Investigating the Health and Welfare of Rainbow Trout in Recirculating and Flow-through Aquaculture Systems

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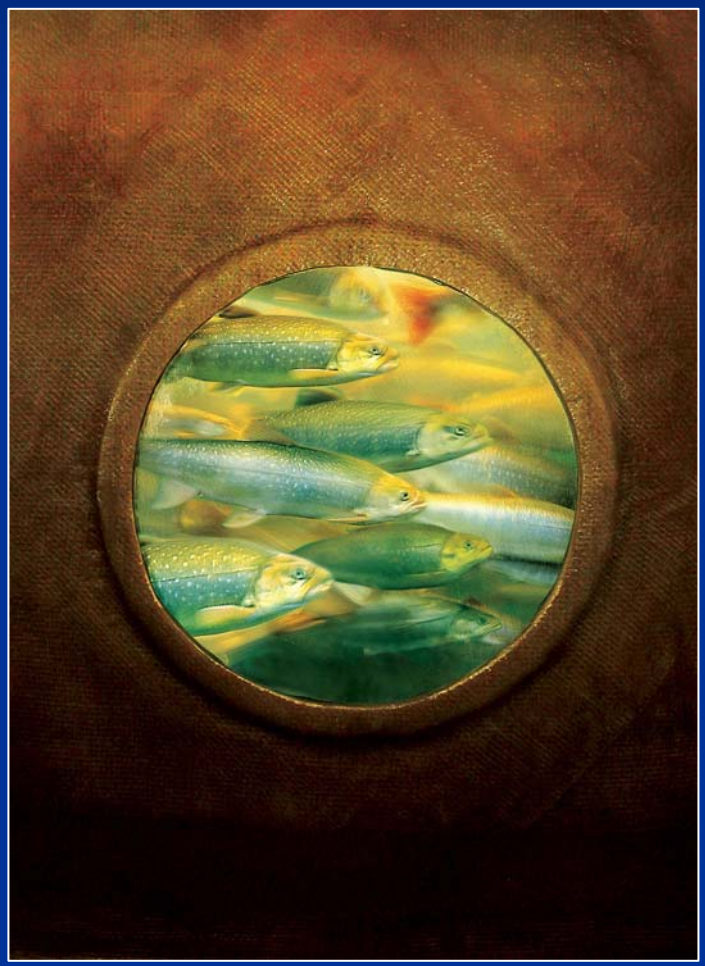
Presentation Outline

- **Summaries of two studies conducted at The Freshwater Institute:**
  1. Evaluating the health of rainbow trout in recirculating systems operated under high feed and low flushing conditions
  2. A factorial study assessing performance, fin condition, and fillet quality of rainbow trout reared at two different densities and fed fishmeal- or grain-based diets

Previous Observations at The Freshwater Institute



- During **low flushing** (1-2% water exchange) and **high feeding** (1.3-2.0 kg/d per m^3/d makeup flow):
  - Elevated morbidity and mortality
  - Unknown causation
  - No infectious causes suspected
  - Water quality parameters within safe limits

Study Objectives

- Investigate this potential barrier for RAS managed with high feeding rates; specifically:
  1. Assess fish performance in high vs. low makeup water exchange systems
  2. Assess fish health and welfare in these conditions through
     i. organ histopathological evaluation
     ii. plasma chemistry analyses
     iii. fin condition assessments
  3. Monitor changes and differences in water quality between the two treatments

Recirculating Aquaculture Systems

Materials and Methods

Identical Recirculating Aquaculture Systems (6)

Materials and Methods

Small Flow-Through Comparison Tanks (3)

Materials and Methods

- 6300 rainbow trout raised for 6 months
  - Stocked at 6 months of age (approx. 135g)
  - 1000 per RAS plus 100 per flow-through (initial #s)
- 40(min) - 80(max) kg/m^3 density
- 3 RAS with high makeup H_2O (2.6%)
- 3 RAS with low makeup (0.26%)

} Randomized

- Mean feed loadings of 0.39 and 4.1kg/day per m^3/day makeup flow, respectively

Materials and Methods

High Makeup H_2O Exchange vs. Low Makeup H_2O Exchange

Materials and Methods

High Makeup H_2O Exchange vs. Low Makeup H_2O Exchange

Data Collection

- Monthly length/weight sampling
- Daily mortalities
- Tissue sampling every 2 months for histopathological evaluation (5 fish per tank) – **Washington Animal Disease Diagnostic Laboratory**
  - Skin, gill, heart, liver, spleen, swim bladder, anterior and posterior kidney
- End-of-study plasma collection (5 fish per tank) for small animal chemistry panel – **Animal Health Diagnostic Center, Cornell University**
- End-of-study fin quality assessment
- Water quality analysis 2-3 times/week

Results: Water Quality

- Significant differences ($p<0.05$) between high/low makeup:
  - TAN, nitrite, nitrate, $cBOD_5$, TSS, true color, UV transmittance, phosphorus, copper, sulfur

Parameter	High Makeup	Low Makeup	Flow-through
TAN (mg/L)	0.29 ± 0.00	0.48 ± 0.05	0.22 ± 0.01
Unionized Ammonia (mg/L)	0.002 ± 0.000	0.004 ± 0.000	0.001 ± 0.000
Nitrite (mg/L)	0.041 ± 0.005	0.0095 ± 0.005	0.011 ± 0.001
Nitrate (mg/L)	12 ± 0	70 ± 4	2 ± 0
Alkalinity (mg/L)	226 ± 1	214 ± 4	261 ± 0
CO2 (mg/L)	9 ± 0	9 ± 0	13 ± 0
cBOD5 (mg/L)	2 ± 0	6 ± 1	1 ± 0
True Color (Pt-Co units)	11 ± 1	74 ± 9	1 ± 0
UV Transmittance (%)	89 ± 0	53 ± 4	97 ± 0
Phosphorous (mg/L)	0.52 ± 0.01	3.11 ± 0.22	0.08 ± 0.01
TSS (mg/L)	2.7 ± 0.1	6.4 ± 1.2	1.7 ± 0.1
Temperature (oC)	13.2 ± 0.0	13.2 ± 0.1	12.6 ± 0.0
pH	7.53 ± 0.03	7.54 ± 0.04	7.67 ± 0.00
DO (mg/L)	10.0 ± 0.0	10.0 ± 0.1	10.6 ± 0.0

Results: Mortality

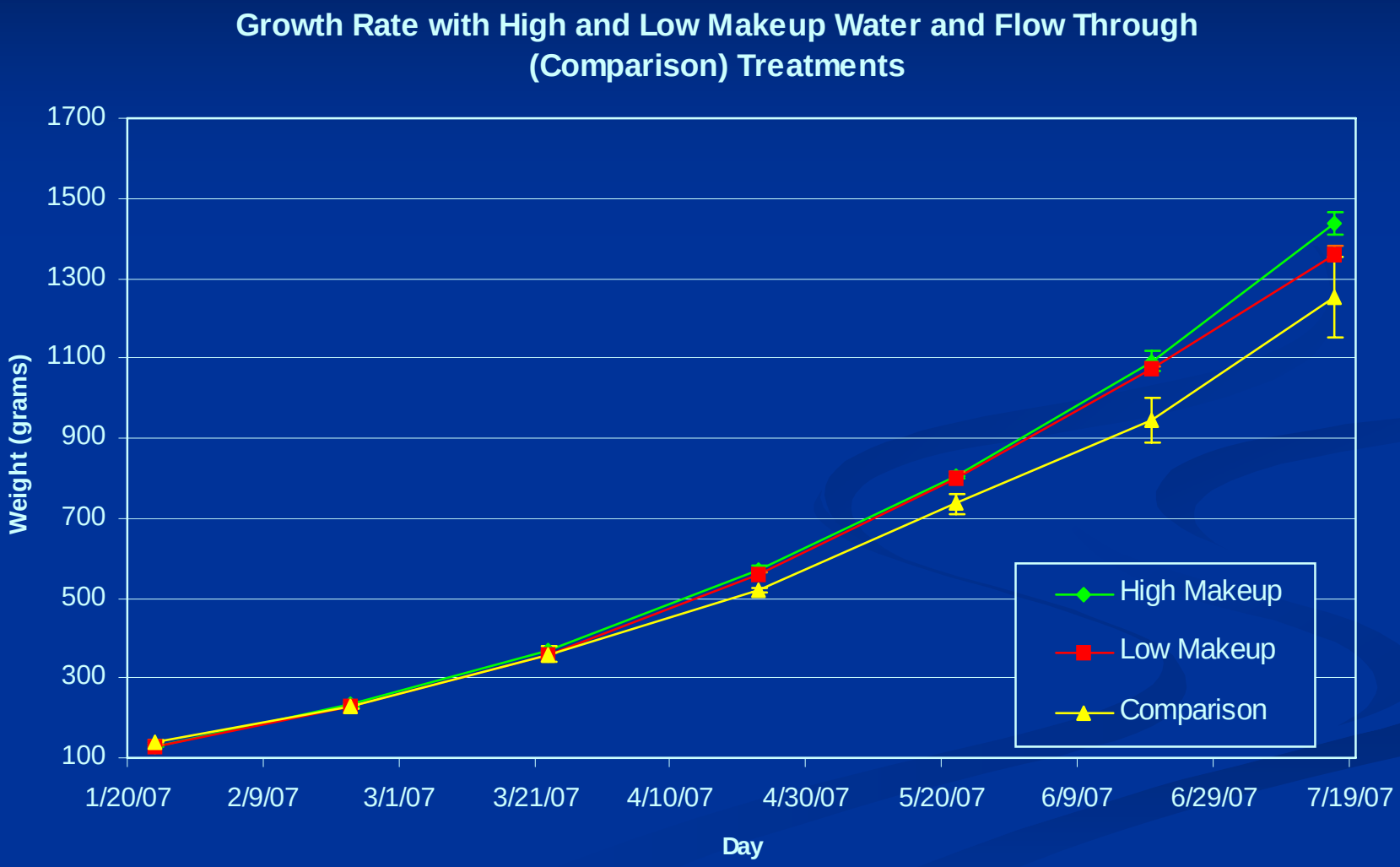
- Mean cumulative mortality risk rates:
  - High Makeup = 0.74%
  - Low Makeup = 1.69%

  } p = 0.0495

  - Flow-through = 1.12%

  } No significant differences

Results: Performance

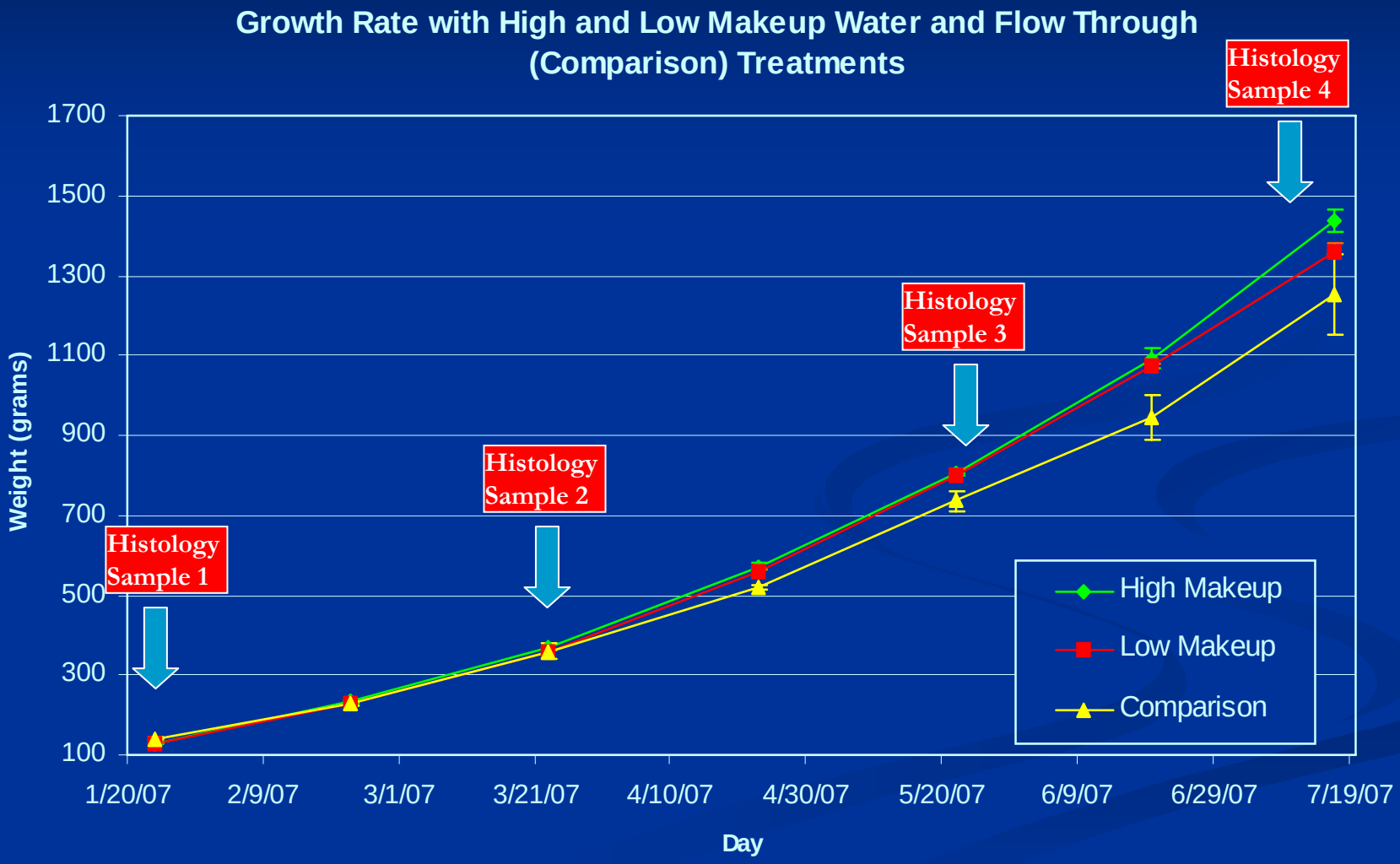


Results: Performance

- **Mean Final Weight:**
  - High makeup: **1401g**
  - Low makeup: **1366g**
  - Flow-through: **1253g**
- **Mean TGC:**
  - High makeup: **2.64**
  - Low makeup: **2.62**
  - Flow-through: **2.56**

No significant differences

Results: Histopathology



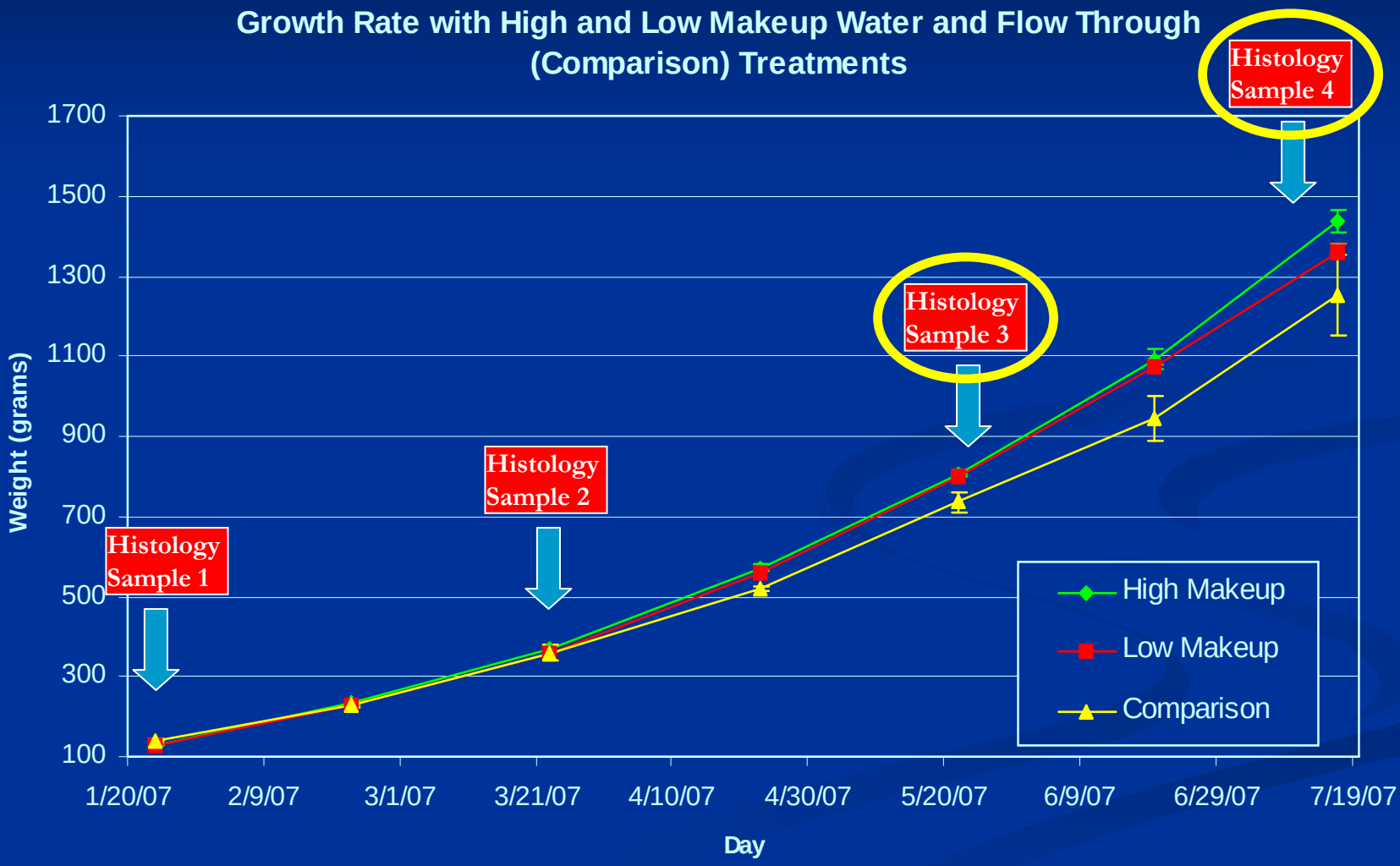
Results: Histopathology

Low (vs. High) makeup H_2O (overall results)

- Bivariate logistic regression models comparing presence/absence of histopathological lesions in fish reared in high vs. low makeup RAS (all sampling data combined)

Tissue	Odds Ratio	95% Conf. Int.	p-value
Gill	0.48	(0.16, 1.41)	0.179
Liver	1.64	(0.61, 4.44)	0.327
Heart	1.00	(0.45, 2.21)	1.000
Spleen	***1.57***	***(1.02, 2.42)***	***0.042***
Anterior kidney	0.48	(0.17, 1.34)	0.162
Posterior kidney	***0.34***	***(0.21, 0.57)***	***<0.000***
Swim bladder	-	-	-
Skin	***2.92***	***(1.76, 4.85)***	***<0.000***
> 1 tissue affected	1.23	(0.64, 2.36)	0.539
> 2 tissues affected	1.12	(0.58, 2.18)	0.734

Results: Histopathology



Results: Histopathology

Low (vs. High) makeup H_2O (individual samplings)

- Bivariate logistic regression models comparing presence/absence of histopathological lesions in fish reared in high vs. low makeup RAS (all $p<0.10$ models shown)

Tissue	Sample (1-4)	Odds Ratio	95% Conf. Int.	p-value
Liver	**3**	7.00	(1.00, 49.23)	0.051
Skin	**4**	4.33	(1.58, 11.91)	0.004

Results: Histopathology

Recirc. (vs. Flow-Through) (overall results)

- Bivariate logistic regression models comparing presence/absence of histopathological lesions in fish reared in recirculating vs. flow-through systems (all sampling data combined)

Tissue	Odds Ratio	95% Conf. Int.	p-Value
Gill	1.64	(0.70, 3.84)	0.254
Liver	0.52	(0.23, 1.16)	0.111
Heart	***2.11***	***(1.44, 3.10)***	***<0.000***
Spleen	***2.47***	***(1.56, 3.90)***	***<0.000***
Anterior kidney	0.74	(0.20, 2.73)	0.647
Posterior kidney	0.76	(0.22, 2.60)	0.667
Swim bladder	2.03	(0.24, 17.56)	0.513
Skin	1.92	(0.60, 6.15)	0.274
> 1 tissue affected	1.37	(0.71, 2.65)	0.345
> 2 tissues affected	1.61	(0.85, 3.05)	0.147

Results: Histopathology

Recirc. (vs. Flow-Through) (individual samplings)

- Bivariate logistic regression models comparing presence/absence of histopathological lesions in fish reared in recirculating vs. flow-through systems (all $p<0.10$ models shown)

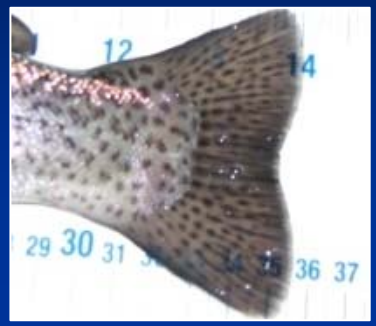
Tissue	Sample (1-4)	Odds Ratio	95% Conf. Int.	p-Value
Gill	4	2.67	(0.88, 8.12)	0.084
Liver	3	0.17	(0.05, 0.55)	0.003
Spleen	3	4.97	(1.29, 19.17)	0.020
> 1 tissue affected	4	4.57	(1.08, 19.26)	0.038
> 2 tissues affected	4	6.00	(0.88, 40.94)	0.067

Results: Plasma Chemistry

Parameter	Treatment	Mean	SE	Significance*
Potassium	High Makeup	2.37	0.84	B
(mEq/L)	Low Makeup	2.41	0.83	
	Flow-through	1.73	1.06	
Chloride	High Makeup	124.1	0.80	A, B
(mEq/L)	Low Makeup	131.0	1.24	
	Flow-through	122.1	1.04	
Urea Nitrogen	High Makeup	15.9	0.62	A, B
(mg/dL)	Low Makeup	19.0	0.80	
	Flow-through	<2	0.00	
Phosphate	High Makeup	12.6	12.6	B
(mg/dL)	Low Makeup	12.7	12.7	
	Flow-through	14.6	14.6	
Glucose	High Makeup	78.1	5.27	B
(mg/dL)	Low Makeup	76.4	6.99	
	Flow-through	88.1	4.60	

* Significance: **A** = $p<0.05$ High Makeup vs. Low Makeup; **B** = $p<0.05$ Recirc. vs. Flow-through

Results: Caudal Fin Assessment



Good Condition

Moderate Condition

Poor Condition

	Mean Prevalence at End-of-Study		
	Good	**Moderate**	**Poor**
High Makeup	96/111 (87%)	9/111 (8%)	6/111 (5%)
Low Makeup	70/111 (63%)	21/111 (19%)	20/111 (18%)
Significance	**$p<0.05$**	**$p<0.05$**	**$p<0.05$**

Conclusions

- Unable to replicate previous observations
- Fish performed surprisingly well under high feed and low flushing conditions
- Some statistically significant **subclinical** (histopathological lesions, plasma chemistry) and **clinical** (caudal fin condition, mortality) differences noted between treatments
  - Did not affect fish performance **during study period**

A Factorial Study Assessing Performance, Fin Condition, and Fillet Quality of Rainbow Trout *Oncorhynchus mykiss* Reared at Two Different Densities and Fed Fishmeal- or Grain-based Diets

Fin Erosion

- Common problem seen in many species of farmed fish
- Still poorly understood; complex, multifactorial etiology
- Both density and nutritional factors considered important in its development
- Observed at The Freshwater Institute in rainbow trout in both recirculating and flow-through systems

Study Objectives

Investigate the associations of diet and density with:

- Fin condition
- Fish performance
- Fillet quality

Materials and Methods

2X2 Factorial Study Design

Two diets:

Traditional Fish Meal-based

Grain-based

Two rearing densities:

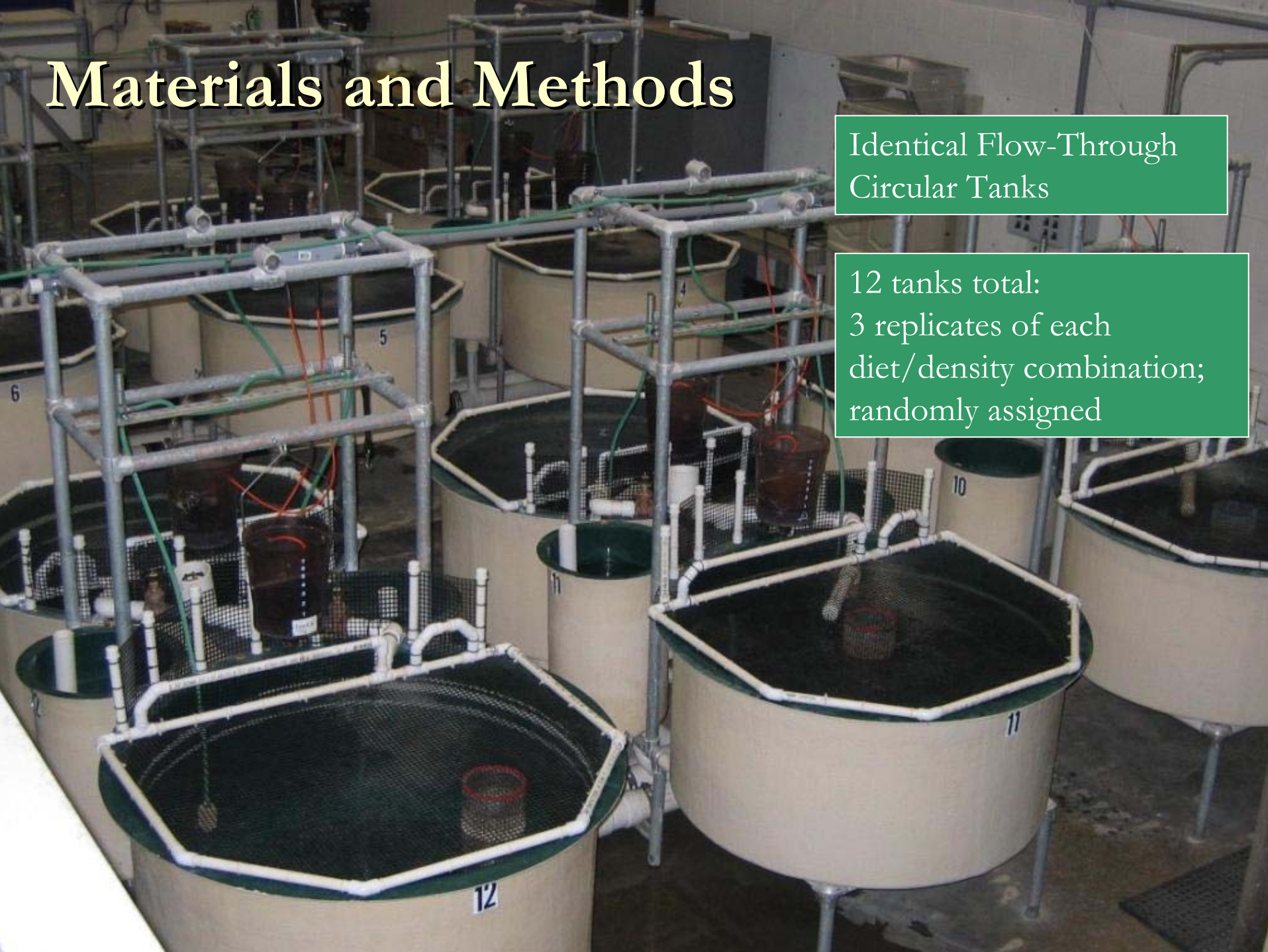
High: 60-80 kg/m^3

Low: 20-40 kg/m^3

Materials and Methods

- Both diets:
  - Formulation developed by Rick Barrows
  - Same caloric content
  - Small sizes produced at the Feed and Nutrition Laboratory (USFWS Bozeman Fish Technology Center, MT)
  - Larger sizes produced by Silver Cup Feed (Nelson & Son Inc., Murray, Utah)

Materials and Methods



Identical Flow-Through Circular Tanks

12 tanks total:
3 replicates of each diet/density combination; randomly assigned

Materials and Methods

- Rainbow trout raised from sac-fry to 650-900g in 312 days in 500L flow-through tanks (~12.5°C)
- Daily mortality and monthly length/weight data
- End-of-study assessment:
  - Fin indices (all rayed fins; fin length standardized by fork length)
  - Visceral evaluation: visceral index, liver index, spleen index
- Fillet quality: cook yield, texture, pesticide and PCB content

Results

- Proximate analyses:

Fish Meal-Based Diet	
Moisture	5.77%
Protein	41.1%
Fat	13.26%
Fiber	1.29%
Ash	8.95%

Grain-Based Diet	
Moisture	6.02%
Protein	49.6%
Fat	17.7%
Fiber	0.19%
Ash	6.24%

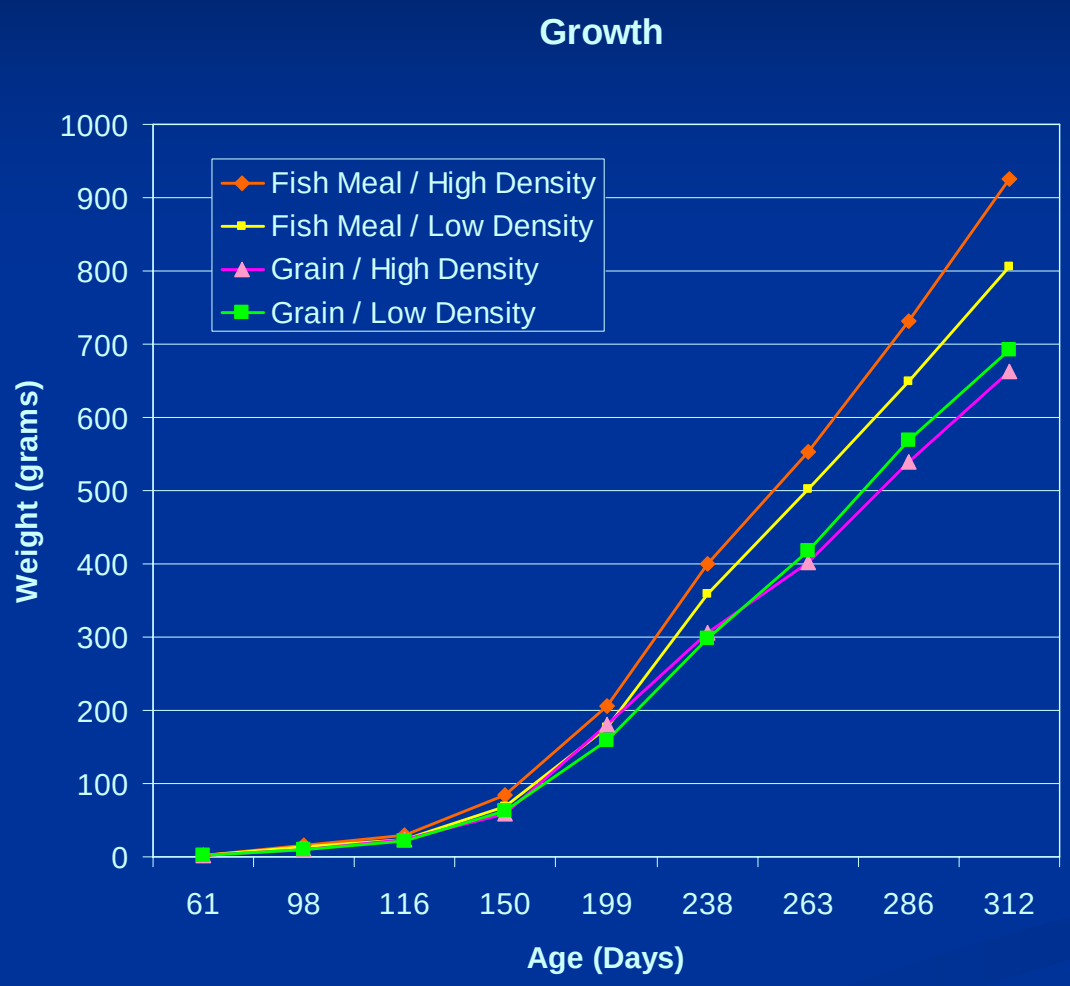
Results

- Fish appearance:
  - Enhanced coloration of skin and fillet in fish fed grain-based diet
  - Small amount of pigment added to grain-based diet, so difficult to determine true association of grain-based ingredients with coloration

Fish Meal-based diet

Grain-based diet

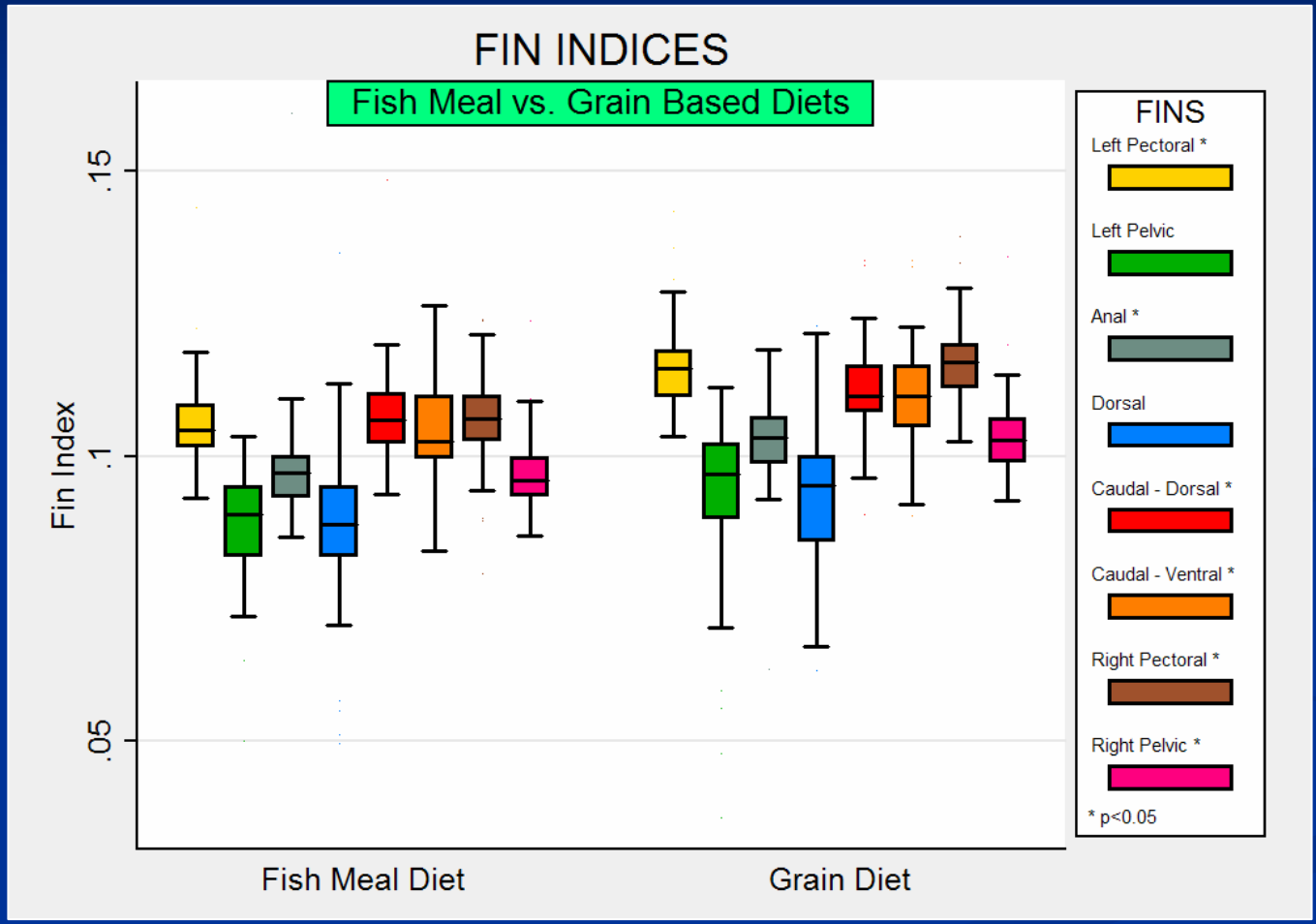
Results: Performance



Parameter	Fish Meal Diet Mean ± SD	Grain Diet Mean ± SD	p-value
Weight (g)	866.1 ± 72.04	677.1 ± 68.67	0.001
Length (mm)	364.9 ± 10.88	345.1 ± 8.315	0.005
FCR	1.163 ± 0.047	1.059 ± 0.079	0.018
TGC	2.457 ± 0.066	2.374 ± 0.124	0.180
Mortality Risk Rate	0.032 ± 0.008	0.022 ± 0.005	0.025

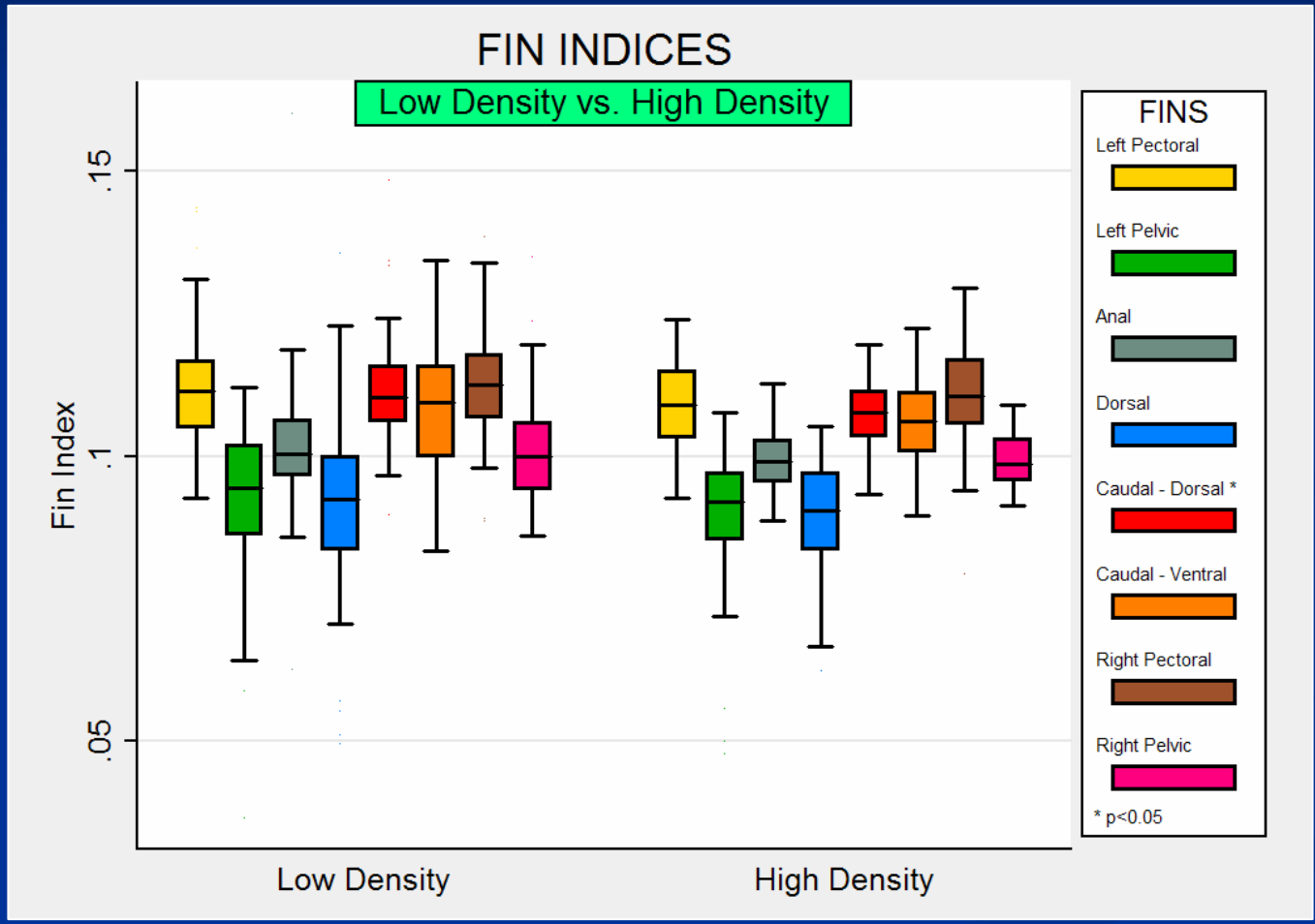
	High Density	Low Density	
Weight (g)	794.1 ± 150.9	749.1 ± 85.73	0.538
Length (mm)	359.3 ± 16.06	350.7 ± 10.94	0.304
FCR	1.101 ± 0.112	1.122 ± 0.046	0.674
TGC	2.442 ± 0.089	2.389 ± 0.119	0.400
Mortality Risk Rate	0.026 ± 0.009	0.0263 ± 0.008	0.696

Results: Fin Quality and Diet



Fin	Fish Meal Diet Mean ± SD	Grain Diet Mean ± SD	p-value
Left pectoral	0.108 ± 0.009	0.117 ± 0.009	0.001
Left pelvic	0.088 ± 0.009	0.096 ± 0.016	0.067
Anal	0.099 ± 0.013	0.104 ± 0.010	0.005
Dorsal	0.088 ± 0.009	0.095 ± 0.014	0.137
Caudal, dorsal	0.109 ± 0.009	0.113 ± 0.009	0.025
Caudal, ventral	0.105 ± 0.008	0.112 ± 0.010	0.002
Right pectoral	0.107 ± 0.008	0.118 ± 0.007	0.001
Right pelvic	0.097 ± 0.008	0.105 ± 0.008	0.001

Results: Fin Quality and Density



Fin	Low Density Mean ± SD	High Density Mean ± SD	p-value
Left pectoral	0.113 ± 0.010	0.109 ± 0.008	0.269
Left pelvic	0.092 ± 0.014	0.089 ± 0.012	0.320
Anal	0.101 ± 0.012	0.099 ± 0.005	0.328
Dorsal	0.092 ± 0.017	0.090 ± 0.010	0.568
Caudal, dorsal	0.111 ± 0.009	0.107 ± 0.006	0.031
Caudal, ventral	0.109 ± 0.010	0.106 ± 0.008	0.263
Right pectoral	0.112 ± 0.009	0.111 ± 0.008	0.590
Right pelvic	0.101 ± 0.009	0.100 ± 0.005	0.543

Results: Visceral Evaluation

Parameter	Fish Meal Diet Mean ± SD	Grain Diet Mean ± SD	p-Value
Visceral index	0.143 ± 0.018	0.117 ± 0.016	0.001
Liver index	0.015 ± 0.002	0.012 ± 0.001	0.001
Spleen index	0.0012 ± 0.0005	0.0012 ± 0.0006	0.601

Parameter	High Density Mean ± SD	Low Density Mean ± SD	p-Value
Visceral index	0.131 ± 0.017	0.129 ± 0.025	0.791
Liver index	0.013 ± 0.002	0.013 ± 0.002	0.831
Spleen index	0.0013 ± 0.0005	0.0012 ± 0.0005	0.499

Results: Fillet Quality

Parameter	n	Fish Meal Diet Mean ± SD	Grain Diet Mean ± SD	p-Value
Fillet yield (%)	60	49.7 ± 2.10	50.5 ± 1.60	0.123
Cook yield (%)	60	84.5 ± 2.14	84.7 ± 1.82	0.847
Texture (g/g wt)	60	337.1 ± 71.4	330.0 ± 45.5	0.727
DDE * (ug/g)	18	0.0059 ± 0.0019	0.0064 ± 0.0031	0.779
PCB * (ppm)	18	0.0069 ± 0.0035	0.0048 ± 0.0042	0.145

* DDE and PCB analyzed in high density tanks only

Parameter	n	High Density Mean ± SD	Low Density Mean ± SD	p-Value
Fillet yield (%)	60	50.0 ± 1.60	50.2 ± 2.20	0.731
Cook yield (%)	60	84.6 ± 2.05	84.6 ± 1.93	0.909
Texture (g/g wt)	60	327.8 ± 62.4	339.3 ± 56.8	0.574

Conclusions

Important Findings:

- Diet appeared to be much more influential than density for both fish performance and fin condition
- **Performance:** Fish were significantly larger and had better FCR with fish meal diet, but had significantly higher mortalities
- **Fin condition:** Most fins were significantly more healthy with grain-based diet
- **Fillet quality:** No significant differences in pesticide or PCB content (*low power?*). No significant difference in fillet yield.
- Further assessments:
  - fillet fatty acid profiles
  - intestinal histopathology

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