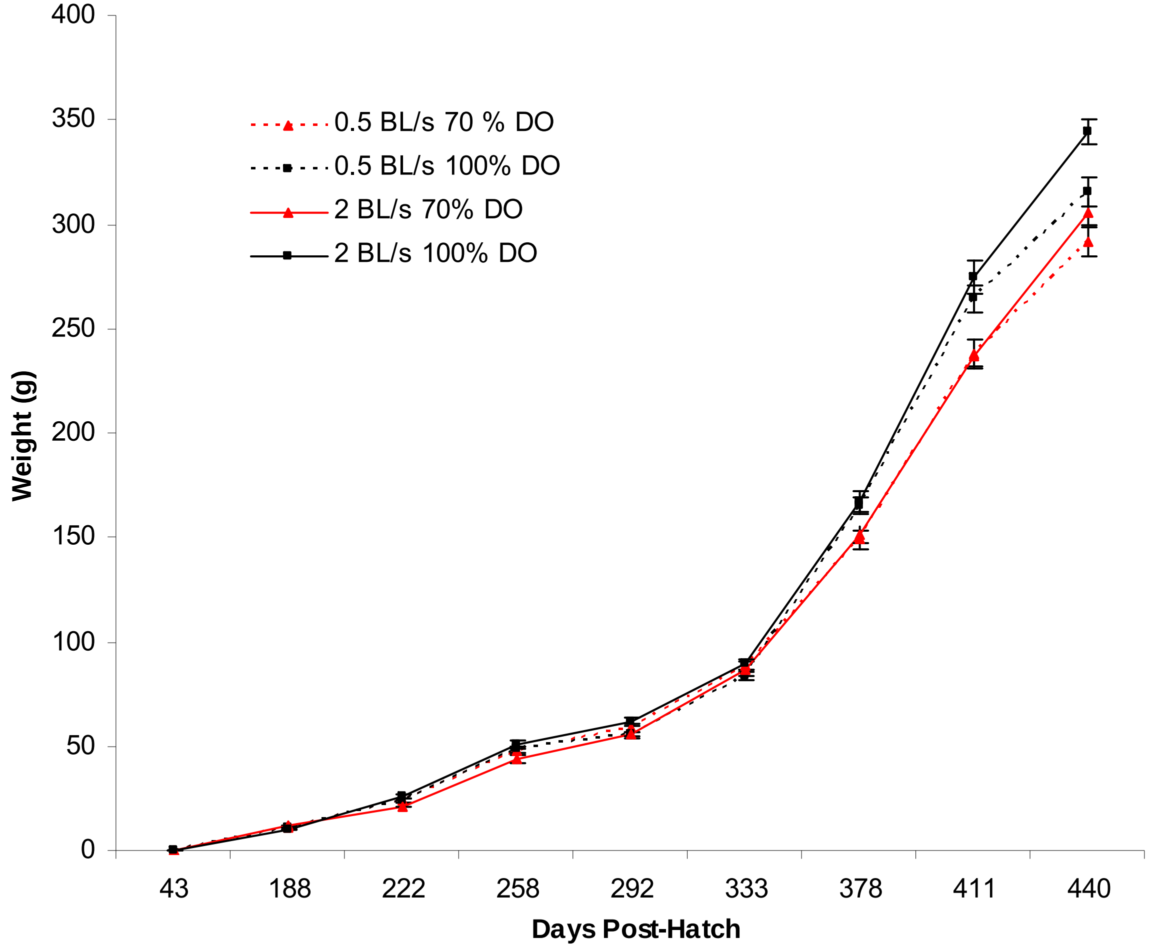
Low: 70% saturation

Exercise

Identical Flow-Through Circular Tanks

<u>12 tanks total:</u>
3 replicates of each
Swimming speed /DO combination

Exercise


0.5 BL/s 70 % DO
0.5 BL/s 100% DO
2 BL/s 70% DO
2 BL/s 100% DO
Weight (g)
400
350
300
250
200
150
100
50
0
43
188
222
258
292
333
378
411
440
Days Post-Hatch

Exercise

	DO 100% saturation		DO 70% saturation	
	2 BL/s	0.5 BL/s	2 BL/s	0.5 BL/s
Weight (g)	344.3 ± 6.3	315.8 ± 7.5	306.0 ± 6.87	292.2 ± 6.9

Treatment	df	F	p-value
Swimming speed	1	9.86	0.0018
Dissolved oxygen	1	18.95	<0.0001
Swimming speed X dissolved oxygen	1	1.35	0.2451

Precocious Males:

2 BL/sec : **6.4%**
< 0.5 BL/sec: **11.5%**

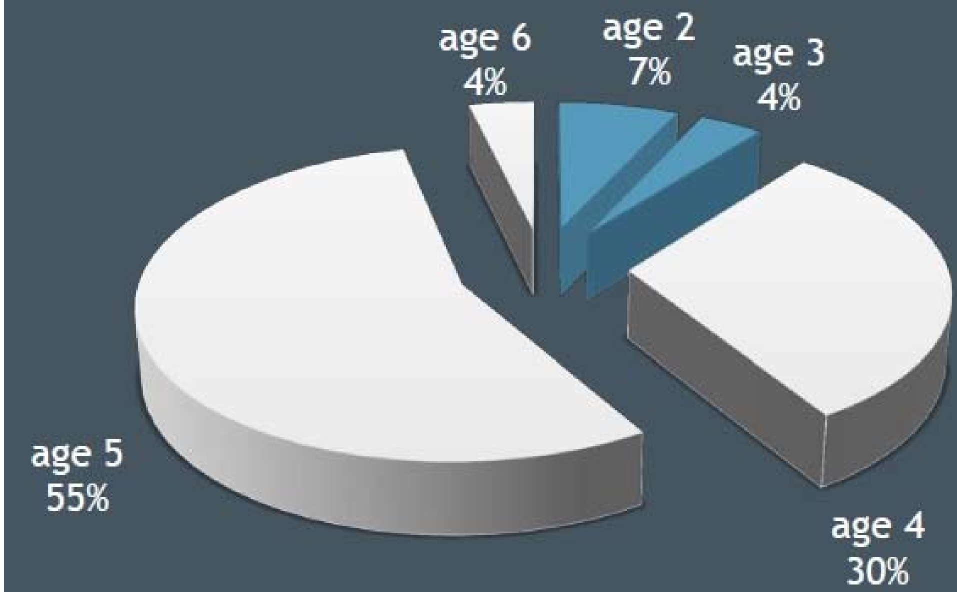
Logistic regression model reporting odds ratios for the probability of precocious males within each treatment group:

Treatment	Odds ratio	(95% CI)	p-value
0.5 BL/s	1.896	(1.121, 3.208)	0.017
70% DO	0.945	(0.546, 1.636)	0.839

Adult returns from 2009 release

Courtesy Joe Miller,
Anchor QEA

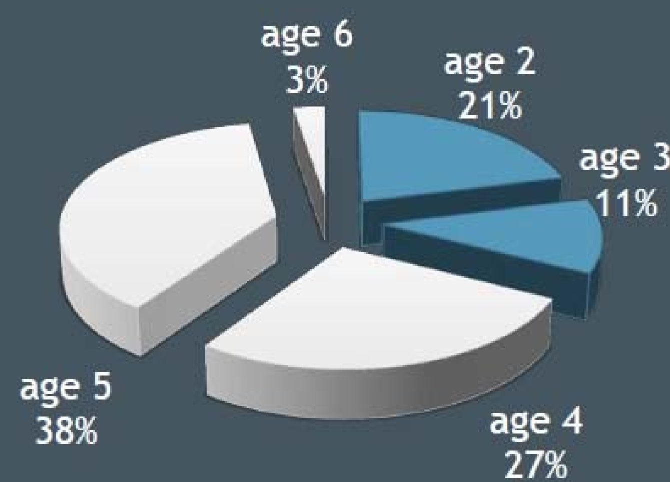
Circular



N = 177

SAR = 1.67 %

Raceway



N = 73

SAR = 0.81%

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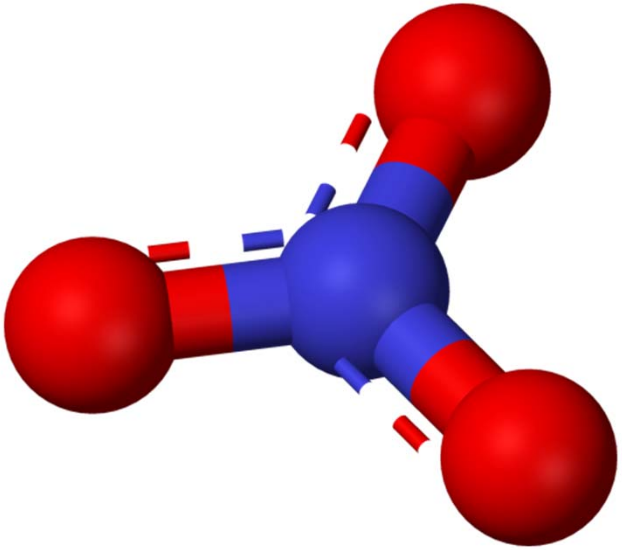
Water Chemistry

High Makeup H_2O Exchange (2.6%)

Low Makeup H_2O Exchange (0.26%)

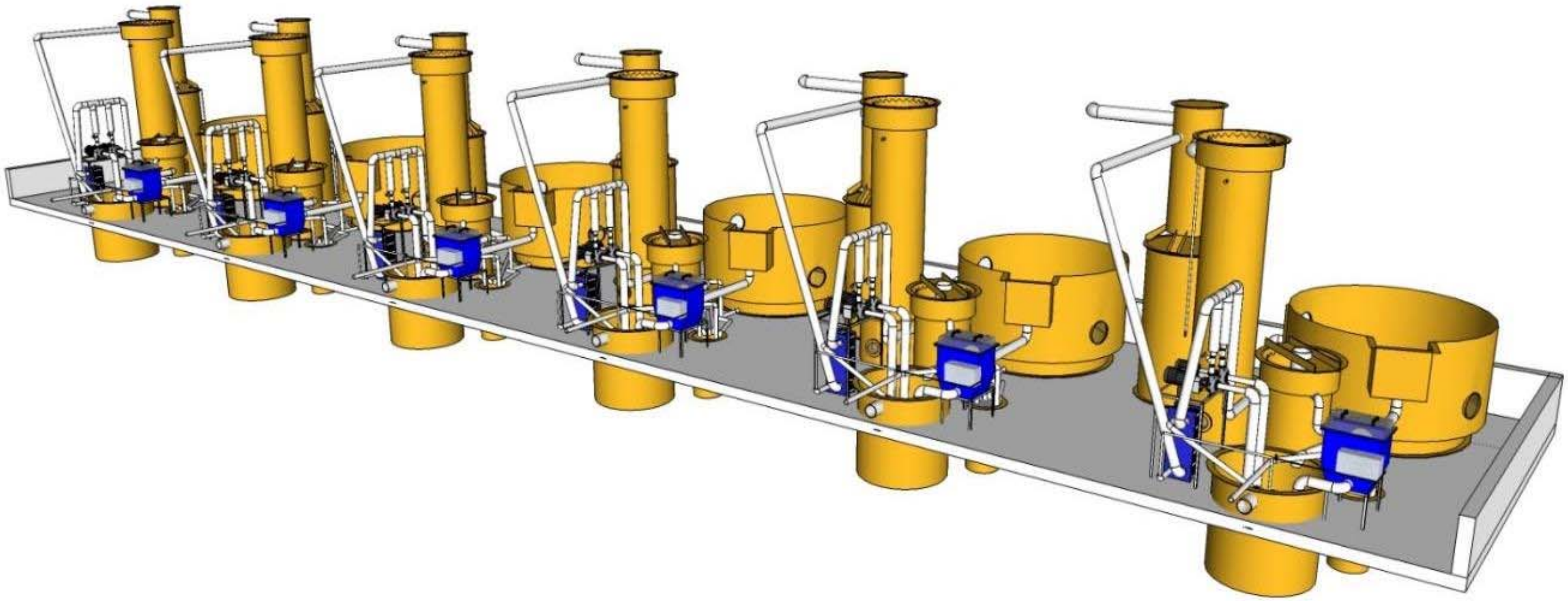
Nitrate nitrogen NO_3-N



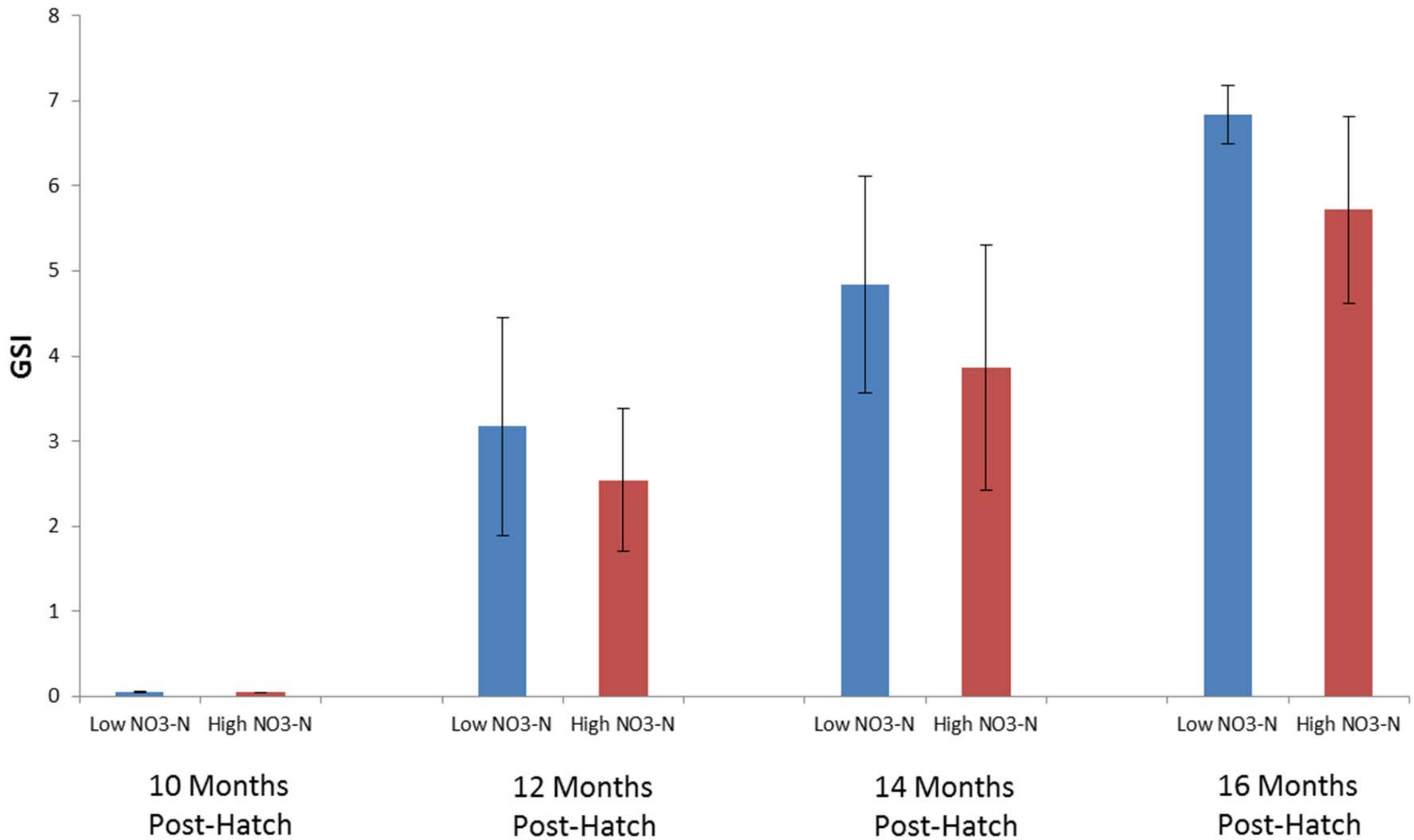
- End product of biofilter nitrification
- Accumulates in RAS relative to flow-through systems
- Has been shown to disrupt endocrine function in a variety of aquatic species
- Can influence plasma sex steroid levels in fish; more research needed

Long-term NO_3-N exposure

- 8-month trial to assess effects of NO_3-N on post-smolt Atlantic salmon
  - 100 mg/L vs. 10 mg/L



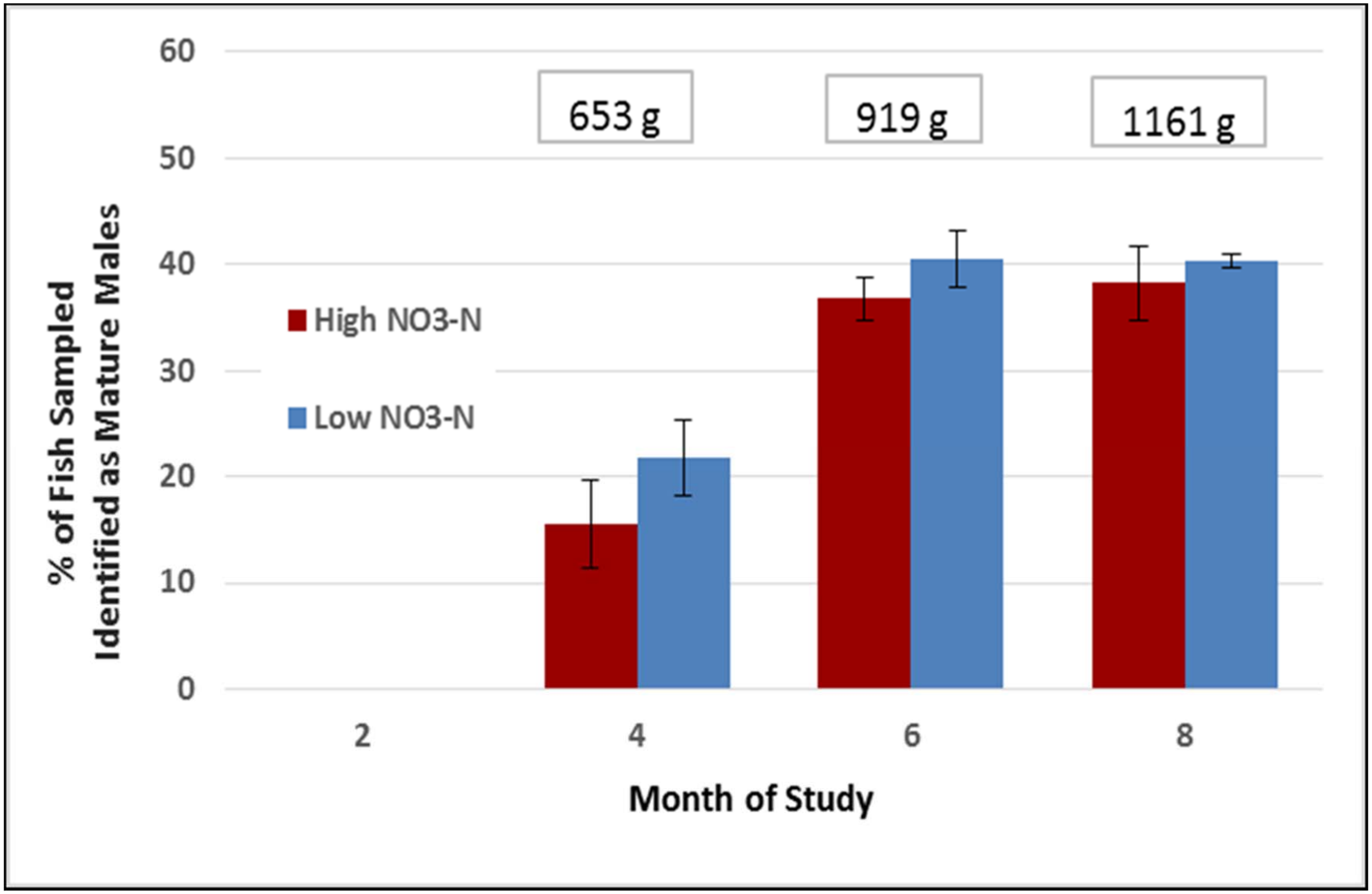
GSI - males


GSI
0
1
2
3
4
5
6
7
8
Low NO3-N
High NO3-N
10 Months Post-Hatch
12 Months Post-Hatch
14 Months Post-Hatch
16 Months Post-Hatch

GSI >0.5%

	% Grilse	
Fish Age	Low NO_3-N	High NO_3-N
10 months	0 (0/7)	0 (0/10)
12 months	67 (4/6)	70 (7/10)
14 months	71 (5/7)	50 (4/8)
16 months	100 (6/6)	78 (7/9)

Grilse based on observation



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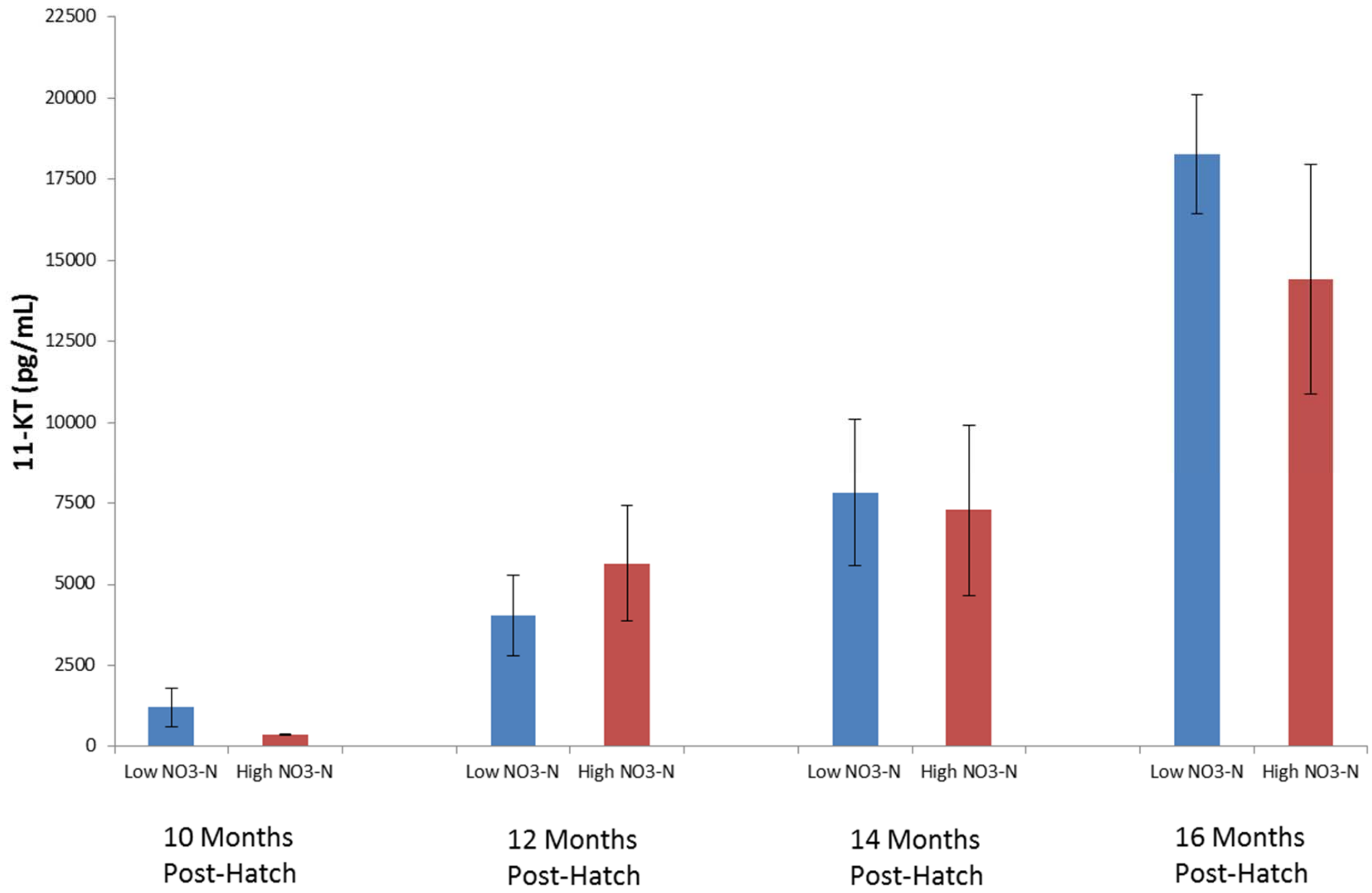
Plasma 11-KT

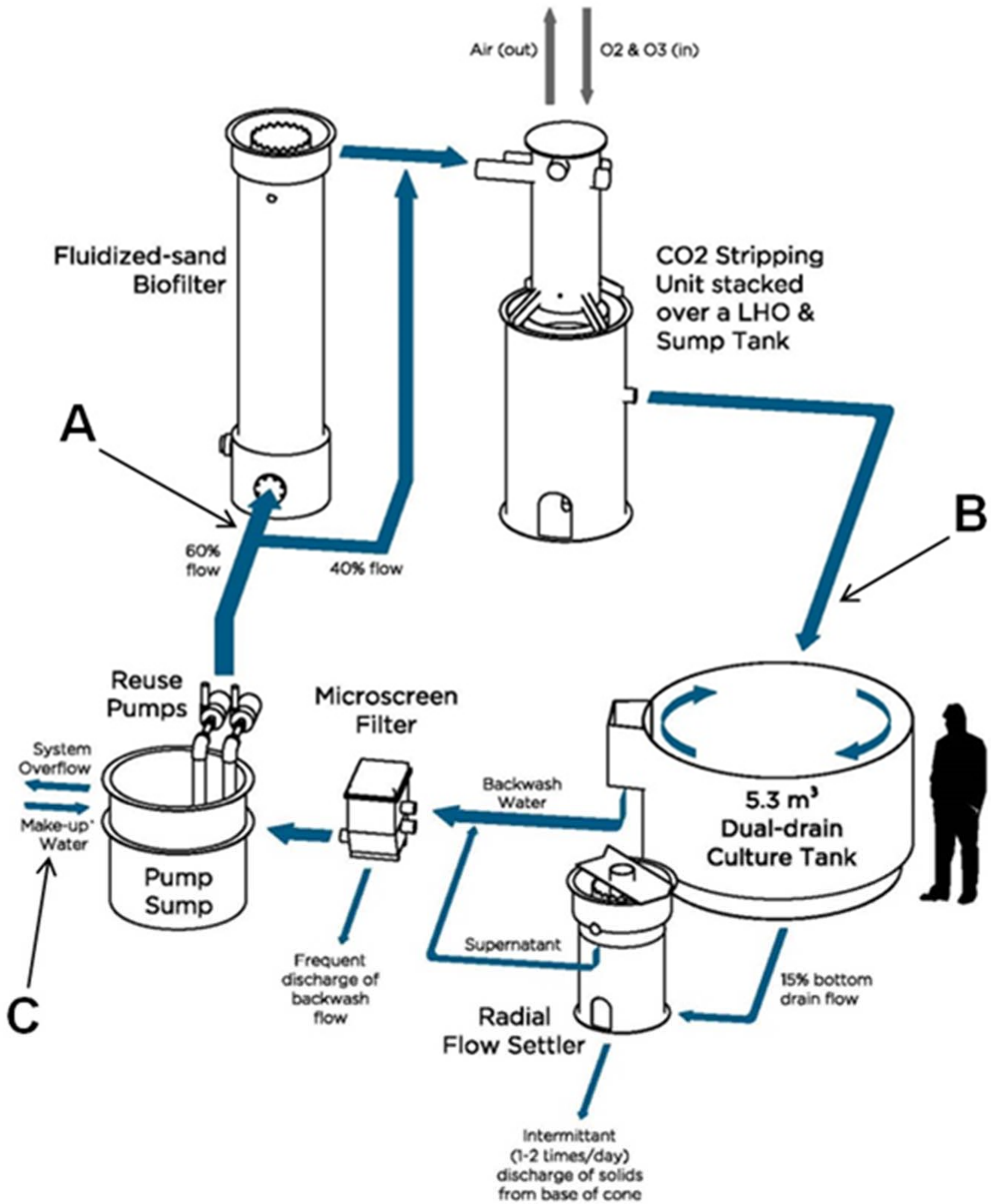


Conclusion

- No quantifiable difference in male maturation between NO_3-N treatment groups

Accumulation of steroid hormones?

e.g. Testosterone, 11-ketotestosterone, estradiol



Triplicate water samples collected from each RAS:

A – pre-water treatment processes
B – post-water treatment processes
C – makeup water influent

EIA quantification

Results

		Water sample location		
Hormone	Exchange rate	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]

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Results

		RAS	
	Sex	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]

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Hormonally active compounds LC-MS

Hormones

- 11-Ketotestosterone
- 17-α Estradiol
- 17-α Ethynylestradiol
- 17-β Estradiol
- 19-Norethindrone
- 4-Androstene-3,17-dione
- Trans-Diethylstilbestrol
- Equilin
- Equilenin
- Estriol
- Estrone
- Norgestimate
- Progesterone
- 6-α–methyl-17-α-hydroxyprogesterone
- Testosterone
- Epitestosterone
- Trenbolone

Hormone Conjugates

- 17 β-estradiol-3-sulfate
- 17 β-estradiol-17-sulfate
- Androsterone sulfate
- Equilenin sulfate
- Equilin sulfate
- Estriol-3-sulfate
- Estriol-17-sulfate
- Estrone-3-sulfate
- Ethinylestradiol-3-sulfate
- Testosterone sulfate
- 17 β-estradiol-17-glucuronide
- Androsterone-glucuronide
- diethylstilbesterol glucuronide
- Estriol-3-glucuronide
- Estrone glucuronide
- Ethenylestradiol-3-glucuronide
- Testosterone glucuronide

Phytoestrogens

- Genestein
- Daidzein
- Formonentin
- Coumesterol
- Equol
- Biochanin A

Mycotoxin

- A-zearalanol

Conclusion

- There is a vast amount of research still required in order to understand and manage this problem
- No concrete recommendations can be made based on existing research
- Best opportunity at present is the potential availability of all-female eggs

Acknowledgements

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