THE CONSERVATION FUND

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Smolt Production in the Future

Sunndalsøra, Norway

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Evaluating the Effects of Nitrate-nitrogen Concentrations with Heart Rate Bio-loggers

John Davidson
Senior Research Scientist
The Freshwater Institute

- Nitrification – Ammonia → Nitrite → Nitrate
- Feeding, water exchange, nitrification, & denitrification affect NO_3-N levels
  - We control NO_3-N via dilution by replacing backwash and RAS overflow
- Establishing a safe nitrate level instructs:
  - Required water use, feed loading rate, and system design criteria

Drum Filter Backwash Spray


RAS Overflow


Fluidized Sand Biofilters


Moving Bed Bioreactors

- Nitrate is less toxic than ammonia and nitrite, but it can negatively affect fish physiology.
- Davidson et al. (2017) - No effects on Atlantic salmon growth, health, or welfare - 10 vs. 99 mg/L NO_3-N
  - 0.1 kg to ~1.2 kg over an 8-month study
- Can we grow salmon post-smolts at >100 mg/L NO_3-N?

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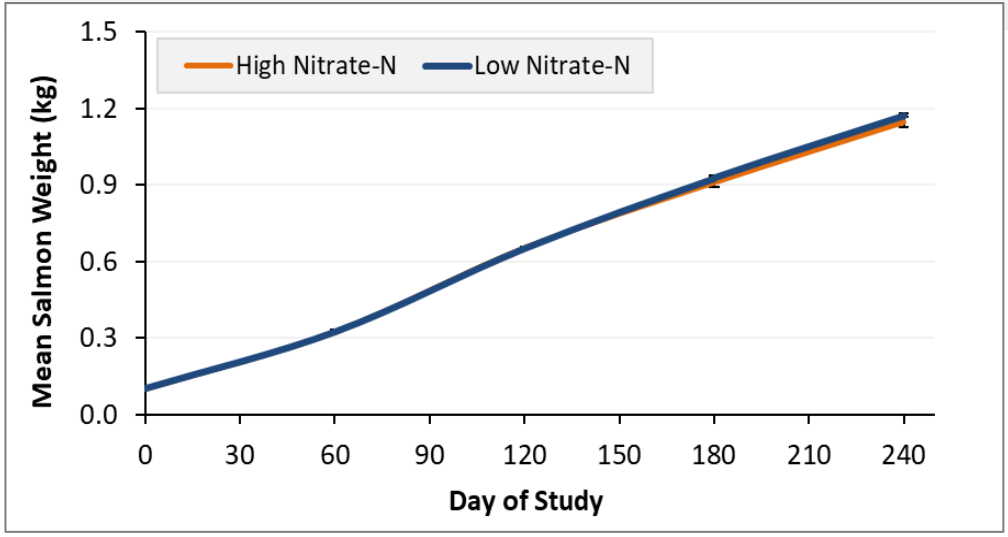
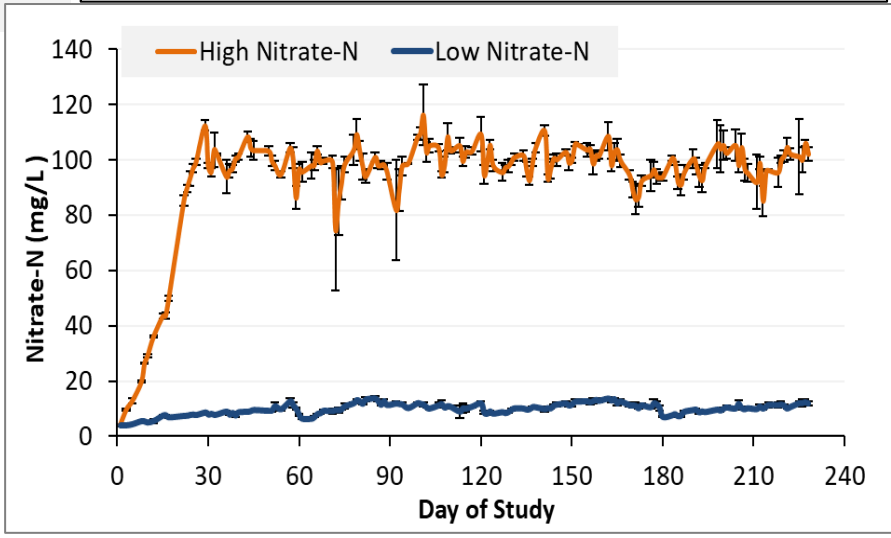
Evaluating the chronic effects of nitrate on the health and performance of post-smolt Atlantic salmon *Salmo salar* in freshwater recirculation aquaculture systems

John Davidson*, Christopher Good, Christina Williams, Steven T. Summerfelt

The Conservation Fund's Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443, United States





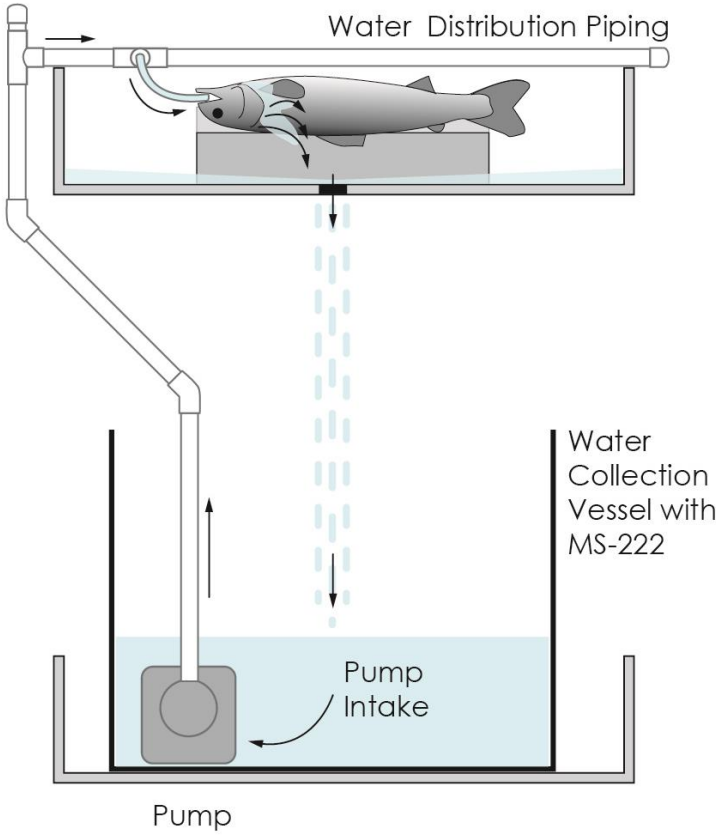
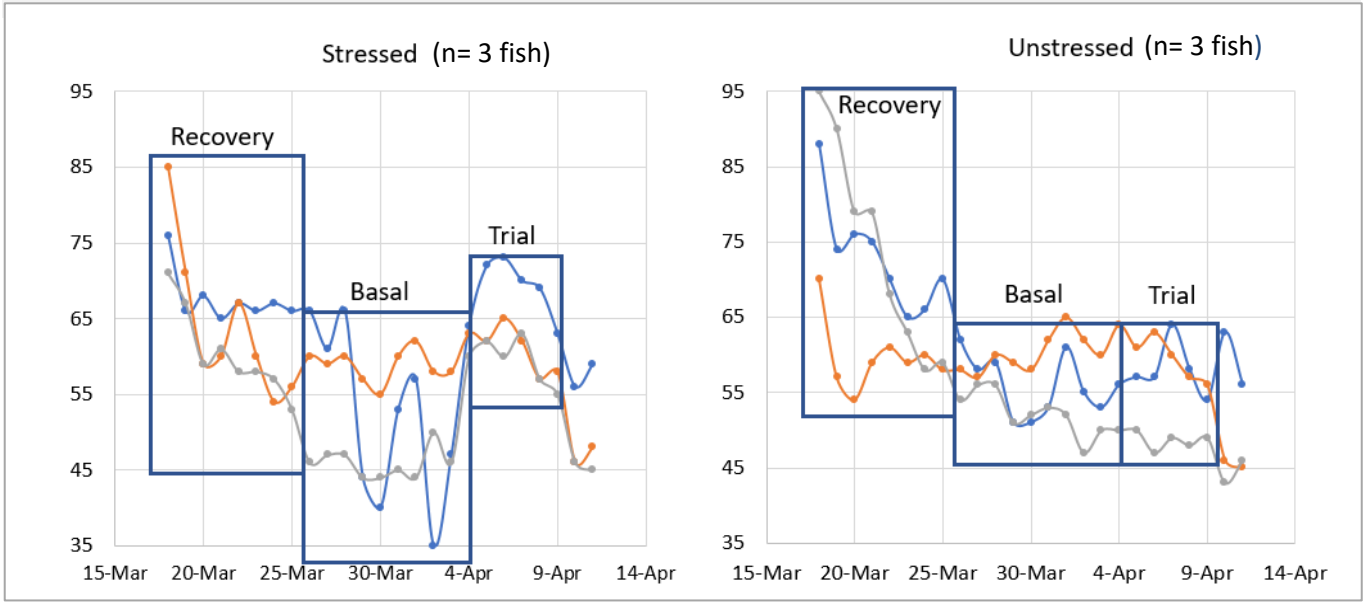
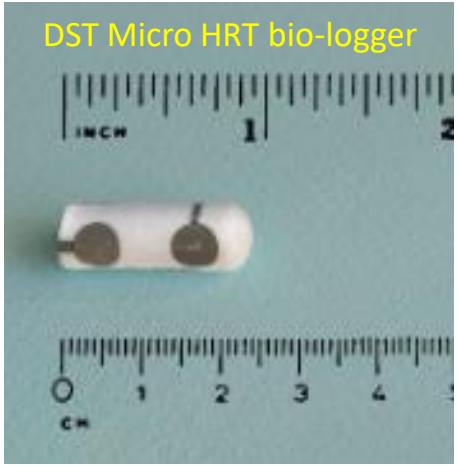


Fig. from Davidson et al (2024). Aquac. Eng. 107, 102461.

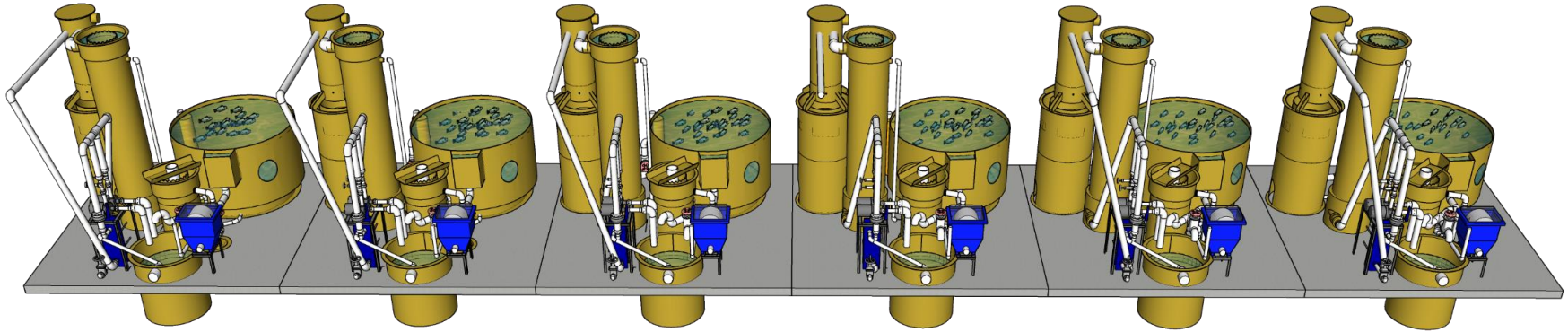
- Heart rate bio-loggers have been used to assess fish stress and the suitability of fish husbandry practices.
  - Not previously used to assess RAS environment effects
- Before the main study, we familiarized ourselves with the bio-logger implant procedure.
- Constructed a surgery table that recirculated oxygenated water containing a mild sedative.

- **Pilot study** - Implanted bio-loggers in six salmon (200 – 250 g)
  - Stocked in two flow-through tanks (3 fish/tank)
- After 3 weeks, we chased fish with a net in one tank to observe changes in heart rate.
- Proof of concept and guidance for the necessary recovery period



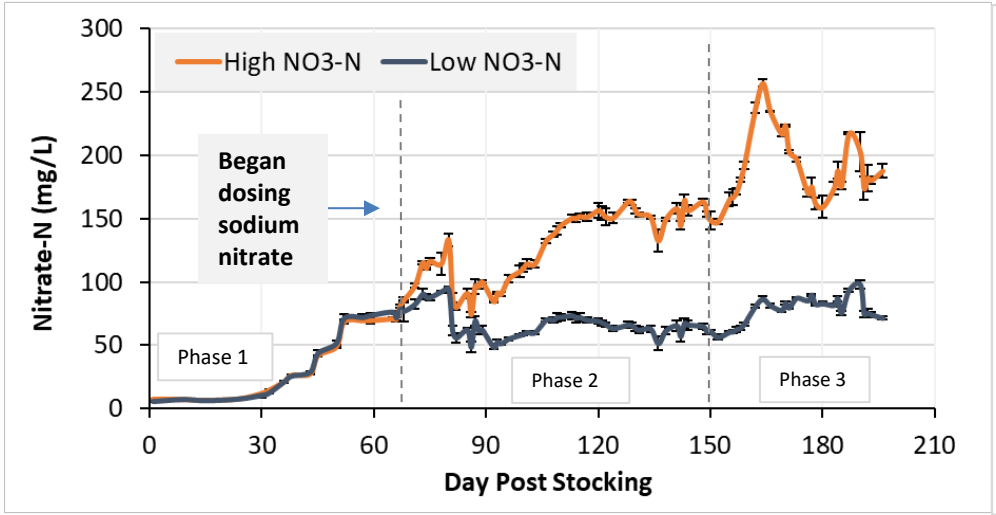


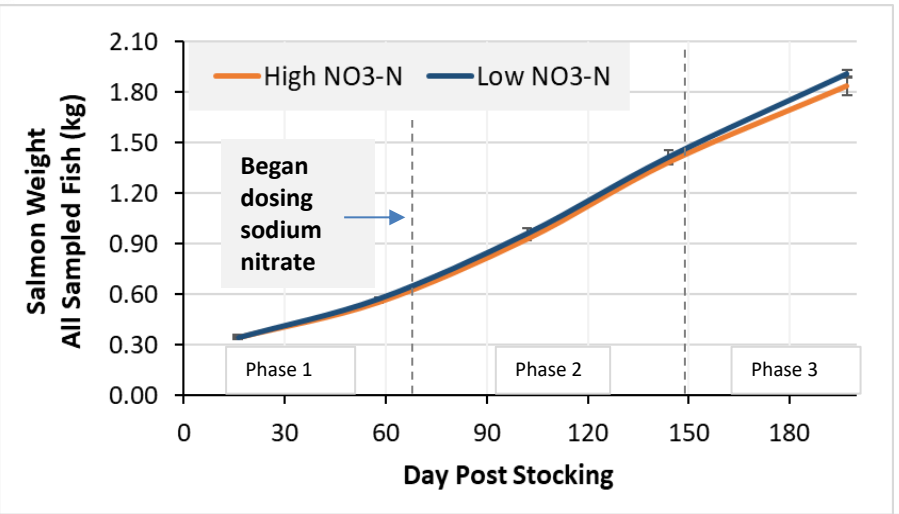
- Six replicated RAS - 5.3 m^3 tanks; 9.5 m^3 total volume
  - 50 - 100 mg/L vs. 100 - 250 mg/L NO_3-N (3 RAS/treatment)
  - 227 all-female Atlantic salmon (79% diploid, 21% triploid), 0.32 kg to begin
  - 6 salmon with bio-loggers per RAS
- Natural NO_3-N accumulation to 50 - 100 mg/L in all RAS
  - ~12-day system hydraulic retention time
- Sodium nitrate dosed via peristaltic pump to achieve higher NO_3-N levels
- Sodium sulfate dosed to balance Na+ and conductivity in the control RAS



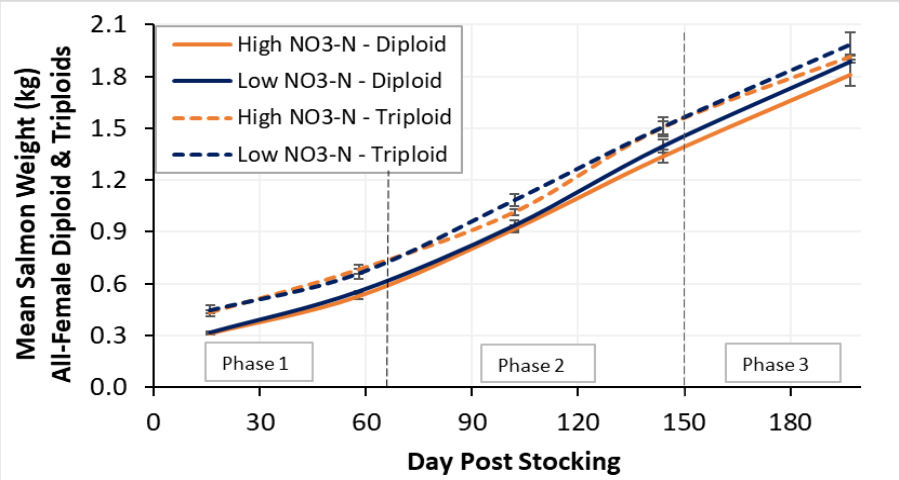
- Water quality control is essential in toxicity studies.
- However, there was a trade-off for balancing ionic conditions.
  - Higher sodium & sulfate in control RAS due to sodium sulfate dosing
  - Non-toxic to salmonids

* *Significant difference*	High NO_3-N	Low NO_3-N
Dissolved Oxygen (mg/L)	10.4 ± <0.01	10.5 ± 0.2
Temperature (°C)	13.0 ± 0.1	12.9 ± 0.1
Alkalinity (mg/L)	151 ± 5	146 ± 1
Hardness (mg/L as $CaCO_3$)	329 ± 1	333 ± 2
Carbon Dioxide (mg/L)	6.1 ± 0.1	6.1 ± <0.1
Dissolved Copper (mg/L)	0.014 ± 0.001	0.016 ± 0.001
Dissolved Potassium (mg/L)	6.3 ± 0.1	6.3 ± 0.1
Total Ammonia Nitrogen (mg/L)	0.23 ± 0.01	0.23 ± <0.01
Nitrite Nitrogen (mg/L)	0.022 ± 0.004	0.014 ± 0.002
Nitrate Nitrogen (mg/L) *	147 ± 1	71 ± 1
Sodium (mg/L) *	217 ± 3	249 ± 2
Sulfate (mg/L) *	35 ± <1	256 ± 2
Specific Conductance (µS/cm) *	1.86 ± 0.01	1.92 ± 0.01
Total Suspended Solids (mg/L)	1.8 ± 0.2	1.6 ± 0.1





- NO_3-N increasing from 100 to ~250 mg/L did not affect post-smolt A. salmon growth (*P*>0.05).
- Trend for slight, albeit non-significant decrease in growth rate above ~150 mg/L NO_3-N
- No effect on all-female diploid or triploid growth

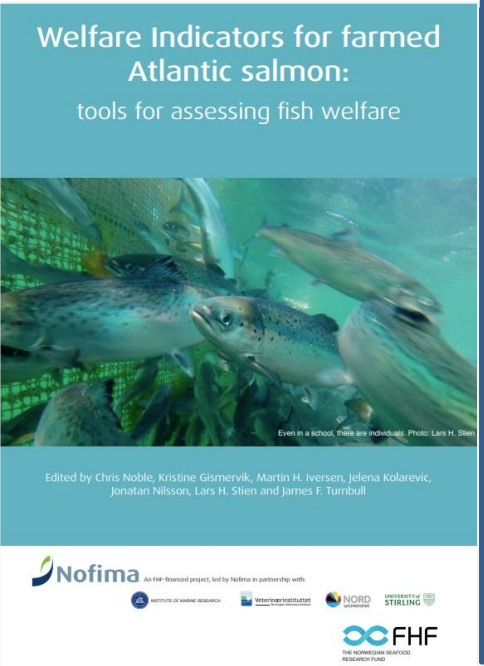


- High survival for both treatments across all study phases
- Trend for higher FCR during Phase 3

	High NO_3-N	Low NO_3-N	High NO_3-N	Low NO_3-N	High NO_3-N	Low NO_3-N
	Phase 1		Phase 2		Phase 3	
Avg. NO_3-N (mg/L)	31 ± <1	31 ± <1	*122 ± 1	67 ± 2	*186 ± 3	76 ± 1
NO_3-N range (mg/L)	8 - 74	7 - 78	108 - 172	45 - 79	135 - 261	53 - 105
Mean Weight A-F Diploids (kg)	0.53 ± 0.01	0.55 ± 0.01	1.34 ± 0.04	1.40 ± 0.04	1.81 ± 0.07	1.89 ± 0.01
Mean Weight A-F Triploids (kg)	0.68 ± 0.03	0.66 ± 0.03	1.50 ± 0.04	1.51 ± 0.06	1.91 ± 0.02	1.99 ± 0.07
Survival (%)	99.3 ± 0.4	99.7 ± 0.1	99.1 ± 0.1	99.8 ± 0.1	99.8 ± 0.2	100
FCR (economic)	1.01 ± 0.01	0.90 ± 0.06	1.09 ± 0.02	1.08 ± 0.05	1.28 ± 0.08	1.07 ± 0.14
Thermal Growth Coefficient	2.26 ± 0.06	2.46 ± 0.10	2.65 ± 0.01	2.65 ± 0.07	1.62 ± 0.13	1.74 ± 0.18
Maximum Fish Density (kg/m^3)	39.6 ± 0.6	41.3 ± 0.9	58.3 ± 0.2	60.1 ± 1.0	75.2 ± 1.3	79.3 ± 1.5
A-F Diploid External Signs of Maturity (%)	7.6 ± 3.1	7.3 ± 1.2	6.6 ± 2.3	9.8 ± 4.5	7.3 ± 2.7	4.6 ± 2.3

No differences in welfare metric scores

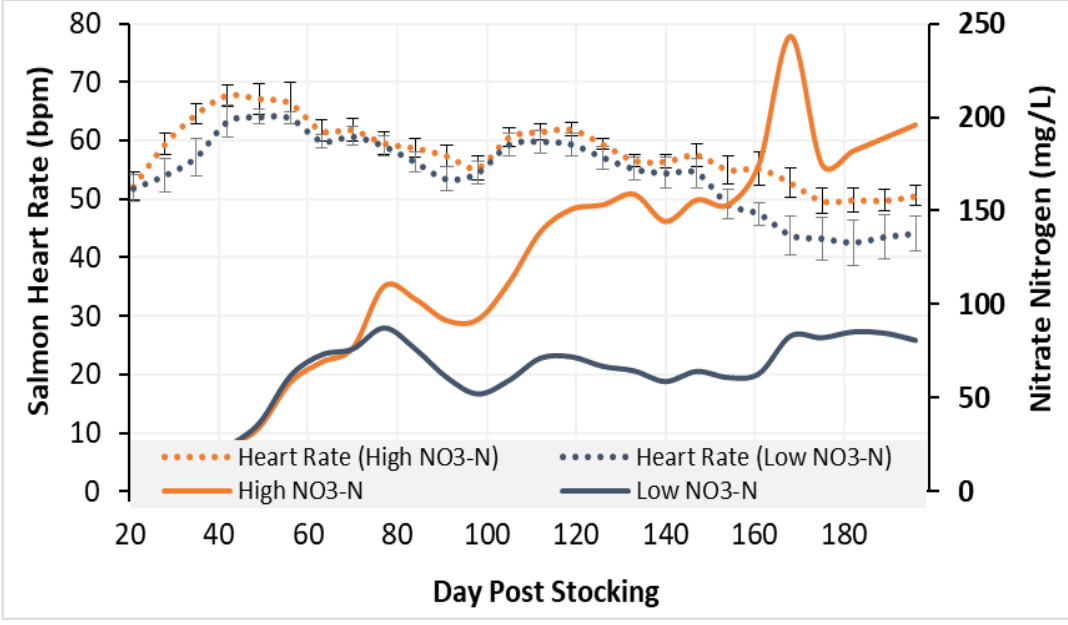
- Fins, cataracts (P>0.05)

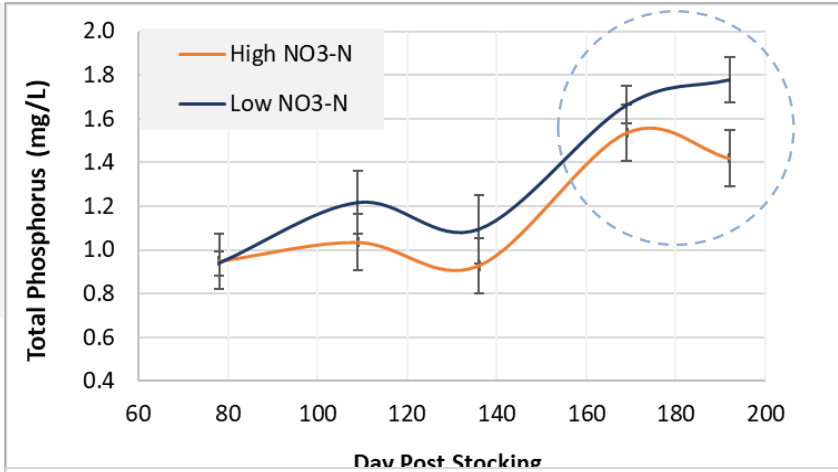


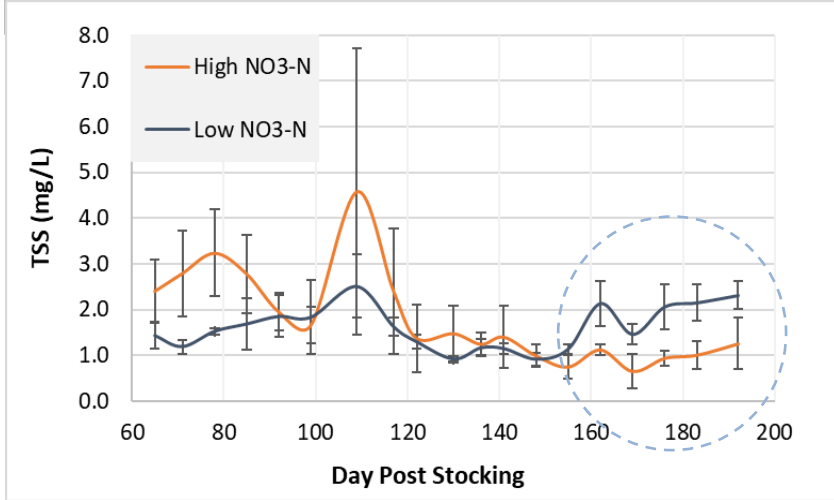
Treatment	Welfare Metric	Baseline	Phase 1	Phase 2	Phase 3
High NO_3-N	Dorsal Fin Score	1.2 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8 ± <0.1
Low NO_3-N	Dorsal Fin Score	1.2 ± 0.1	1.7 ± <0.1	1.7 ± 0.1	1.8 ± 0.1
High NO_3-N	Caudal Fin Score	0.8 ± <0.1	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.1
Low NO_3-N	Caudal Fin Score	0.8 ± <0.1	1.4 ± 0.1	1.4 ± 0.1	1.2 ± <0.1
High NO3-N	Left Eye Cataracts	0.1 ± <0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Low NO3-N	Left Eye Cataracts	0.1 ± <0.1	0.5 ± <0.1	0.3 ± 0.1	0.4 ± 0.1
High NO3-N	Right Eye Cataracts	0.4 ± 0.1	1.5 ± <0.1	1.2 ± 0.1	1.4 ± 0.1
Low NO3-N	Right Eye Cataracts	0.5 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.1 ± 0.1

Salmon Heart Rate & Overlapping Responses

- Trend for increased heart rate >150 mg/L NO_3-N
- Higher plasma hematocrit (%PCV) and hemoglobin (g/dL) (P<0.001) and chloride (P=0.037) - high NO_3-N RAS – Day 180
- Water quality suggested reduced feeding – high NO_3-N RAS







Conclusions

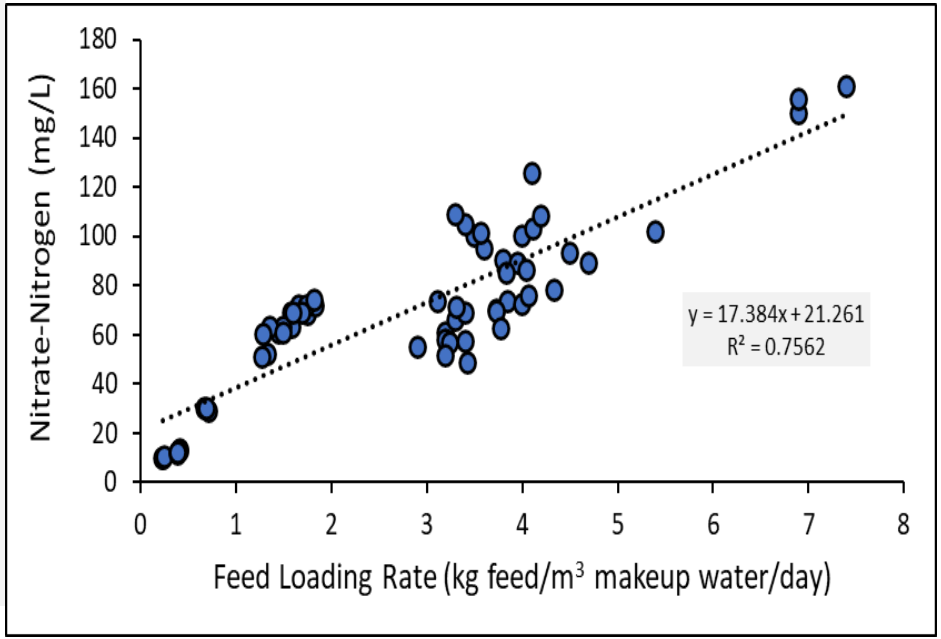
- Under these conditions *(e.g., freshwater, hard water, fish size),* NO_3-N up to 250 mg/L did not affect salmon growth or survival (*P*>0.05).

- Trends for faster heart rate, higher FCR, and different blood chemistry observed at >150 mg/L NO_3-N
  - A salmon farmer would likely culture fish at NO_3-N levels of no observable effect

- Higher feed loading and less water required/ kg feed
  - 278 L makeup water/ kg feed at 100 mg/L NO_3-N
  - 143 L makeup water/ kg feed at 150 mg/L NO_3-N

- But what about nitrogen discharge?

- Depends on site-specific variables and decisions:
  - Water availability, discharge requirements
  - Decouple denitrification to reduce complexity
  - Effluent treatment with membrane biological reactors, wetlands, aquaponics



Acknowledgments

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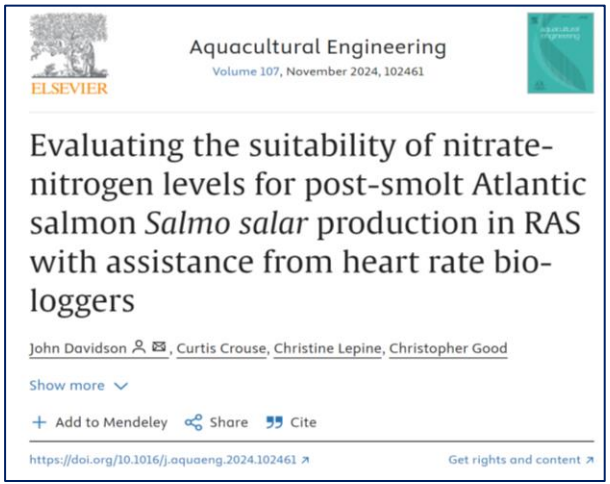
- All experimental protocols complied with the Animal Welfare Act (9CFR) and were approved by the Freshwater Institute's Animal Care and Use Committee.

- Special thanks to our Fish Production, Water and Environmental Chemistry, Operations, and Engineering teams for their support during this project.



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Questions?

John Davidson
Senior Research Scientist
jdavidson@conservationfund.org

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