#### THE CONSERVATION FUND

America's Partner in Conservation

### Fish Health & Welfare in Closed Containment Systems



**Christopher Good** 

#### Advantages of Closed Containment

With biosecure source water and specific pathogen-free eggs:

- No vaccinations required
- Reduction or elimination of antibiotic and chemotherapeutant usage
- More environmental control to reduce stress





#### Advantages of Closed Containment



#### Advantages of Closed Containment

#### **Opportunistic pathogens**

still need to be considered:

- Bacterial gill disease
- Columnaris
- Fungal infections
- Protozoans

Control conditions to prevent opportunists from gaining "upper hand"



#### Stress Reduction



#### **Stress Physiology**

- Stressful stimuli result in increased circulating cortisol levels
- Elevated cortisol leads to immunosuppresion:
  - Decreased serum bactericidal activity
    - Complement, lysozyme
  - Impaired phagocytosis
  - Decreased white cell count
  - Decreased antibody production

#### Stress Reduction

America's Partner in Conservation

#### Types of stress

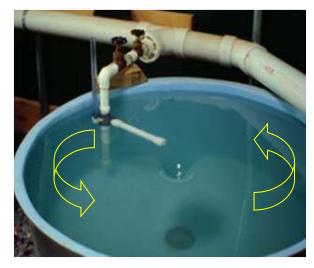
- Chemical
  - Poor diet, suboptimal water quality
- Biological
  - Pathogens, aggression, crowding
- Physical
  - Light, sound, temperature
- Husbandry
  - Handling, transport, treatments

#### Stress Response:

- Depends on the magnitude and duration
- Stressors are often cumulative

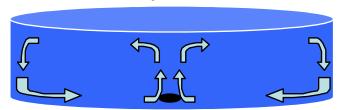
### **Environmental Management:** Circular Tanks

- Tank hydraulics can be optimized
- Solids flushing can be very rapid, < 1-3 min
  - Tanks are self-cleaning
  - Fresh fecal pellets are flushed before they breakdown
  - Solids are not stored in the culture tank primary flow



primary rotating flow

secondary radial flow



### **Environmental Management:** Circular Tanks

### **Outcomes:**

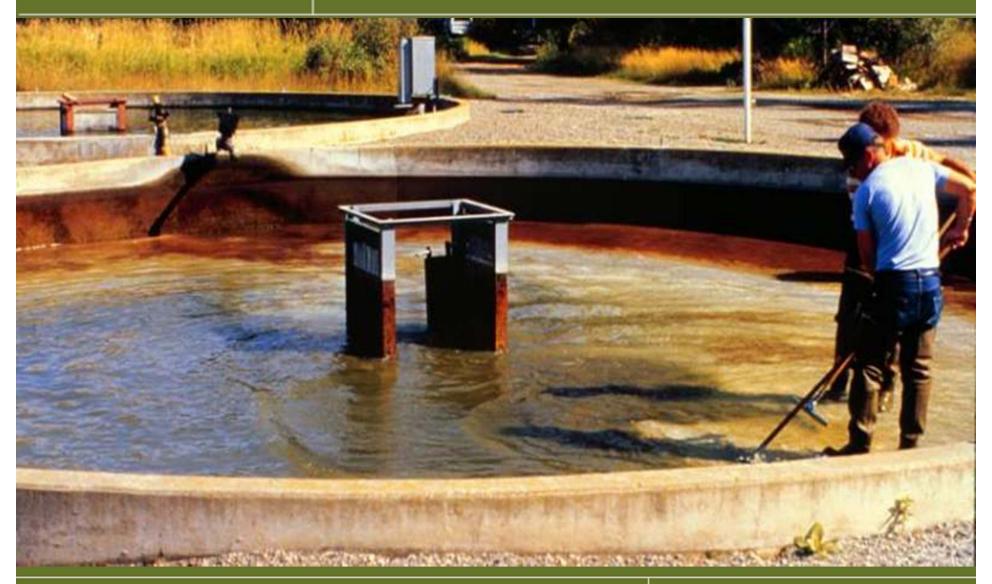
Excellent
solids control
and
uniform water
quality
throughout tanks



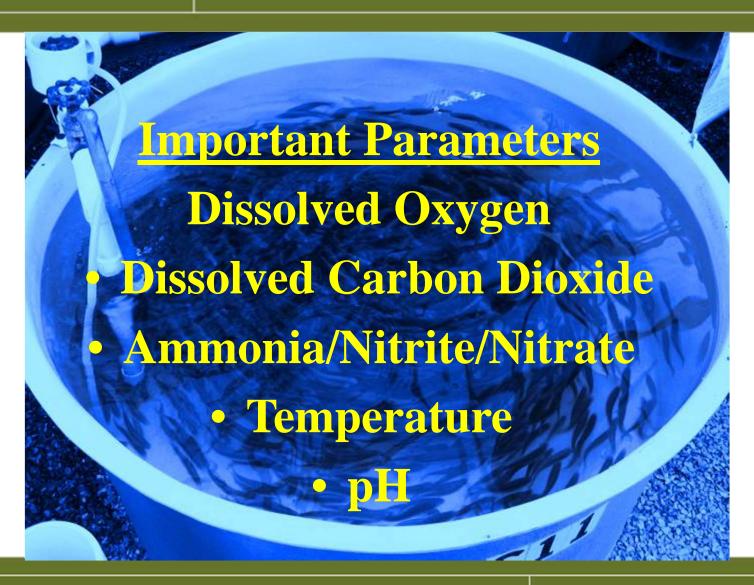
#### THE CONSERVATION FUND

America's Partner in Conservation

### Circular Tanks Without Rotation



### Water Quality: RAS



### Dissolved Oxygen

- Maintaining optimal DO critical in intensive recirculation systems
- O<sub>2</sub> poorly soluble in water
- Max. DO rarely exceeds 10-12 mg/L in H<sub>2</sub>O
- For resting salmonids:
  - 5-20 L H<sub>2</sub>O/kg body wgt/hour irrigation rate
  - 40-60/minute ventilation rate
    - Energy cost: at least 10% of O<sub>2</sub> consumed

### Dissolved Oxygen

- DO consumption rates:
  - Approx. 100 mg O<sub>2</sub>/kg body wgt/hour (resting)
  - As high as 800 mg O<sub>2</sub>/kg body wgt/hour (actively swimming)
- 85-100% saturation of O<sub>2</sub> required to maintain maximum growth rates in salmonids
- Pure oxygen can be added to system water through low-head oxygenators
  - Can maintain tank H<sub>2</sub>O at saturation

### Dissolved CO<sub>2</sub>

- America's Partner in Conservation
  - Salmonids produce 0.96 1.10 g CO<sub>2</sub> per gram of O<sub>2</sub> consumed
  - Inverse relationship of CO<sub>2</sub> with pH
  - Long-term exposure to elevated CO<sub>2</sub>
    - 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

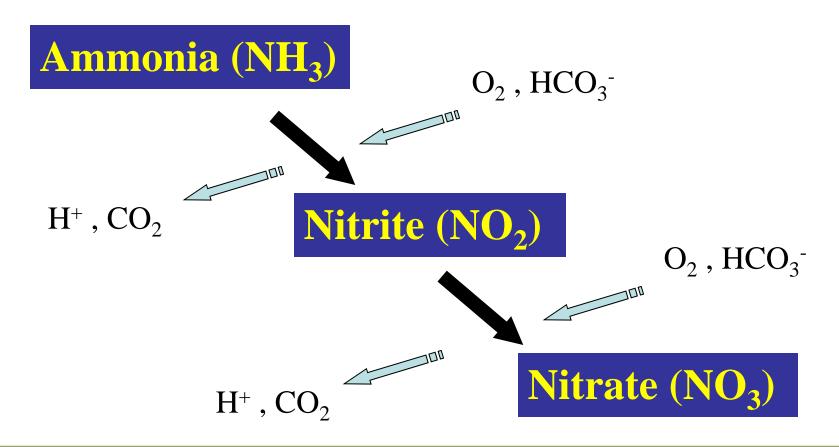
### Dissolved CO<sub>2</sub>

- Growth of salmonids significantly reduced at  $\geq$ 30 mg CO<sub>2</sub>/L
- Maximum limit  $< 20 \text{ mg CO}_2 / L$ 
  - Recent review suggests 10 mg CO<sub>2</sub> / L max for Atlantic salmon

$$NH_3 + H^+ + OH^- \leftrightarrow NH_4^+ + OH^-$$

- Ammonia: end product of protein catabolism
  - Excreted through gills
  - Rate of nitrogen excretion increases with feeding rate
  - Unionized: NH<sub>3</sub> (most toxic)
    - Direct effects on central nervous system
    - Gill damage
    - Osmoregulatory disruption
  - Ionized: NH<sub>4</sub>
  - Balance of unionized vs. ionized: pH dependent

#### **BIOFILTRATION**



- Nitrite ions taken up through gills, bind to hemoglobin to produce methemoglobin
  - Reduction of blood oxygen transport capacity
  - "Brown blood disease"
- Nitrite toxicity influenced by bicarbonate, potassium, sodium, calcium, and most importantly chloride
  - LC<sub>50</sub> nitrite: 0.24–12.20 mg/L depending on chloride concentration
  - Chloride cells in gills do not distinguish chloride from nitrite
- Recommended limit: <1 mg/L</li>
  - < 0.1 mg/L in soft water

- Very low toxicity, and not normally a concern
- Literature: Mortalities occur when levels exceed 1000 mg/L
- Causes non-specific osmoregulatory failure
- Recent evidence at FWI that near-zero exchange RAS (with elevated nitrate) are associated with mortality, deformities, and erratic swimming

### Temperature

- Ectothermic animals
- Increased T = increased metabolism
- Directly effects fish physiological processes:
  - Respiration rate
  - Feed efficiency and assimilation
  - Growth
  - Behavior
  - Reproduction
- Different pathogens thrive at different temperatures

#### Temperature

America's Partner in Conservation

- Water temperature manipulation very important during early life stages of Atlantic salmon:
  - Egg incubation at >8°C can induce skull and vertebral deformities in fry
  - Increased rearing temperature associated with increased vertebral deformities by 60g in size
    - $12^{\circ}C 2\%$
    - 14°C 13%
    - 16°C 15%
    - 18°C 22% Baeverfjord et al.





- 6.5 8.5 recommended range
- 4.8 > pH > 9.2 can damage and kill salmonids
- pH also has a significant influence on toxicities of e.g. ammonia, hydrogen sulfide, and heavy metals
- In RAS, biofiltration gradually consumes the pH-buffering capacity (i.e. alkalinity) of system water
- Therefore, very important to monitor changes and add sodium bicarbonate when necessary

### **Environmental Management: Density**

- Maximum biomass for a given flow rate determined by:
  - Fish metabolic rate e.g. oxygen consumption
  - Water quality e.g. DO, waste treatment or dilution
- Increased density, in general
  - Reduced feed intake
  - Reduced FCR
  - Reduced growth
  - Reduced body and fin condition
  - Increased risk of clinical disease outbreaks

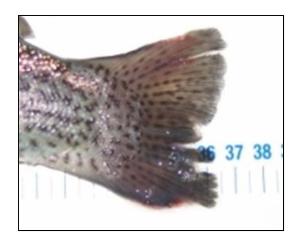
### **Environmental Management: Density**

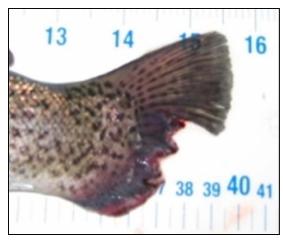
- Inconsistent findings in the scientific literature
- For Atlantic salmon, no consistent density effect up to around 80 kg/m<sup>3</sup>
- Some studies report reduced growth at densities higher than 80 kg/m<sup>3</sup>

### **Environmental Management: Density**

### **Fin Erosion**

- Common welfare index
- For rainbow trout, optimum densities lie between 40 80 kg/m<sup>3</sup>
- Need further research on this and other welfare indices for Atlantic salmon





### **Environmental Management: Optimum Swimming Velocity**

- Optimum swimming velocity in circular tanks
  - = (0.5 to 2.0) x (fish body length)/second



- Velocities in a 'donut-shaped' region about tank center are reduced:
  - allows fish to select a variety of swimming speeds

### **Environmental Management: Optimum Swimming Velocity**

- Advantages of ideal swimming speed:
  - Increased growth rate & protein synthesis
  - Less aggression
  - Enhanced immune function
  - Improved flesh texture



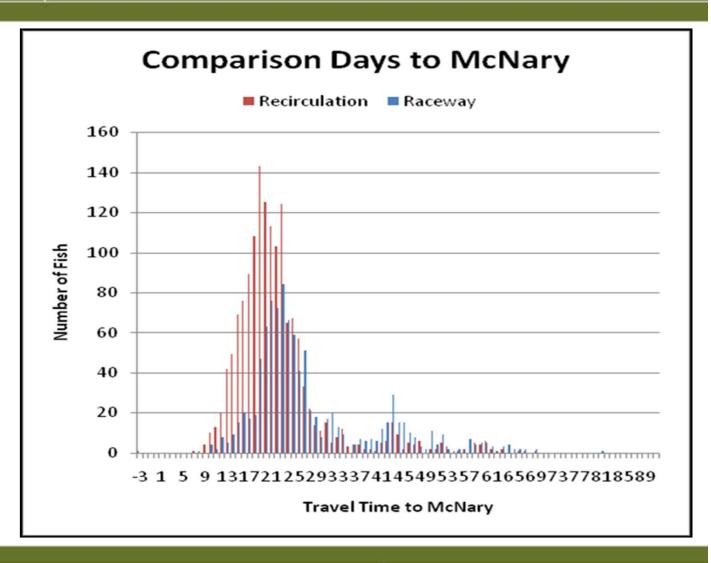


America's Partner in Conservation

### **Environmental Management: Optimum Swimming Velocity**

# Downstream migration of Chinook salmon

Exercised fish out-migrated faster and in greater numbers than raceway fish



### Atlantic salmon *Salmo salar* growout in freshwater closed-containment systems:

- (1) effects of high and low dissolved carbon dioxide
- (2) effects of strain and photoperiod manipulation
- (3) effects of swimming speed and dissolved oxygen

#### STUDY 1

Long-term effects of high (20 mg/L) and low (10 mg/L) carbon dioxide exposure on Atlantic salmon

Aside from fish health implications....

### **Economic Considerations**

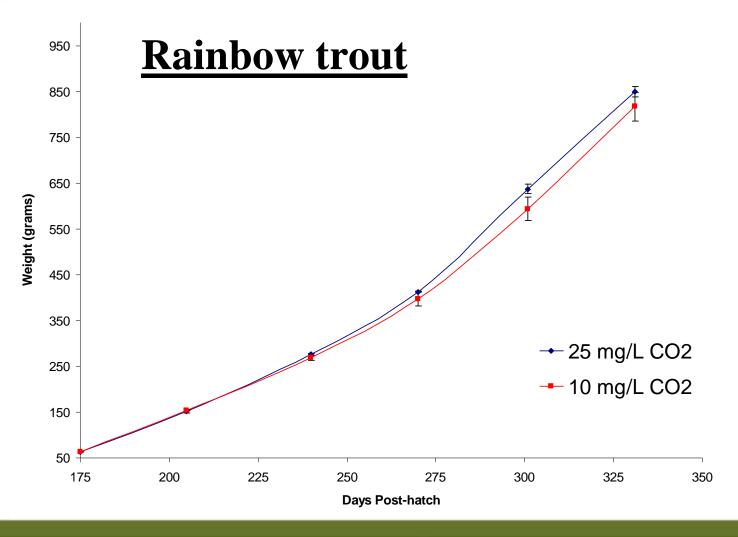
- Decreasing tank CO<sub>2</sub> concentrations requires pumping more water flow & installing a larger stripping unit (cascade column or aerated basin)
  - increases fixed costs
  - increases variable costs to pump water

#### THE CONSERVATION FUND

America's Partner in Conservation

### **Current Research at The Freshwater Institute**

Similar study completed with rainbow trout (Good et al. 2010 Aquacultural Engineering)



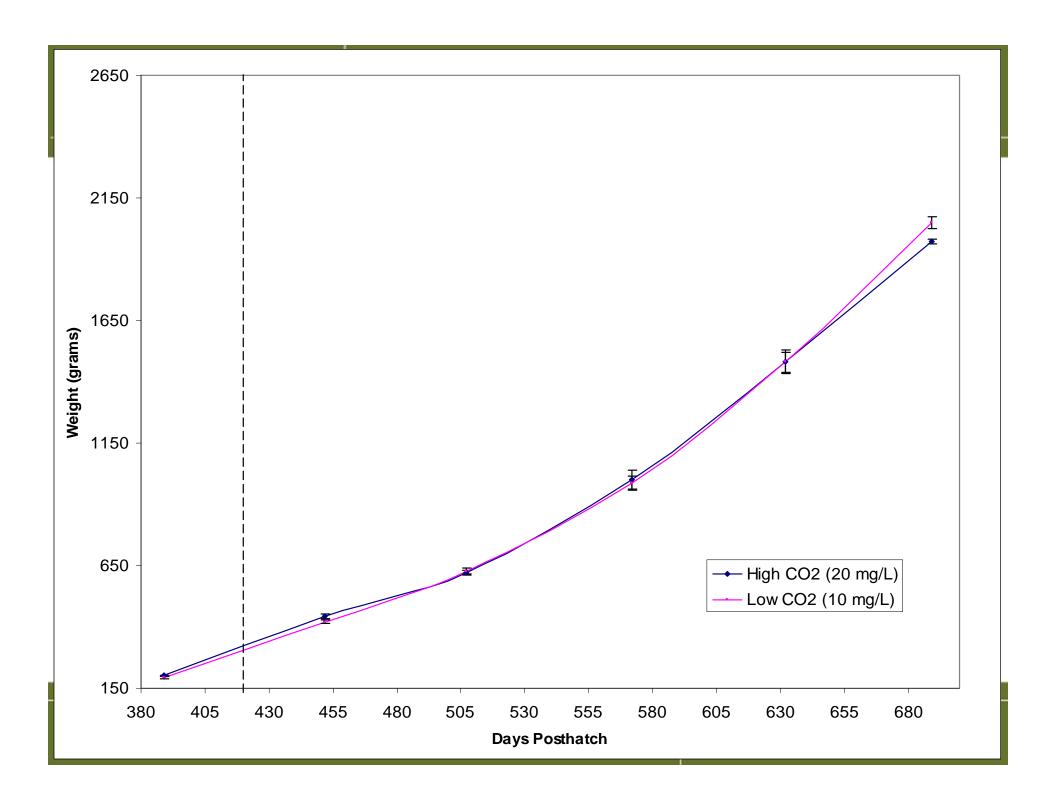
### **Atlantic salmon: Ongoing Data Collection**

- Bi-monthly length/weight sampling
- Daily mortalities
- Gill tissue and plasma collected at increasing intervals
- Kidney tissue collected for histopathology evaluation

#### December 2010

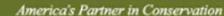
- Age: 689 d post-hatch
- High CO<sub>2</sub>: 1974 ± 10 g
- Low  $CO_2$ :  $2050 \pm 23$  g
- CO<sub>2</sub> beginning to show effects on fish growth

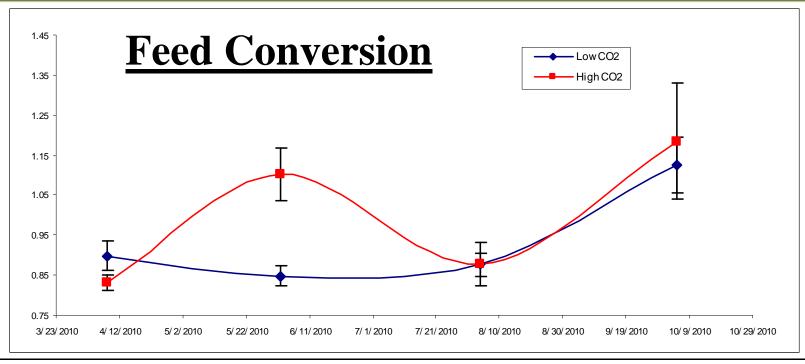




#### THE CONSERVATION FUND

### Current Research at The Freshwater Institute





	4/8/10	6/2/10	8/4/10	10/5/10	Grand
Low CO <sub>2</sub>	0.90	0.85	0.88	1.12	0.94
High CO <sub>2</sub>	0.83	1.10	0.88	1.18	1.00

### **End-of-study sampling:**

- Multiple tissue histopathology
- Blood gas and chemistry
- Fin indices
- RT-PCR of gill tissue for carbonic anhydrase and Rhcg mRNA

#### STUDY 2

## Effects of strain and photoperiod manipulation on Atlantic salmon

### **Objectives**

■ Determine how Atlantic salmon strain and photoperiod manipulation (to produce smoltification) influence growth, processing attributes, and sexual maturity to 26 months post-hatch in <u>freshwater</u> RAS.

#### **Materials and Methods**

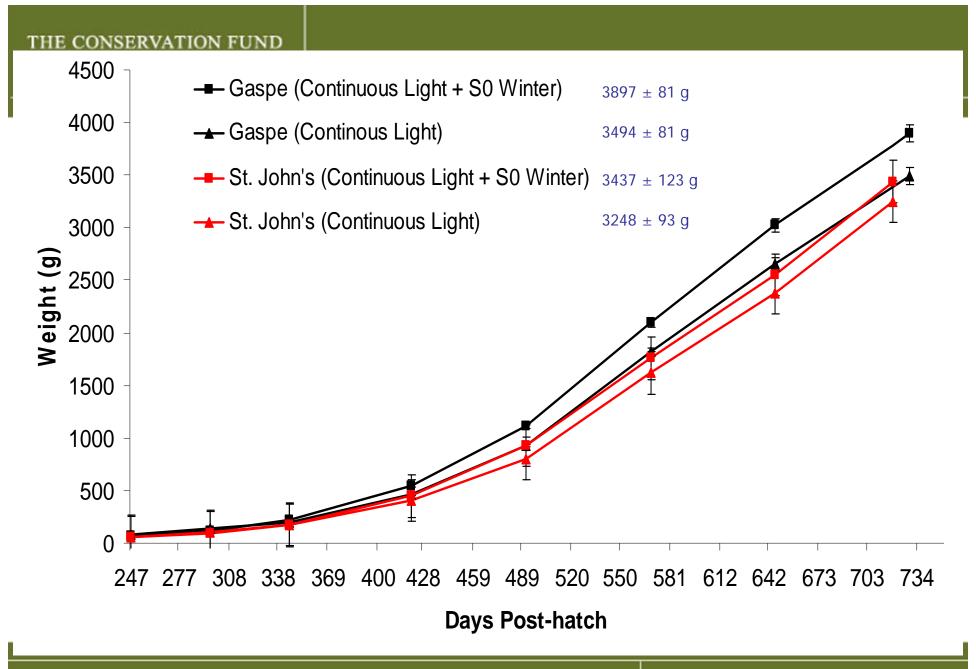
- 2X2 Factorial study
- Two Atlantic salmon strains:
  - 1. St. John's
  - 2. Gaspe
- Two early rearing light regimes:
  - 1. Continuous light
  - 2. Continuous light with  $S_0$  winter
    - 12h light, 12h dark for six weeks, then return to constant light



- Three 10 m³ culture tanks in one freshwater reuse system
- Both strains and photoperiod treatment fish co-mingled within each tank for n=3 replication

#### Results so far...

- Performance differences for both strain and lighting regime are already apparent
- Gaspe strain outperforming St. John's
- Salmon the underwent  $S_0$  winter are statistically (p<0.05) larger within Gaspe strain

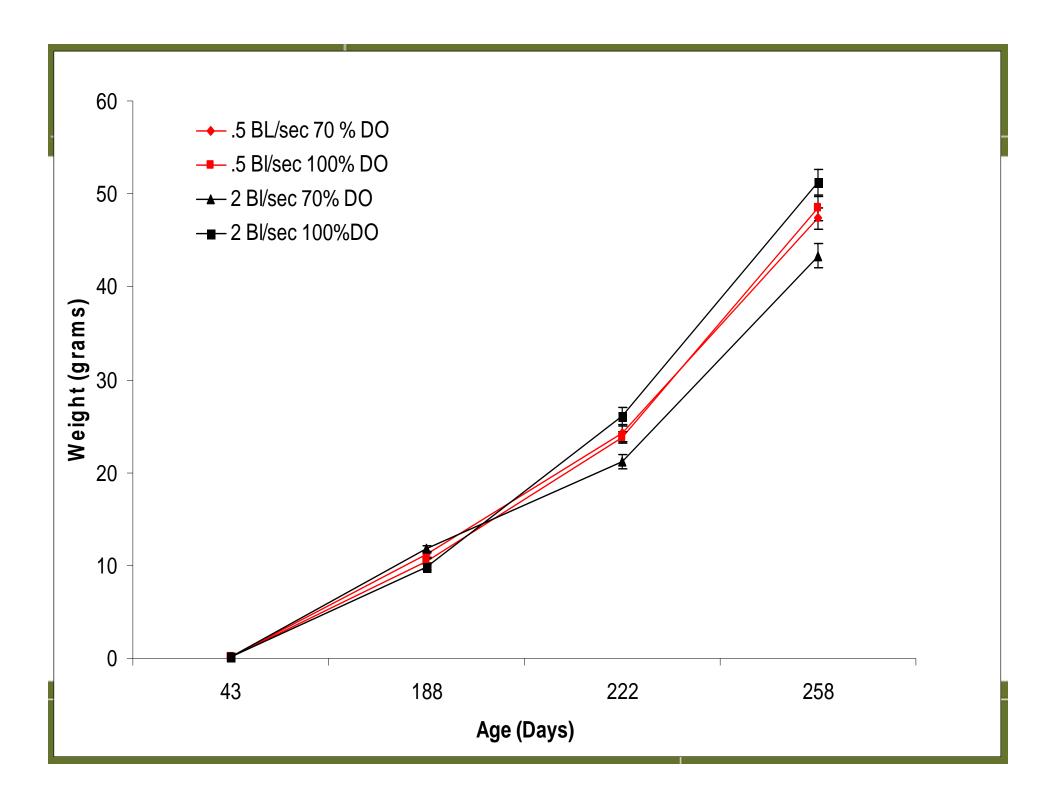


### **Further Data Collection**

- Gill ATPase analysis
- Plasma growth hormone assays
- Histopathology
- Blood gas / chemistry
- Processing attributes: fillet yield, fillet proximate analyses, fatty acid profiles, visceral indices, gonadal indices
  - 22-, 24-, and 26-months post hatch

#### STUDY 3

Effects of swimming speed (2 BL/s vs. 0.5 BL/s) and dissolved oxygen (100% vs. 70% saturation) on Atlantic salmon



### **Acknowledgements**

Nick Zacharias, Phil Backover, Lauren Jescovitch, Tre Kidwell

All research was supported by the Agriculture Research Service of the United States Department of Agriculture, under Agreement No. 59-1930-5-510.

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

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