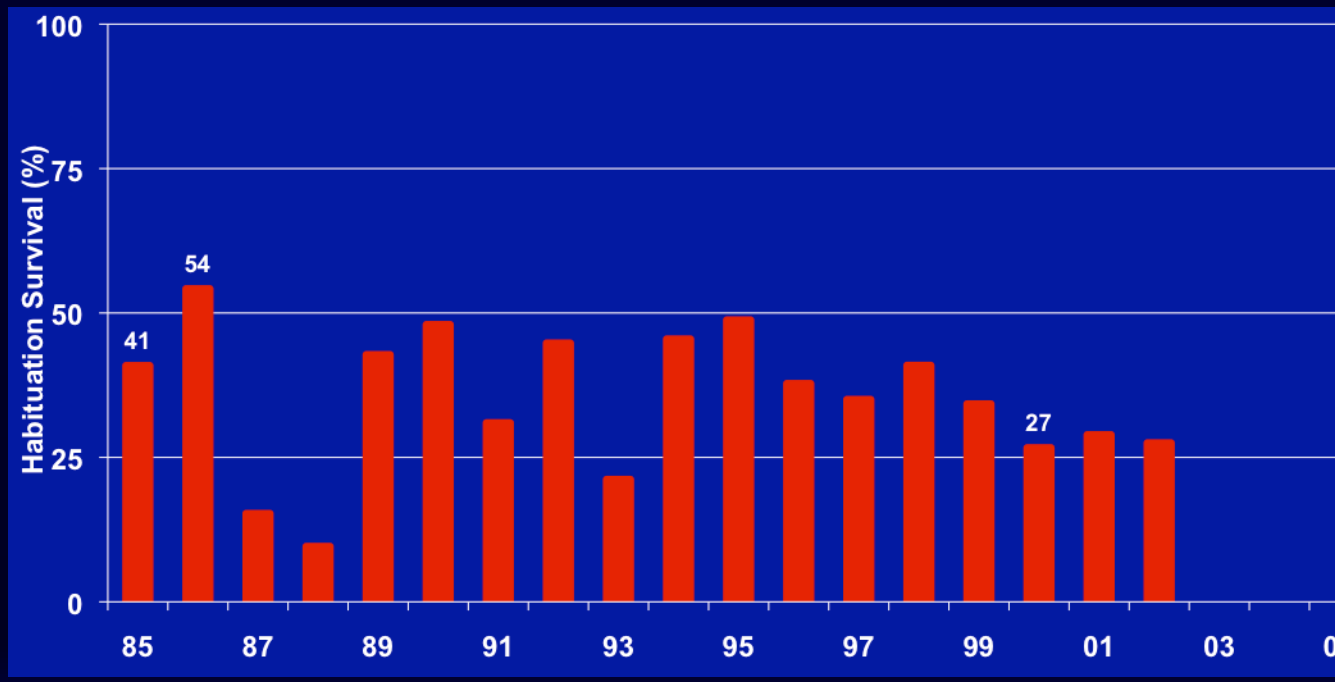
Phase II - Habituation

29 years of walleye habituation

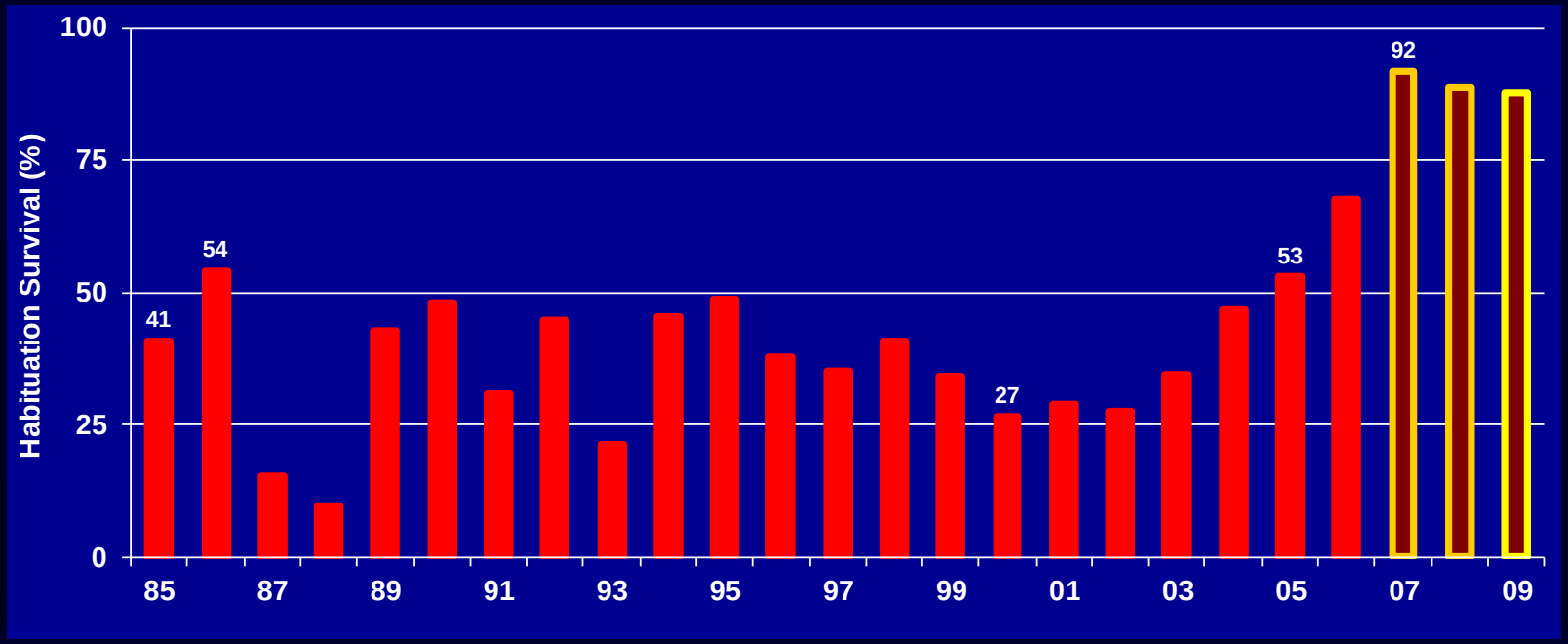
Survival



- **Bottleneck of the process.**
  - **Low survival.**
  - **Unpredictable.**
  - **Inefficient.**

29 years of walleye habituation

Survival



- We gained control of the process.
  - Dark room environment, in-tank lights.
  - Quality habituation diet – Otohime.
  - Optimize fingerling size – 0.5 g.

Paradigm shift

As a result of research:

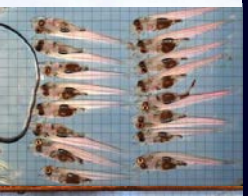
Plan for habituation success and not failure.

Scientific fish culture based on data.

89.5% Survival in three years.

Phase II - Habituation







Process of converting fingerlings from live prey to commercial diets.

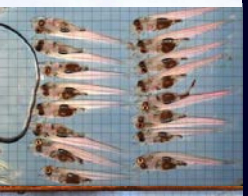
Key developments:

- **Fish size**
- **Environment**
- **Diets and feeding**
- **Disease management**



Habituation Methods







Fish source: RFH one acre plastic lined ponds.

Stocked into indoor raceways.

Raceway Density 2003: 185 fish/ ft^3 (20,000/raceway).

2004: 139 fish/ ft^3 (15,000/raceway).

2014: 125 fish/ ft^3 (13,500/raceway)

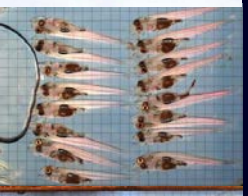
Based on triplicate check weights (f/lb) of pond fng's

Formerly: Fish confined to rear 2/3 (108 ft^3) for 17 days.

Raceway volume 162 ft^3 or 1,200 gallons

Habituation Methods





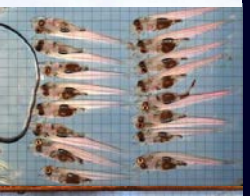


Daily Husbandry

- Monitor dissolved oxygen levels.
- Initial flow rates
  - Initially set at 15 gpm (0.75 exchanges/hr), increased to maintain effluent DO ≥ 5.0 mg/l.
- Raceways cleaned with a broom daily, water lowered to remove waste.
- Collect and evaluate mortalities:
  - Sick?... Starvation?...Cannibalism?...
- Treat disease if necessary.
- Fill feeders.
- Leave them alone.

Habituation Methods







Feeding regime:

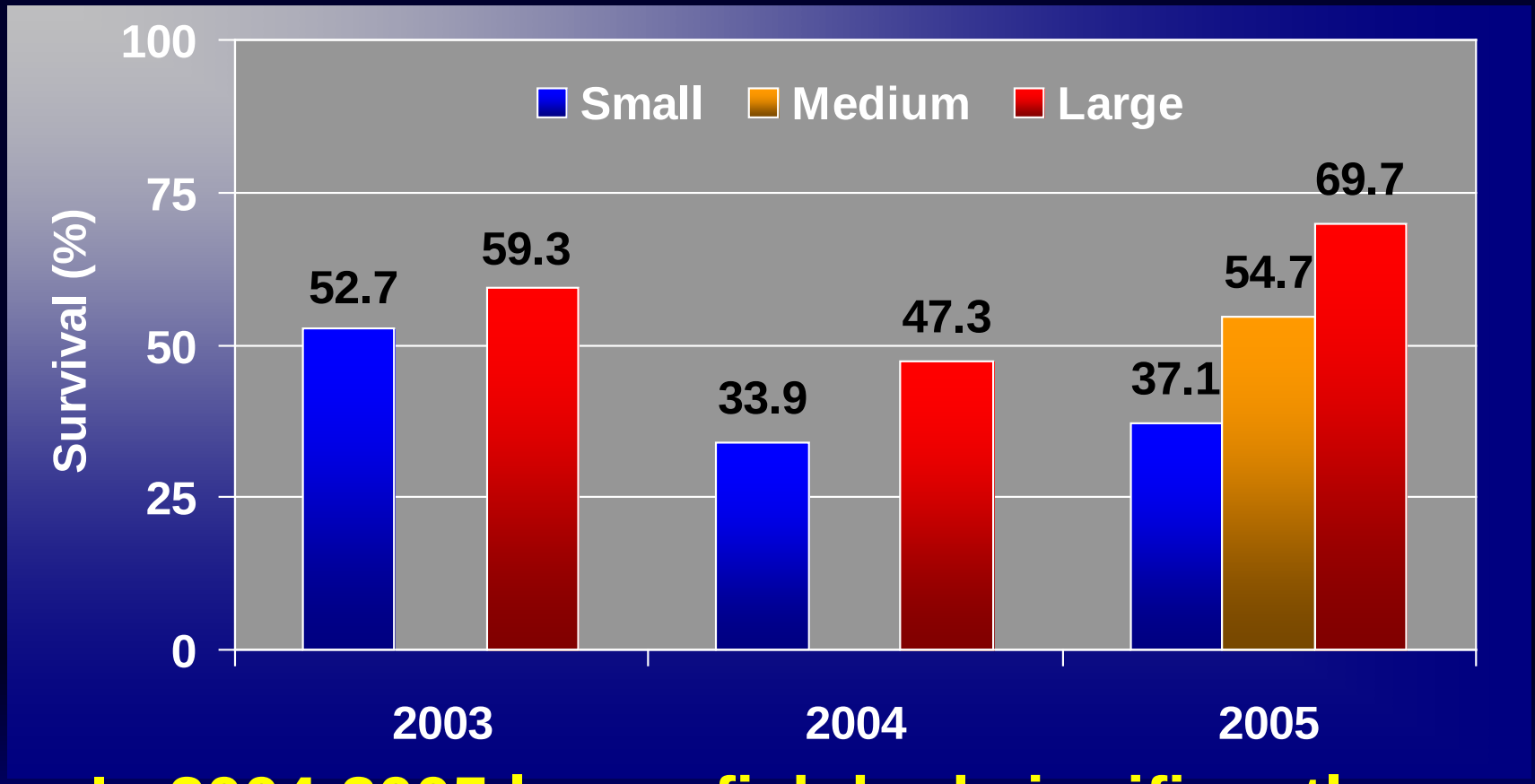
Day	Diet
1-10	Habituation diet
11-17	Transition – blended diets
17-28	Walleye Grower 9206 1.0 mm.

Frequency: 5 minute intervals, 22 h/d

Rate: 10% body weight per day throughout study.

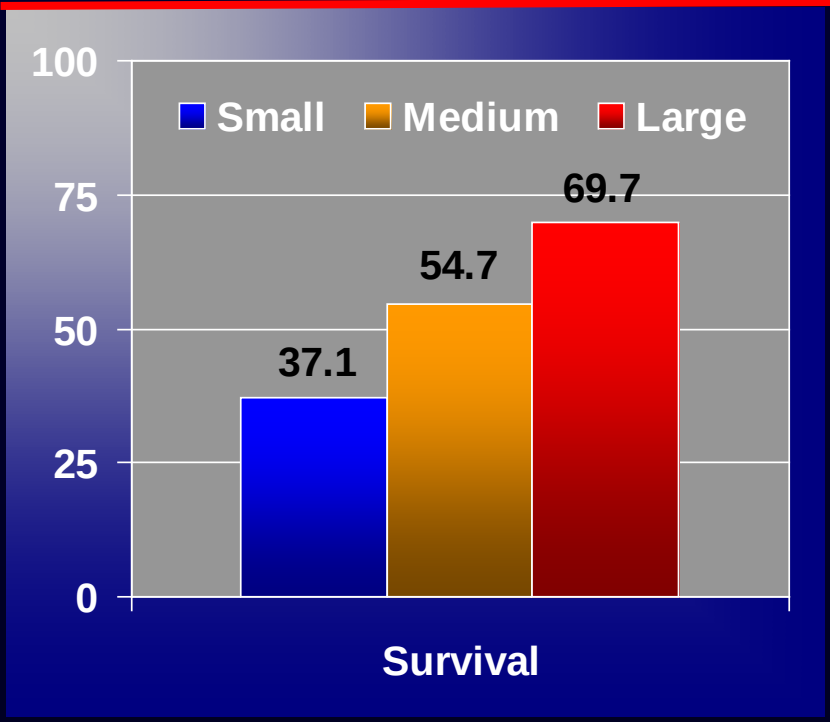
Feeders calibrated twice per week to reflect growth and mortality.

Phase II – Habituation: Influence of Fish size



In 2004-2005 larger fish had significantly higher survival during habituation.

Phase II – Habituation: Fish Size.



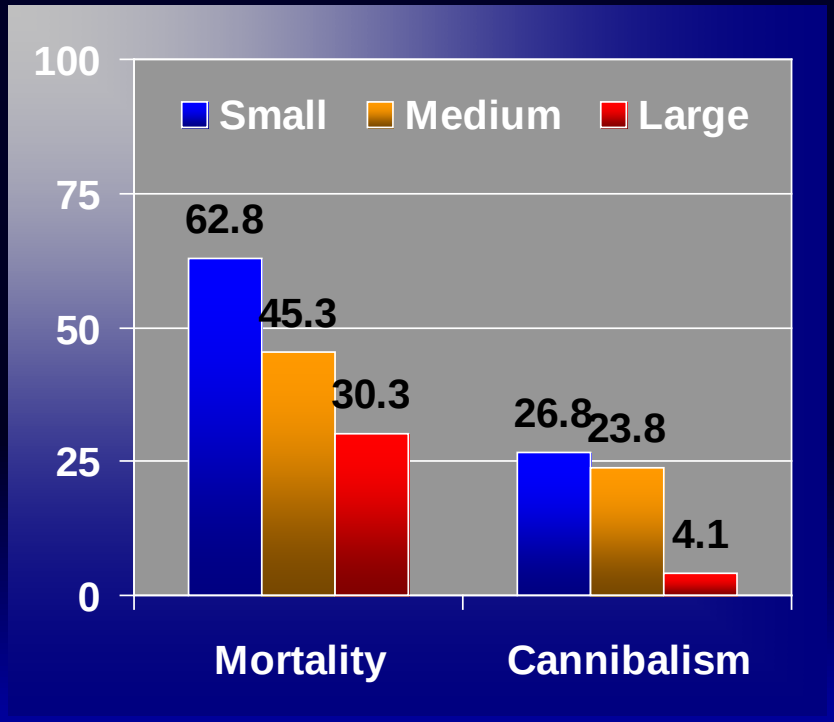
Initial	Small	Medium	Large
Fish/lb	0.32	0.44	0.53
L (mm)	35.7	39.4	41.7
S.E. L	0.246	0.363	0.315
W (g)	0.32	0.42	0.53

2005 study

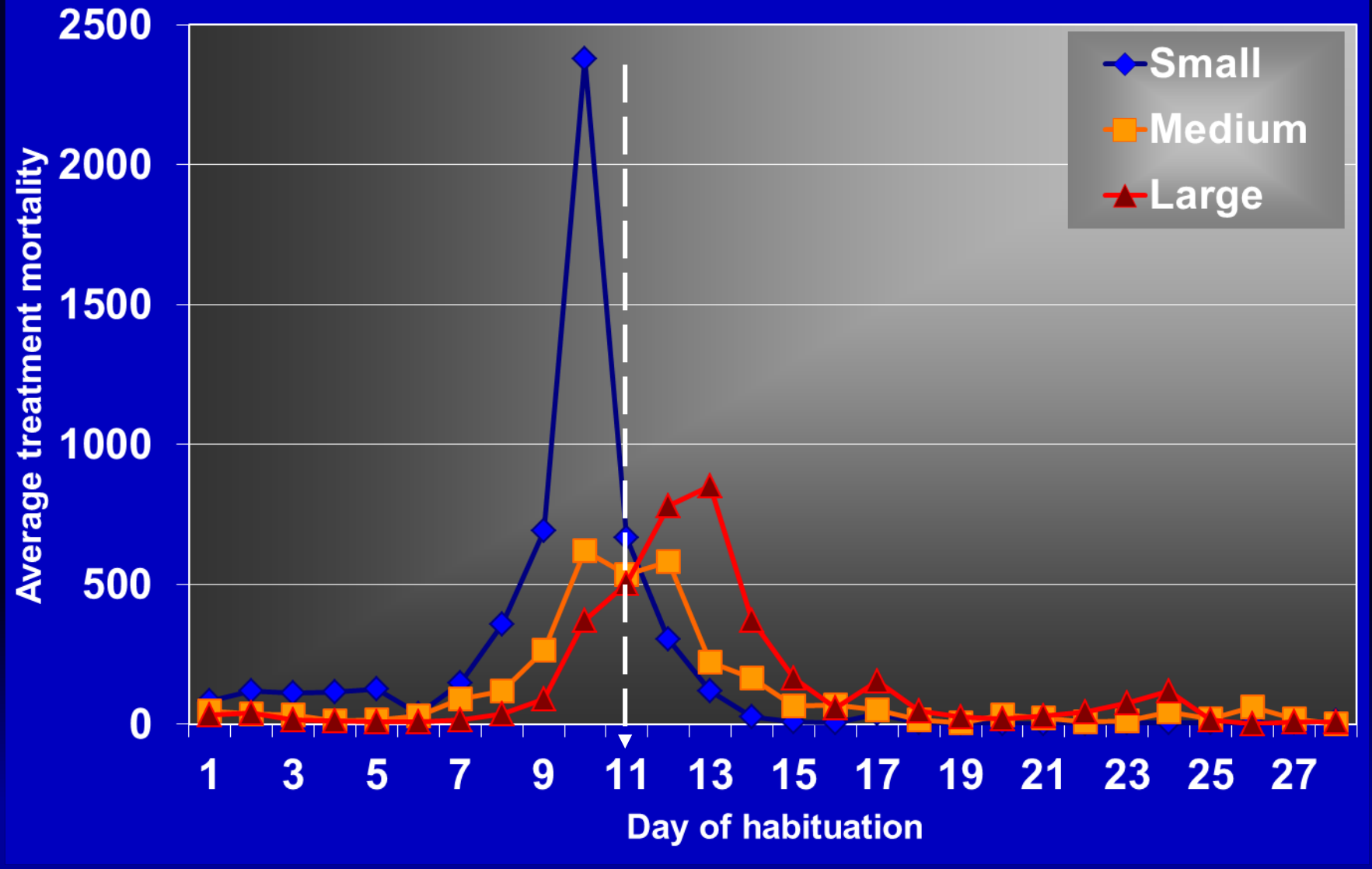
Significant difference

Survival P<0.0001

- **Mortality P<0.0001**
- **Cannibalism P<0.0038**

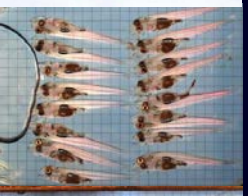


Phase II – Habituation: Pattern of mortality by size group

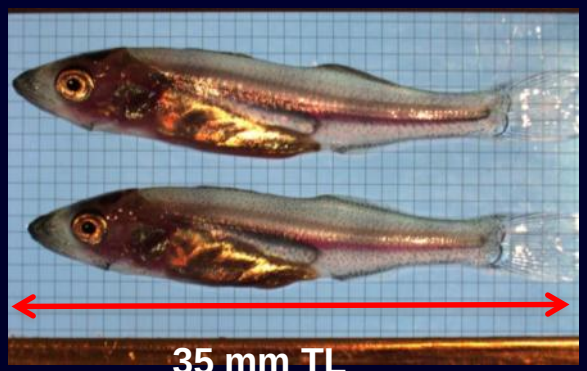


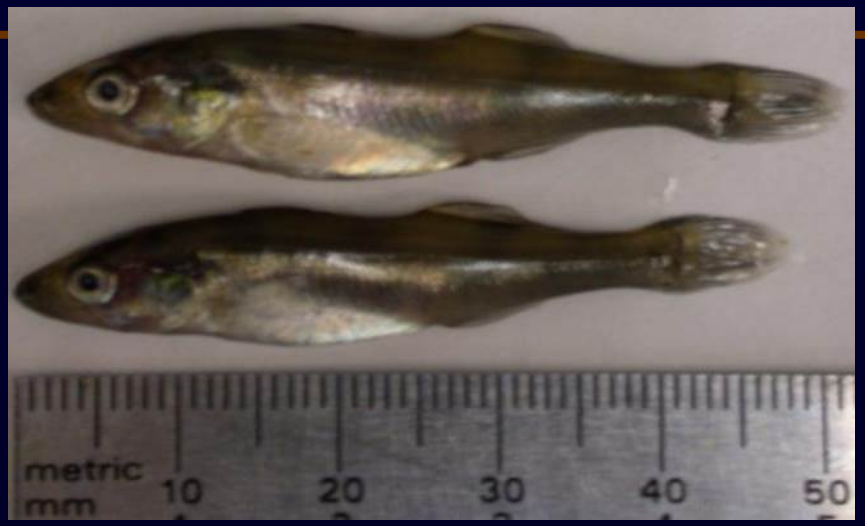
Why >0.56g?











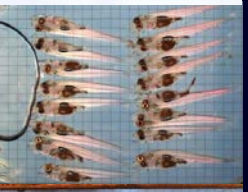
800/lb = >42 mm; 1000/lb= 37 mm.

- **Habituation of pond reared fingerlings best above 0.57 g. (about 42 mm).** Johnson and Rudacille (2010).
- **Scale development initiated at 24 mm complete at 45 mm.**
  - Priegel (1964)
- **Mechanical damage allows entry of Columnaris.**
  - Huissain and Summerfelt (1991)

Theory: fingerlings larger than 0.57 g are fully scaled and therefore more resilient to handling.

Habituation Diet



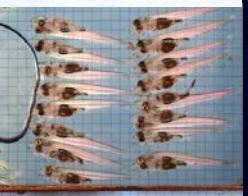




- Habituation - Phase II
  - Process of training fish to eat artificial diet.
  - Habituation diet fed 10 days.
  - The critical period in our production.
- BioKyowa FFK C-1000 *was* our choice for fingerling habituation.
  - Good survival, growth.
  - Expensive, imported.
- 2001: Importation ban on BioKyowa FFK feeds due to Mad Cow disease in Japan.

Habituation Diet





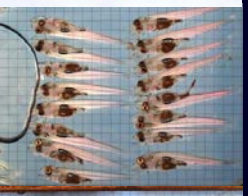


Search for a diet…

- Palatability from marine ingredients (krill or other seafood).
  - Krill hydrosylate coating on a commercial feed increased walleye growth 30% over uncoated feed (Kolkovski et al 2000).
- Prefer a domestic or readily importable.
  - Most US diets are for catfish or salmonids.
  - EU prohibited the feeding of processed terrestrial animal protein to farmed animals.

Habituation Diet





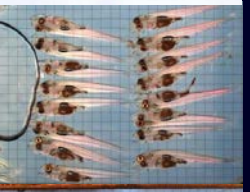


Round 1 – Habituation diet trial:

- **BioVita FF #2** (BioOregon, Inc., Warrenton, OR).
  - Contains Krill. Made in US.
- **Nutra 2000 #2** (Skretting, Inc Vancouver, BC, Can.)
  - Contains Crustacean meal. Made in Canada.
- **Lansy CW 8/12** (INVE Aquaculture Inc, Ogden UT).
  - Contains Fish products. Made in Belgium.
- **Nutra HP 1.0** (Skretting, Inc)
  - Contains Crustacean meal. Made in France
- **EPAC CW 8/12** (INVE Aquaculture Inc)
  - Modification of Lansy CW.
- **Gemma 1.0** (Skretting, Inc)
  - Contains Crustacean meal. Made in France

Habituation Diet





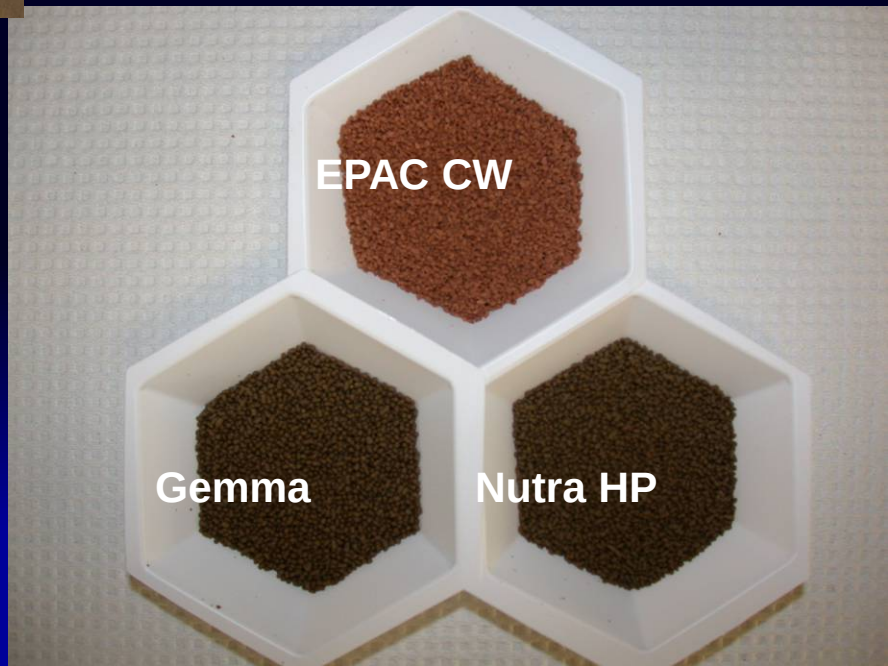


Compare commercial feeds as a habituation diet for pond reared walleye fingerlings.

- Conducted at Rathbun Fish Hatchery
- Two culture seasons, 2003-4.
- Monitor survival (primary factor) and growth.
- 28-day production scale studies.

2003 Diets

2004 Diets



Results

2003 Survival of walleye fingerlings after 28 days.

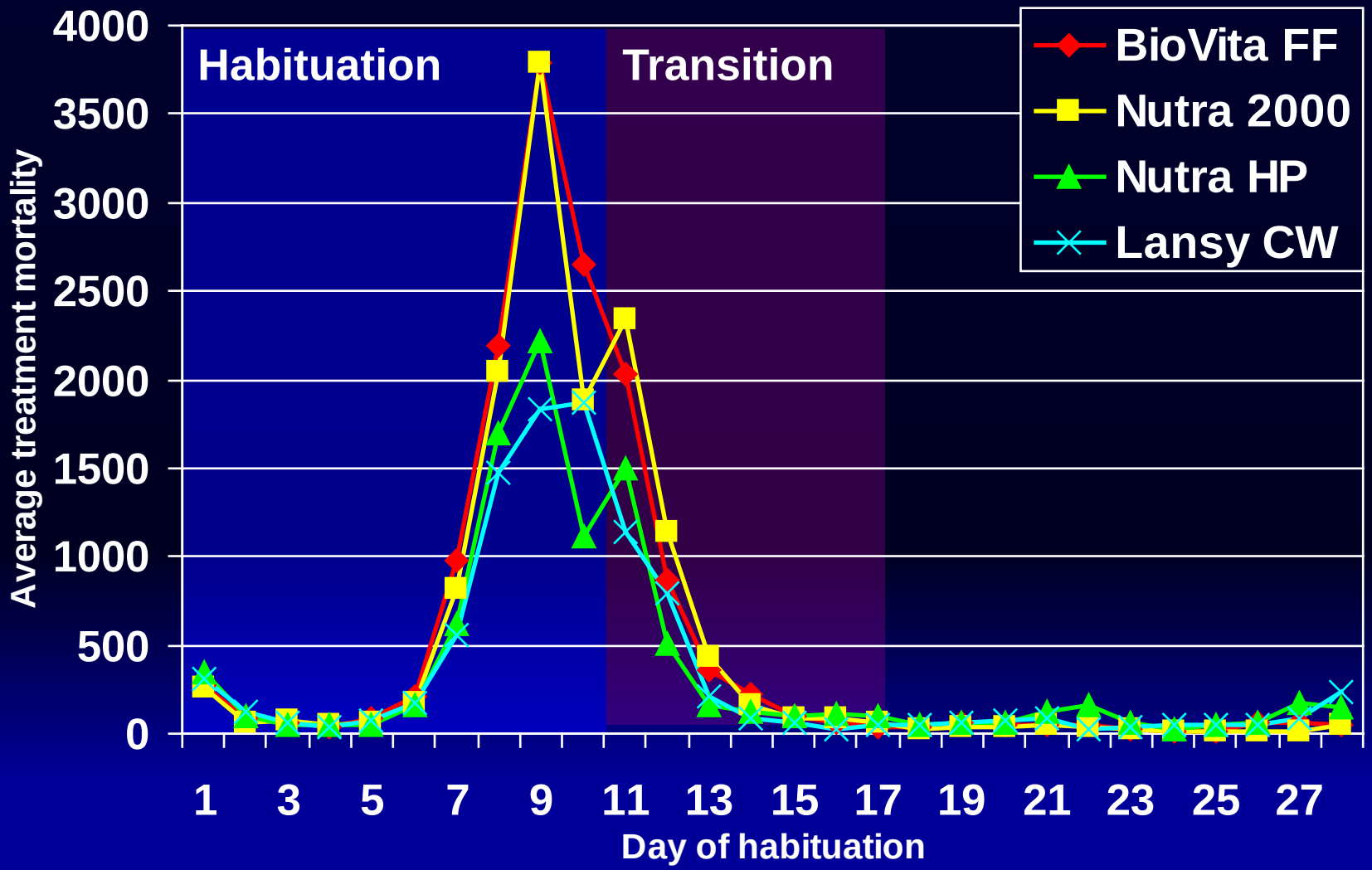
Habituation diet	Survival (%)
BioVita FF	28.9 a
Nutra 2000	28.3 a
Nutra HP	47.3 b
Lansy CW	52.7 b
P-value	0.0121

2004 Survival of walleye fingerlings after 28 days.

Gemma	53.0 a
Nutra HP	44.0 a
EPAC CW	75.0 b
P-value	0.0015

Results

Average daily mortality of walleye during habituation.



Results

2004 - Initial size and condition of walleye

Pond	L (mm)	W (g)	Wr
23	44.6	0.58	68.0
27	44.1	0.54	65.2
29	47.5	0.69	67.1

Results

2003 Final length, weight, and growth rates.

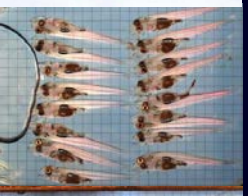
Diet	L (mm)	W (g)	Wr	mm/d
BioVita FF	69.5	3.3	96.7	1.06
Nutra 2000	71.2	3.0	98.9	1.12
Nutra HP	71.5	3.2	94.5	1.13
Lansy CW	71.8	3.4	92.5	1.14
P-value	0.7504	0.7902	0.1504	0.7440

2004 Final length, weight, and growth rates.

Diet	L (mm)	W (g)	Wr	mm/d
Gemma	84.4	5.16	95.1	1.39
Nutra HP	83.7	4.99	94.7	1.37
EPAC CW	83.4	4.99	95.5	1.36
P-value	0.8236	0.8534	0.9342	0.7899

Habituation Diet







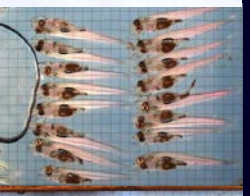
Habituation Diets:

- Highly attractive and palatable.
- Costly, but offered for a short period.
- Significant effect on survival and length.

	Otohime C2	EPAC CW 8/12
Survival (%)	88.3	73.5
Length (mm)	81.8	75.5
Weight (g)	4.5	3.7
$/1000 walleye	$12.66	$6.71

Habituation Diet





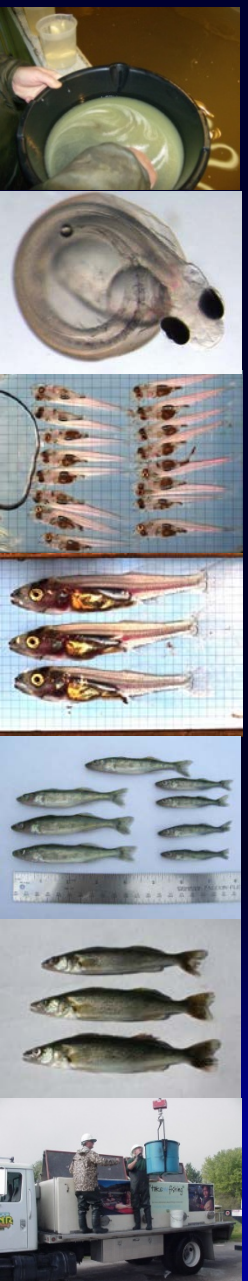


Survival (%) of walleye fingerlings to 28 days.

Diets	2003	2004	2006	2011*
BioVita FF	28.9 a			
Nutra 2000	28.3 a			
Nutra HP	47.3 b	44.0 a		
EPAC CW	52.7 b	75.0 b	73.5 a	
Gemma		53.0 a		
O.range				39.4a
Otohime			88.3 b	76.6b

2003, 2004 and 2006 studies conducted in hatchery tanks
*2011 Diet study conducted in research tanks

Habituation Environment



Sensitive to Light

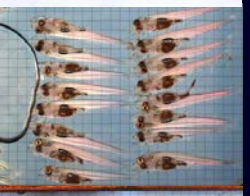
- Eyes are very sensitive to light
- Seek to escape light or shadows

Dark Room Environment

- Eliminates shadows
- Fish attracted to feed around submerged light

Phase II - Habituation







Dark - room Environment

- No overhead lighting eliminates shadows
- Submerged lights further reduce shadows

	Overhead lighting	Dark Room - Submerged Lights
Survival	37.3	60.7
g/d	0.117	0.147

63% increase in survival

- Increased growth rates
- Five evaluations, all favorable.
- Production practice since 2003

Habituation Environment

Culture Environment references

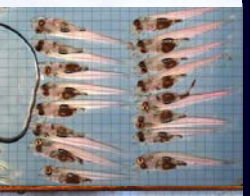
- Walleye are preadapted to life in weak illumination (Moore 1944).
- Nagel (1996) 90% survival with CSL.

Conducted study in the CSL environment.

- 2003: Individual raceway covers
- 2004: Mass raceway covering

Phase II

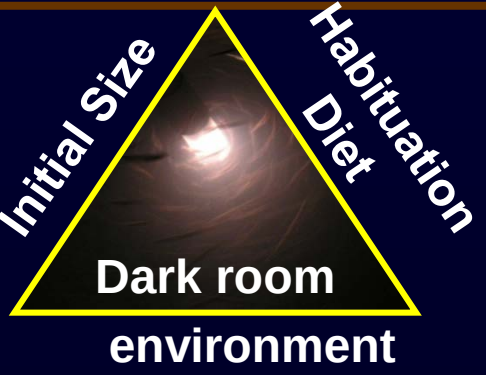


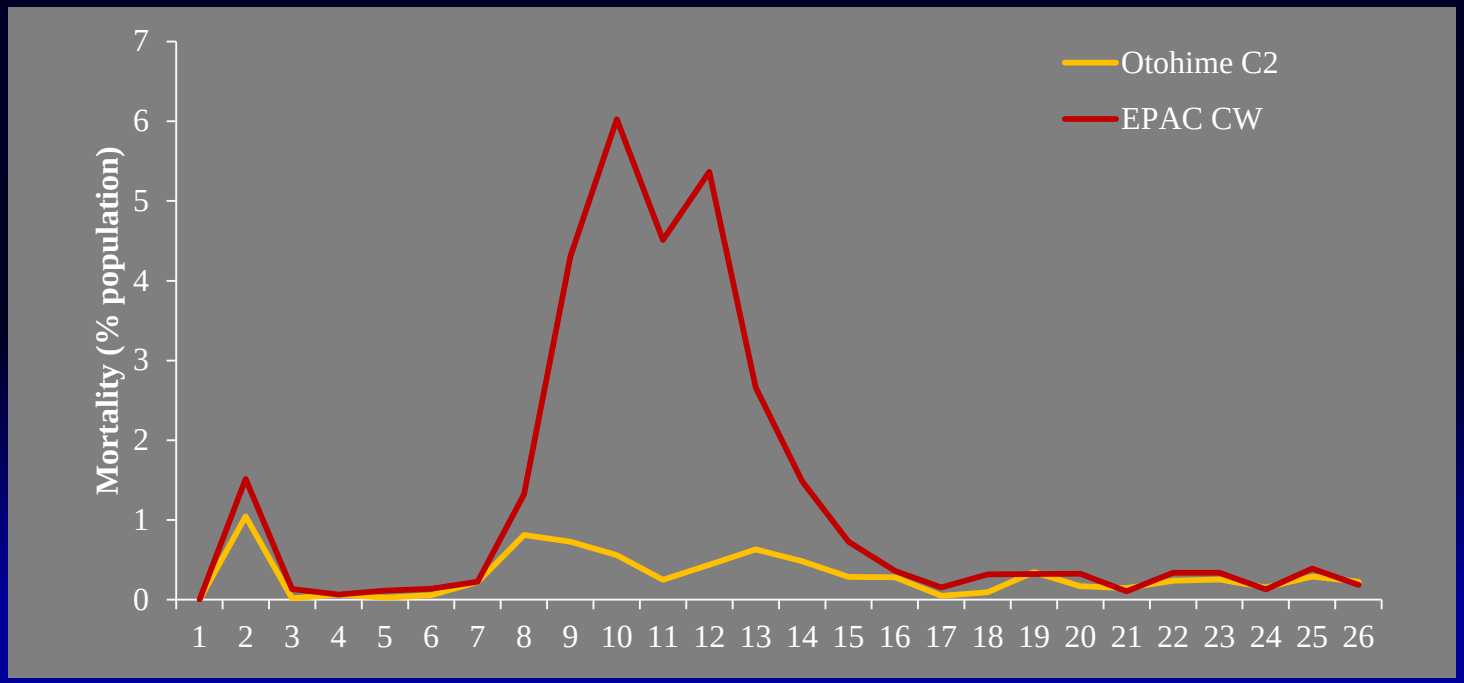




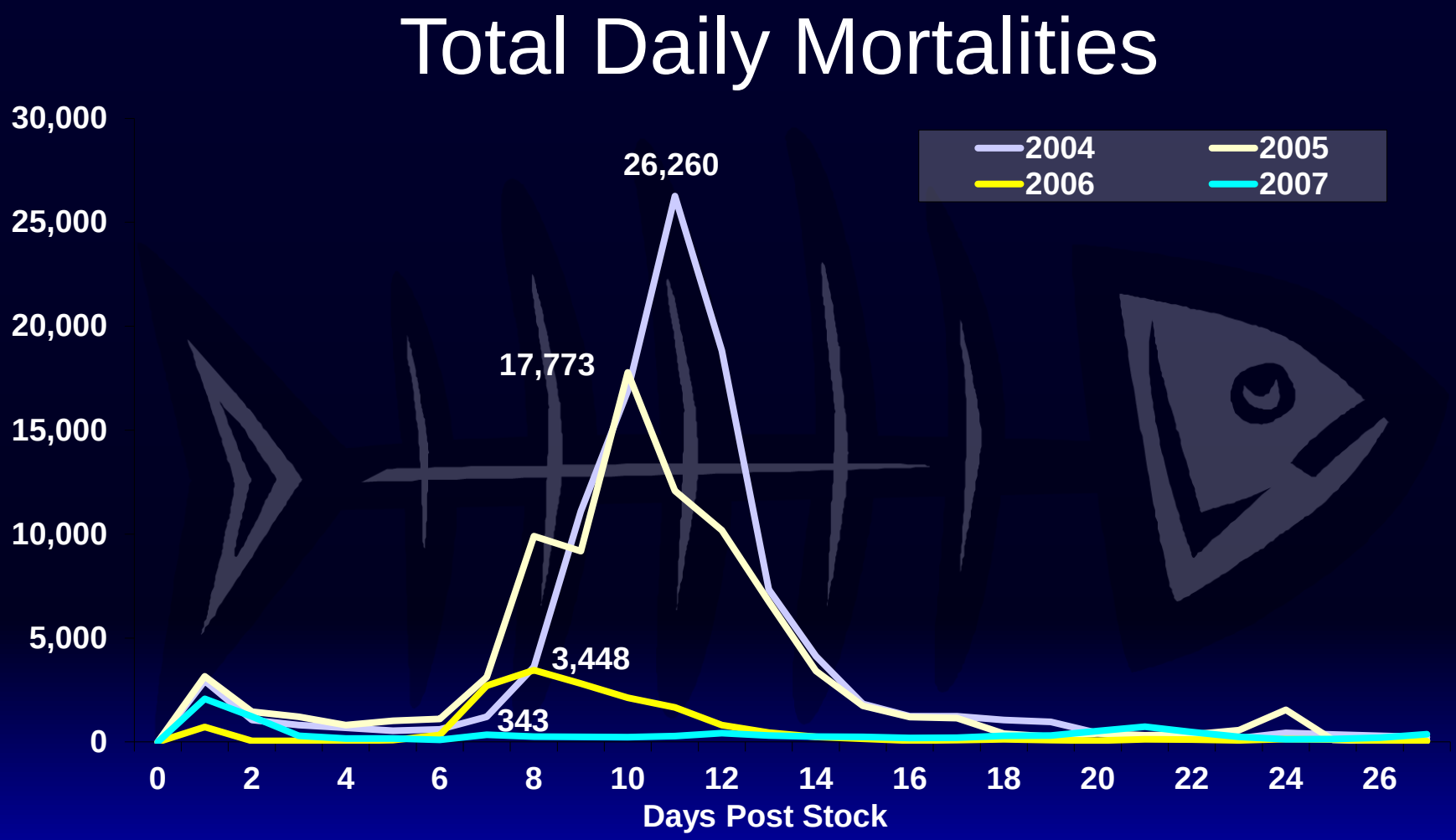
Keys to success:

- 0.57 g fingerling.
- Dark room environment, subm. light.
- Habituation feeding regime:
  - Day 1-10: Otohime C2.
  - Day 11-17: Mix of Otohime C2, Walleye Grower 1. 0.
  - Day 18-35: WG 1.0 to 2.0.



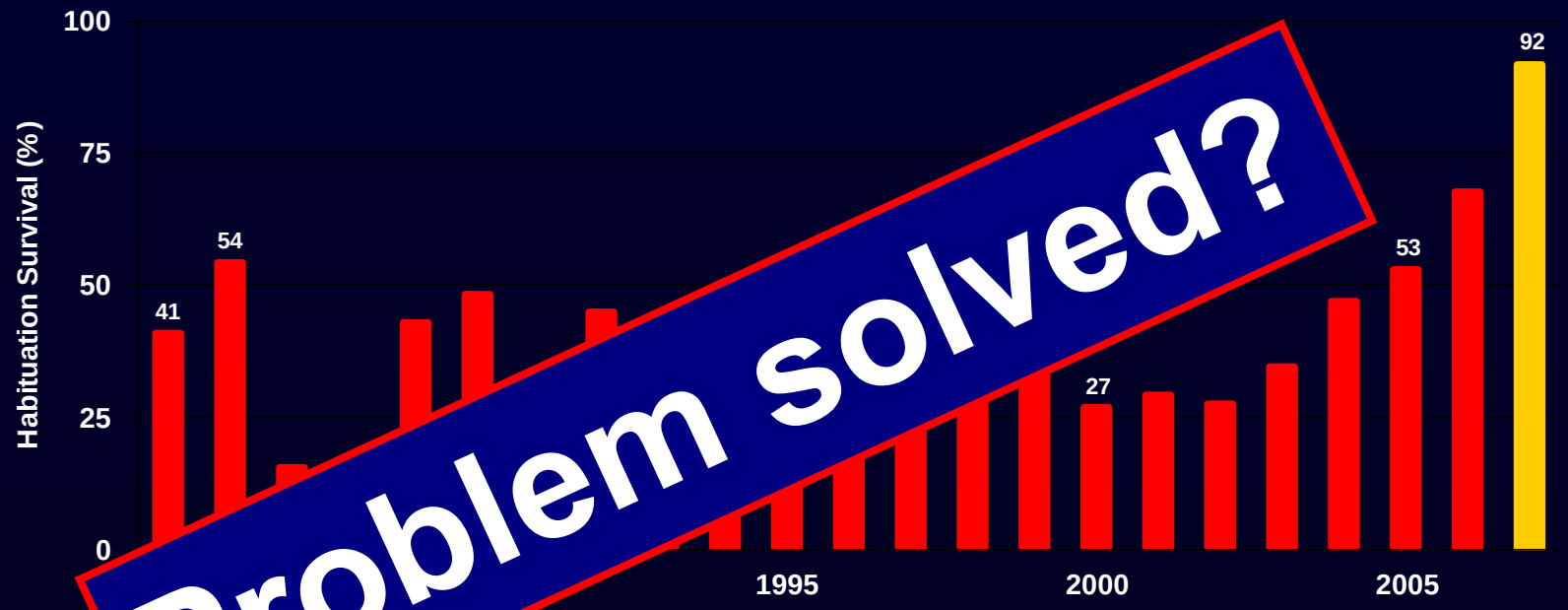


Keys to success: Progress through research



Phase II - Grading

Habituation History



2 2007 Pivotal years

Research to improve culture techniques

- **2.4 fold increase in survival.**
- **Stability and Reliability.**

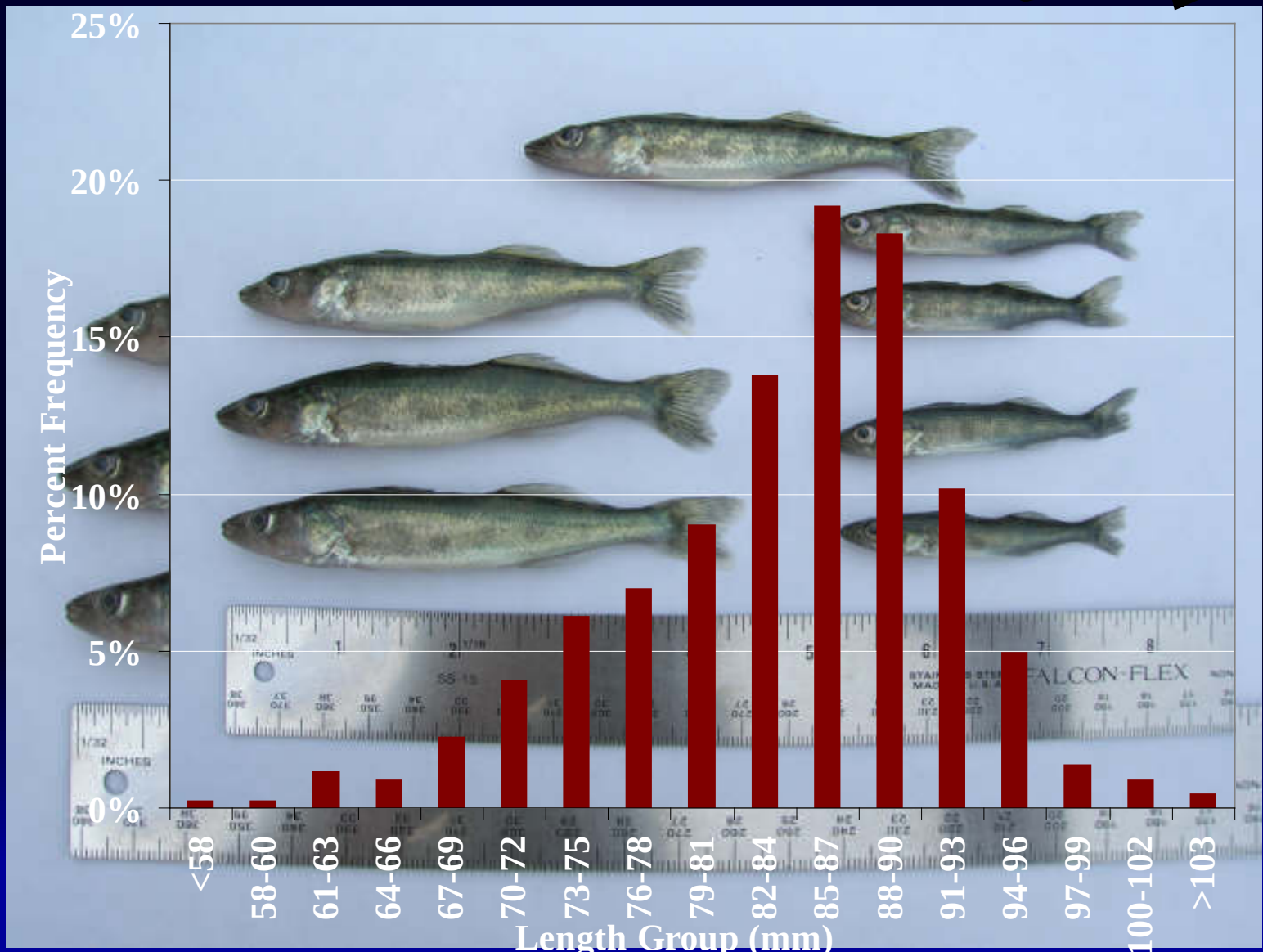
Unexpected problem

Performance:

Growout	2004	2006
FCR	1.87	2.04 ↑
Cannibalism	3.5 %	15.0 % ↑
Survival	92.7 %	83.8 % ↓

Habituation	2004	2006
Cannibalism	19.2 %	4.4 % ↓
Survival	47.0 %	67.6 % ↑

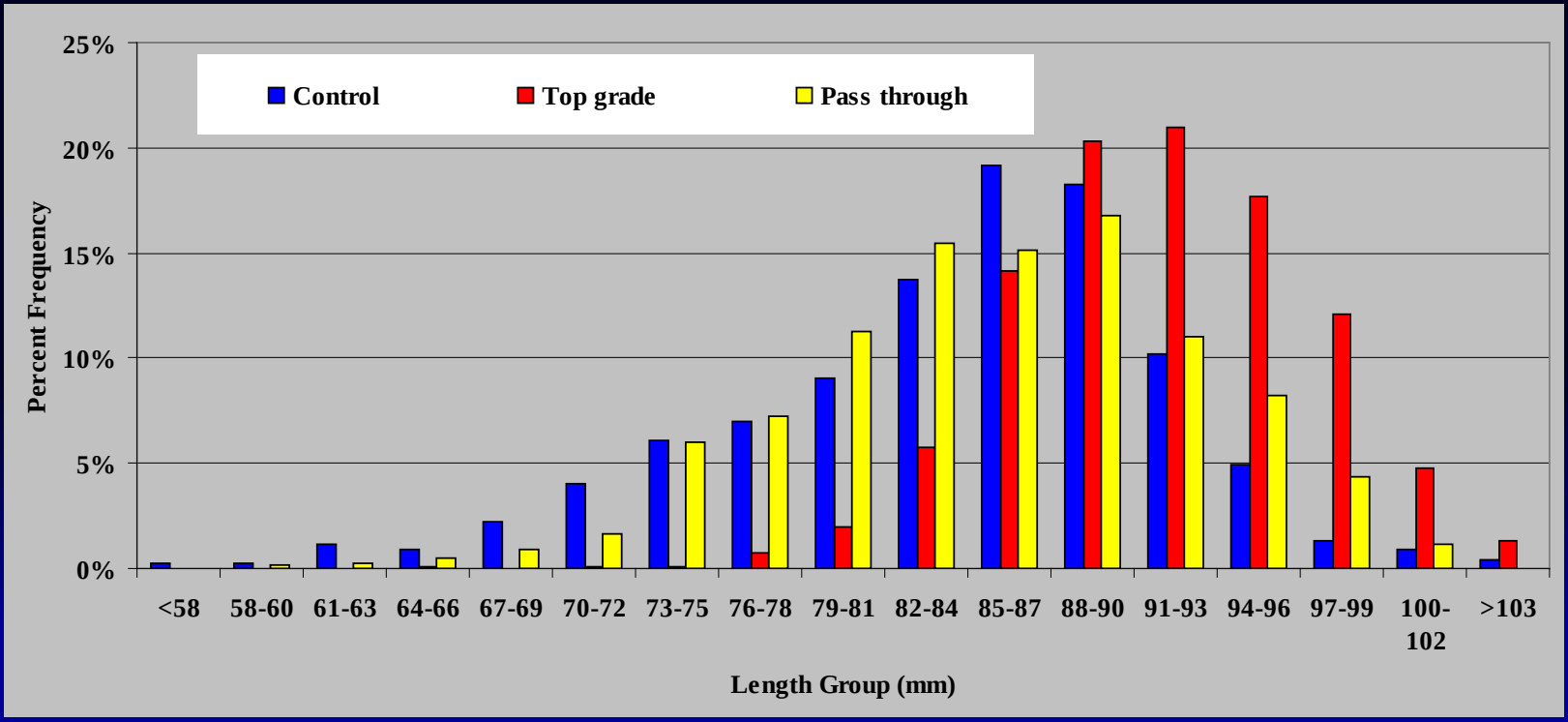
Phase II success?

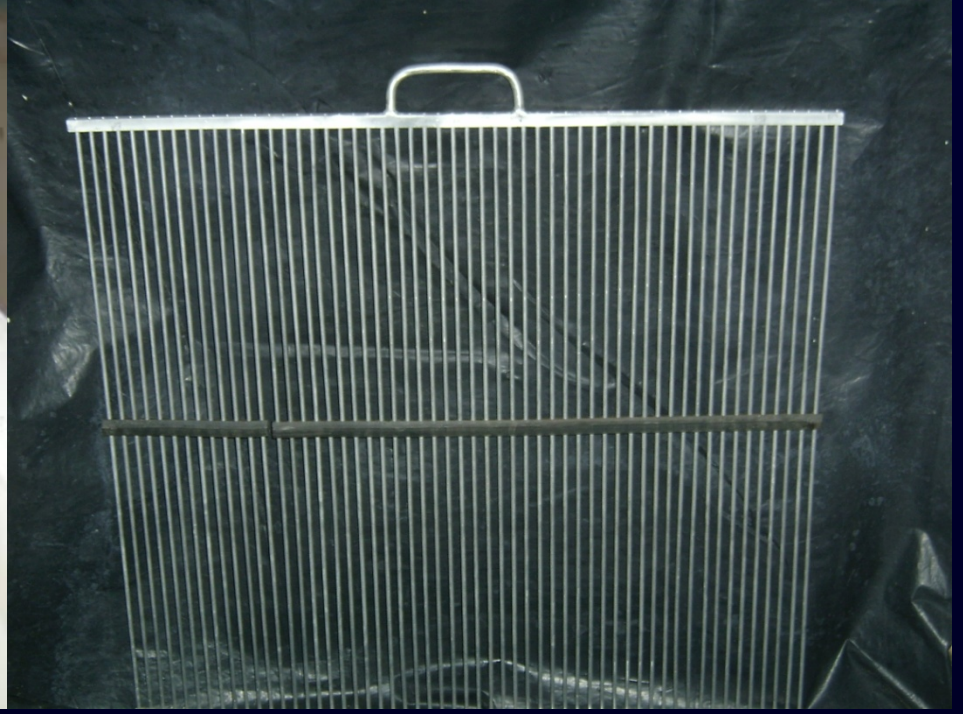


Grading

Evaluate grading

- **Grade between phase II and III.**
- **Comparison of Ungraded, Top 20% Grade, Pass through.**
- **Production scale – 10 outdoor tanks of 22,500 fish/tank.**





2007 - Grading

Significant improvement of production.

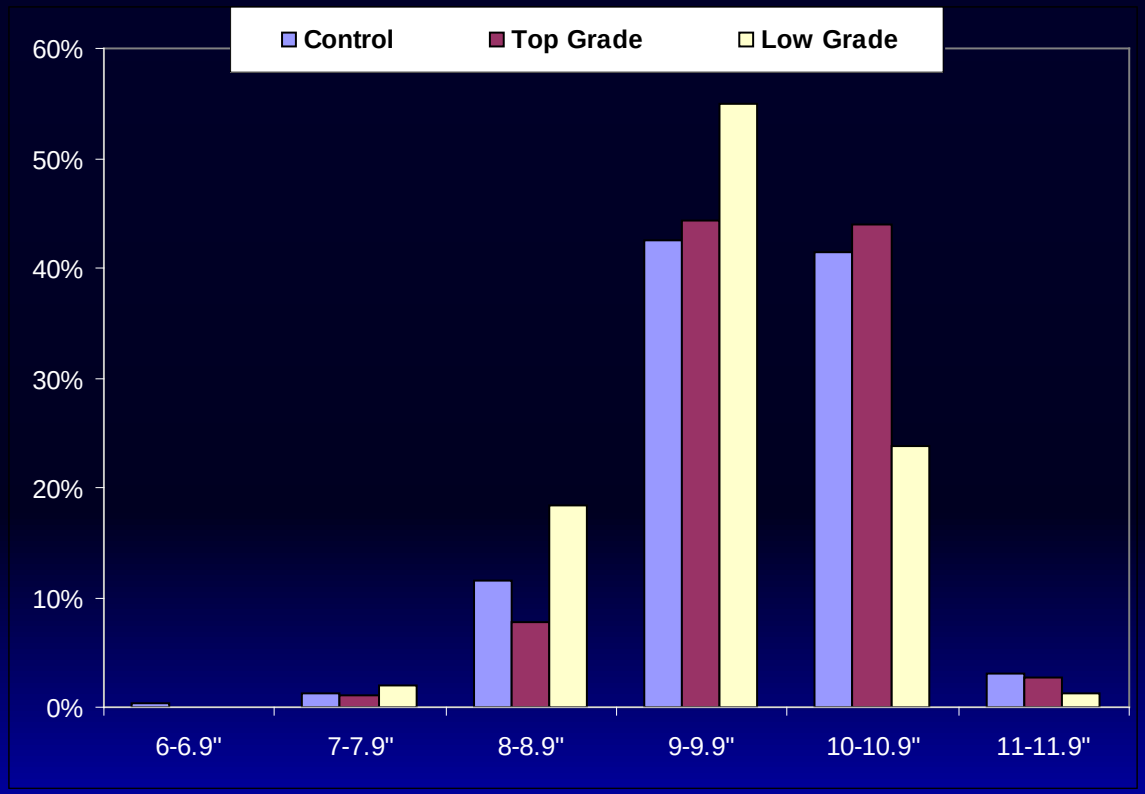
- **Reduced cannibalism by 62%.**
- **Survival increased 20%.**
- **Saved $0.10/fish.**

	Control	Uniformity Graded	Pass-through
Survival (%)	78.6	93.9*	82.5
Mortality (%)	10.4	1.9	9.2
Cannibalism (%)	11.0	4.2*	8.3
Feed conversion ratio	1.8	1.5*	1.8
Feed & Chemical Cost/Fish	$0.53	$0.43	$0.51

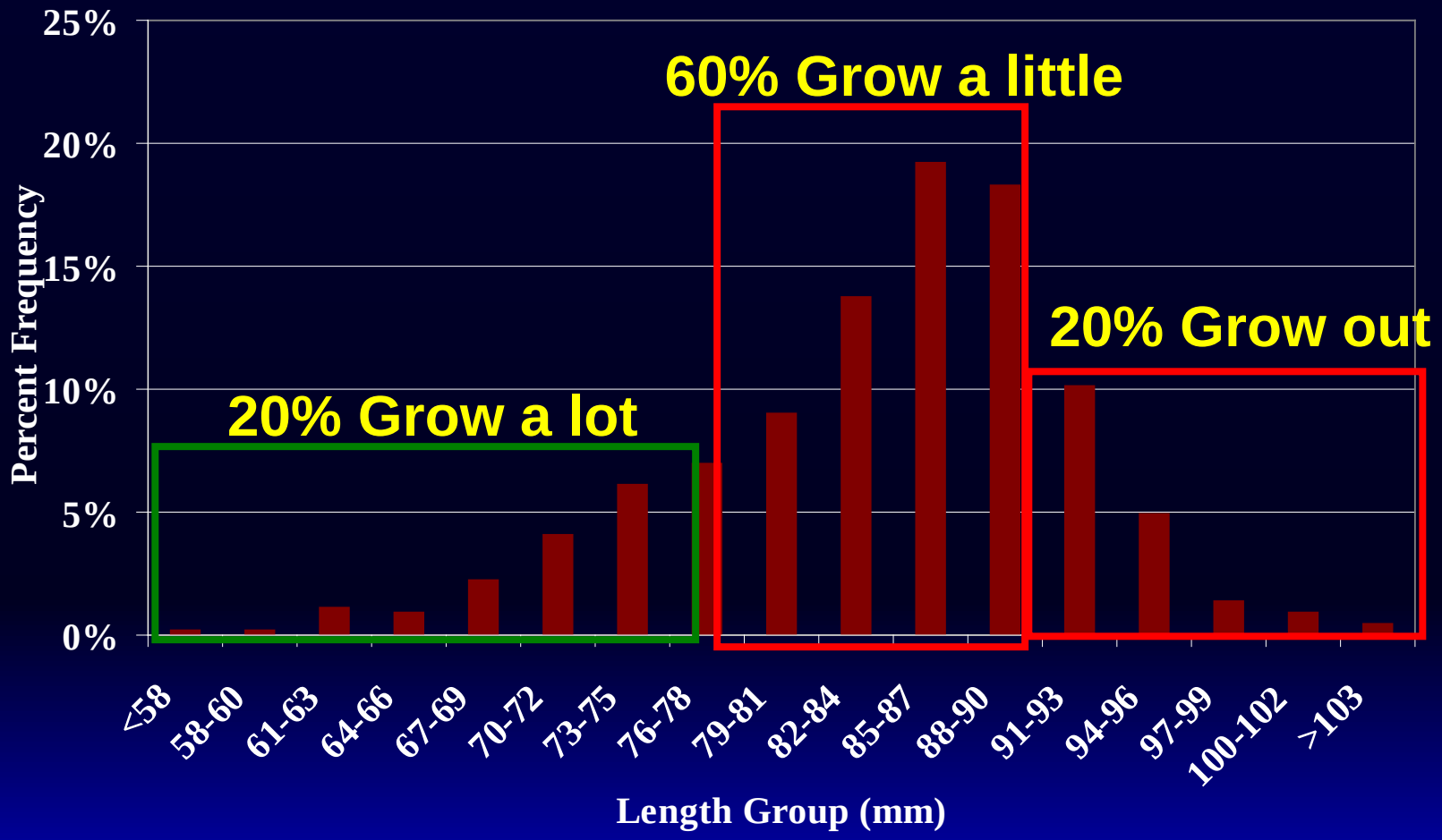
Grading Study - Results

Final length

Control	Top Grade	Low Grade
9.8 in.	9.9 in.	9.6 in.



2008 - Grading Plans



2008 - Grading

Grading was successful!

- **Cannibalism was reduced, same as last year.**
- **Mortality reduced, but not as good as we expected. (!)**

	Top grade	Mid grade	Pass-through
Survival (%)	91.2	87.9	88.9
Mortality (%)	5.5	8.1	7.2
Cannibalism (%)	3.4	4.1	3.9
Feed conversion ratio	1.76	1.60	2.00
Final L (mm)	230	229	223*

Blind Walleye

- Causes: Mechanical damage (handling or rough tanks), disease, gas saturation, sunburn??
- Solutions can be implemented when a cause is defined.
  - Reduce handling, fix saturation columns, etc.

Grading Study

- **Other Important Observations:**

Eye blindness	Control	Top grade	Low grade
Unilateral	5.71%	8.13%	7.17%
Bilateral	0.0%	0.52%	0.82%
All above (total)	5.71%	8.65%	7.99%

- **No significant difference in rate of blindness.**

- **2006 (before grading was implemented)**
  observation 30% blind eyes in one production tank.

Phase III – Growout

Keys to Success

Phase I

- Pond stocking density results in 600-800 fish/lb harvest size.
- Fertilization regime.

Phase II

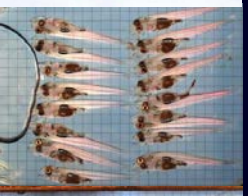
- Dark Room Submerged Light Environment
- Habituation diet – Otohime C2

Phase III

- Size grading
- Feed rates
- Control costs

Phase III







Growth period – July – October

3.75 in. to 9-10 inches

Culture System:

- 10 outdoor circular tanks
- 45,000 gallon, (170 m^3) concrete tanks.
- Flow rate 0.45 exchanges/hour
- Single-pass, surface water.
- Final density 0.15 lb/gal

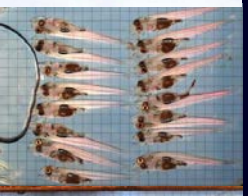
Phase III Stocking

Fish from Phase II are transferred after:

- **trained to formulated diet, WG 9206**
- **graded for size**
- **90 mm in length**
- **Stocking rate = 21,500 fish / tank (126 fish/ m^3).**

Phase III - Husbandry





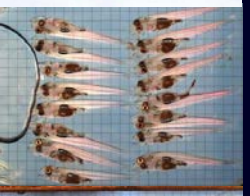


Monitor Dissolved Oxygen
Collect mortality, examine.
Treat disease if necessary
Fill feeders
Calibrate feeders
Leave them alone.

Weekly – sample 5 fish for Ich infestation
Biweekly – sample 25-50 fish Length & Weight


Phase III - Growout

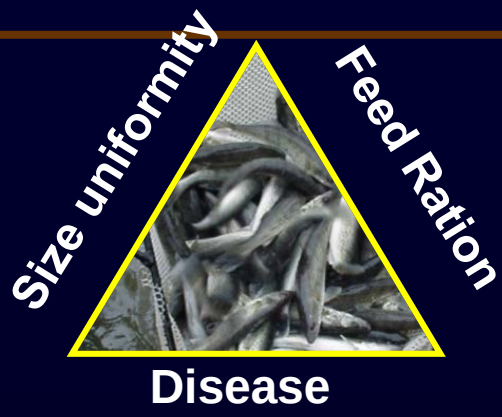






Growout to 9 inches:

- 75% of fish cost incurred.
  - Feed; FCR = 2.0.
  - Therapeutants; Formalin $32,000.



How can we improve efficiency?

Improve FCR

Year	Research Project	FCR	$ Savings/tank
2008	Phase II grading	1.6	$1780
2009	Measure fish, Feed the gain	1.4	$700

Growth rates

23.9 – 25.5ºC Optimal Growth Temperature

- **Fry**
  - **Pond Culture: 1.2 mm/d**
  - **Intensive Fry culture: 1.0 mm/d 18.3º C**
- **Fingerlings: 45 to 90 mm**
  - **1.75 to 2.0 mm/d**
- **Fingerlings: 90 to 230 mm**
  - **1.5 to 1.75 mm/d**

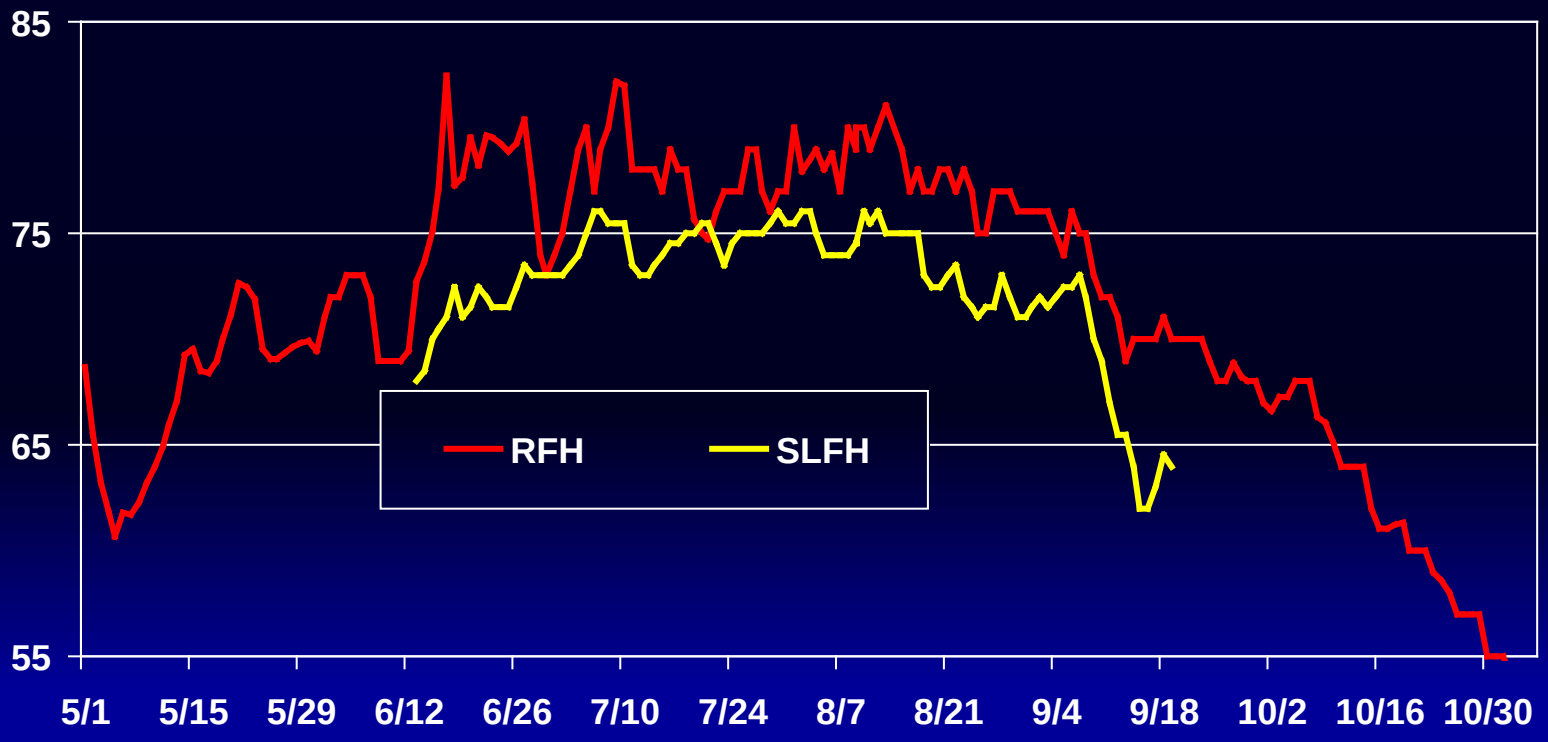
Growth Rates

- **Phase I** – Fry to 45 mm
  - Pond Culture: 1.2 mm/d
- **Phase II** – 45 to 90 mm
  - 1.75 to 2.0 mm/d
- **Phase III** 90 to 225 mm
  - 1.5 to 1.75 mm/d

24-25º C Optimal Growth Temperature

Northern Climates

Rathbun 9-10” in 5 months (73.8°F)
Spirit Lake 6-7.5” in 4.5 months.
Commercial production in heated and enclosed systems.



Challenges to Efficiency

1. Cannibalism

- Grading in Phase II – evaluated in Phase III.

2. Poor feed conversion ratio

- Monitor growth rates
- Feed the gain

3. Formalin use for *Ich* treatment

- Monitor *Ich* population
- Treat according to need

Challenge 1. Cannibalism

As Phase II survival increased

- **Phase III cannibalism losses increased (R^2=0.94)**

2007 Grading Research

- Production scale – 3 outdoor ponds of 22,500 fish/treatment
  - Graded between phases II and III
  - Comparison of uniformity graded and control

2007 Grading Research

Grading significantly improved walleye production efficiency

- **Reduced cannibalism by 80%**
- **Survival increased 15%**

	Control	Graded
Survival (%)	78.6	93.9
Mortality (%)	11.0	4.2
Cannibalism (Unaccounted %)	10.4	1.9
Feed conversion ratio	1.8	1.5
Total Feed and Chemical Cost/Fish	$0.53	$0.43

Grading is now part of our walleye production process

Feed Rates

How much to feed? No standardized rates….

2007 Grading – 1.5 FCR.

Can we do better?

- **1.2 FCR optimum for maximum growth of RbT.**

Methods:

Known growth and mortality rates.

Westers feed equation.

Accurate feeders?

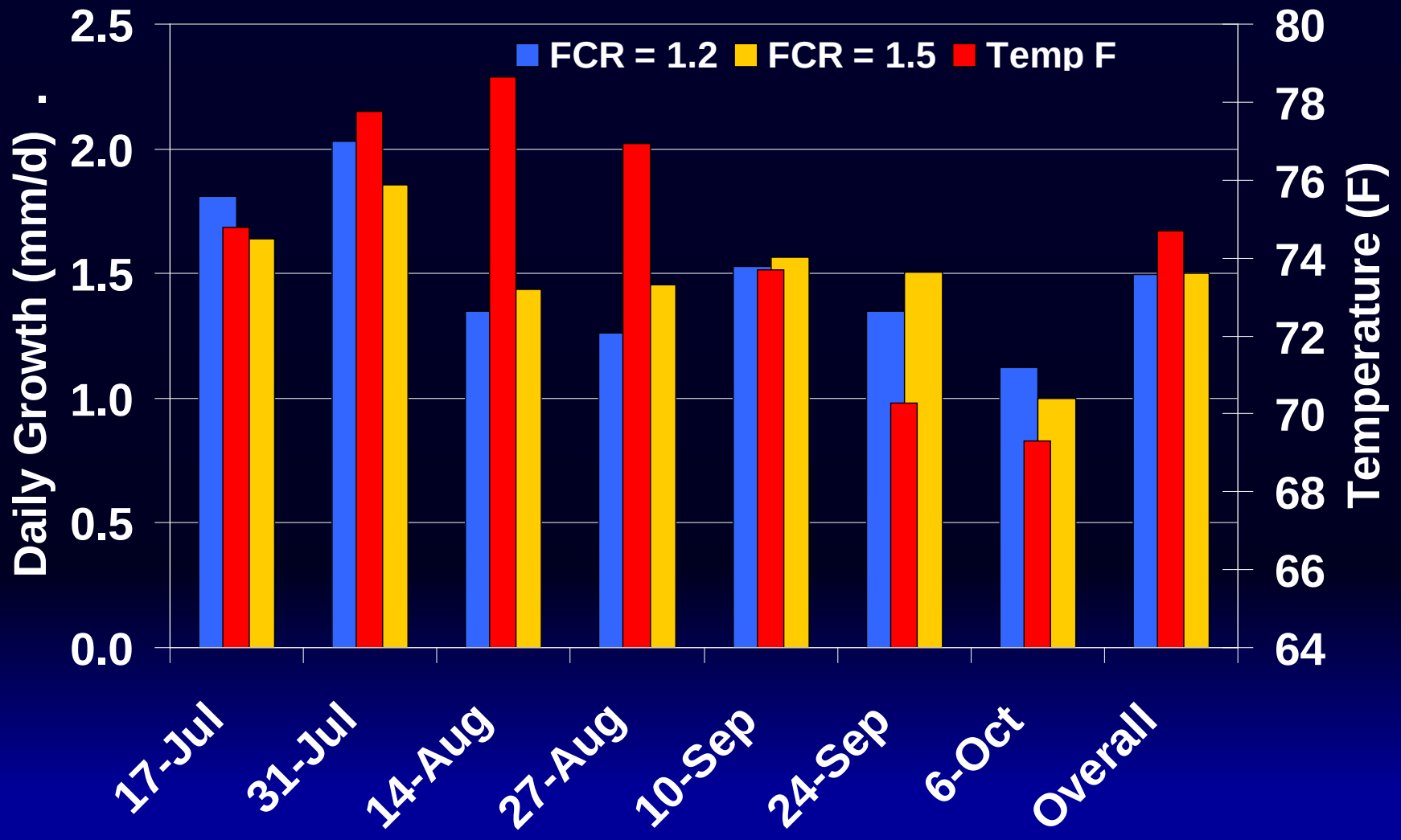
Challenge 2. Feeding

Prior to 2008 – FCR was often 2.0, fish were fed based on projected growth

2008 plan to improve feeding efficiency by:

- Sample fish and measure growth rates
- Developing a feed rate calculation spreadsheet using Westers' equation
- Target a 1.2 FCR

Walleye Growth



2008 Results

- Sampling fish every 2 weeks gave excellent growth data and did not harm the fish
- Feed rate calculation spreadsheet was developed
- Did not achieve goal of 1.2 FCR but made improvement

	1.2 FCR	Control
Actual feed conversion ratio	1.44*	1.90
Survival (%)	89.0	89.8
Final L	229	226
Daily growth (mm/d)	1.5	1.5

Improved FCR resulted in $0.07 savings per fish

2009 Research

Develop a formula to "Feed the gain":

- **Sample for length gain**
- **Translate length gain to weight gain via relative weight (Wr) equation**
- **Feed 1.2 X calculated biomass gain.**
- **Research compared 1.2 and 1.5 FCR**

	A	T	U	V	W	X	Y	Z	AA	AB	AC	AD
1											Predicted	
2		Current	Current	Daily Gain	Predicted	predicted	Weight	Biomass		Daily	Daily	
3	DATE	Length	Weight	Length	Wr	Weight	Gain/fish	Gain (g)	FCR	Feed (g)	Feed (lb)	% BW/d
37	7/26	131.4	18.787	1.50	100.0	19.409	0.622	12820	1.2	15383.9	33.9	3.97
38	7/27	137.3448276	23.04483	1.75	105.6	23.379	0.834	17174	1.2	20609.3	45.4	4.34
39	7/28	139.1	23.379	1.75	105.6	24.233	0.854	17581	1.2	21097.7	46.5	4.35
40	7/29	140.8	24.233	1.75	105.6	25.107	0.874	17990	1.2	21588.1	47.6	4.33
41	7/30	142.6	25.107	1.75	105.6	26.001	0.894	18398	1.2	22077.2	48.6	4.27

2009 Results

	1.2 FCR	1.5 FCR
Feed conversion ratio	1.38	1.59
Survival (%)	83.2	85.5
Final L	230	232
Daily growth (mm/d)	1.5	1.5

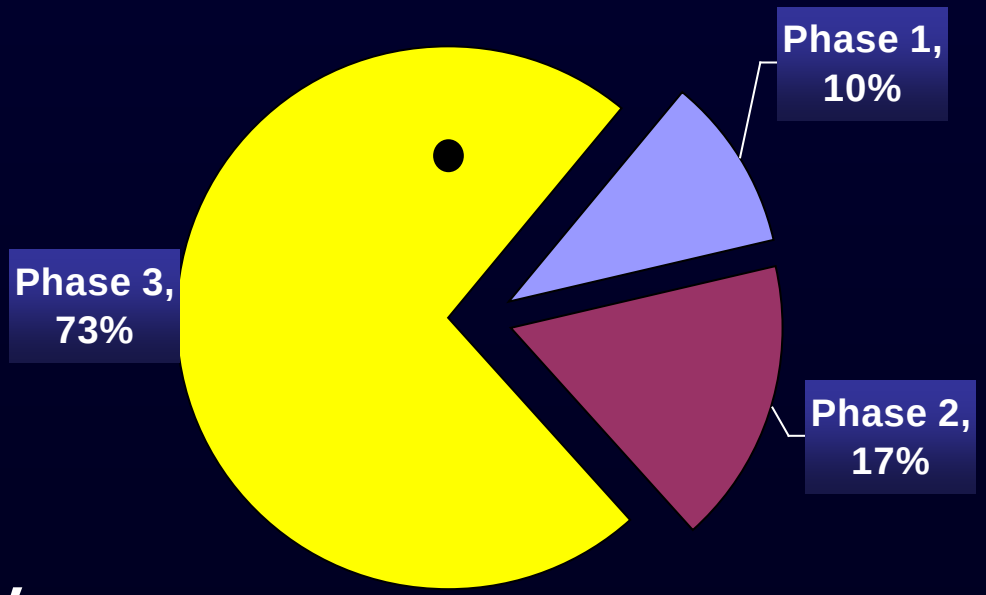
- We currently “feed the gain” at 1.2 FCR.
- Savings - $700 of feed per grow-out pond (1.2 vs 1.5 FCR)

Improved feed efficiency

- **2008 – Grading**
  - **Improved survival, FCR**
  - **$900/tank saved**
- **2009 – Feed calculations**
  - **Improved FCR**
  - **$700/tank saved**

Production Costs

Phase III Costly



Expenses:

Formalin therapy –

Feed: increased fish size –

Avoidable losses:

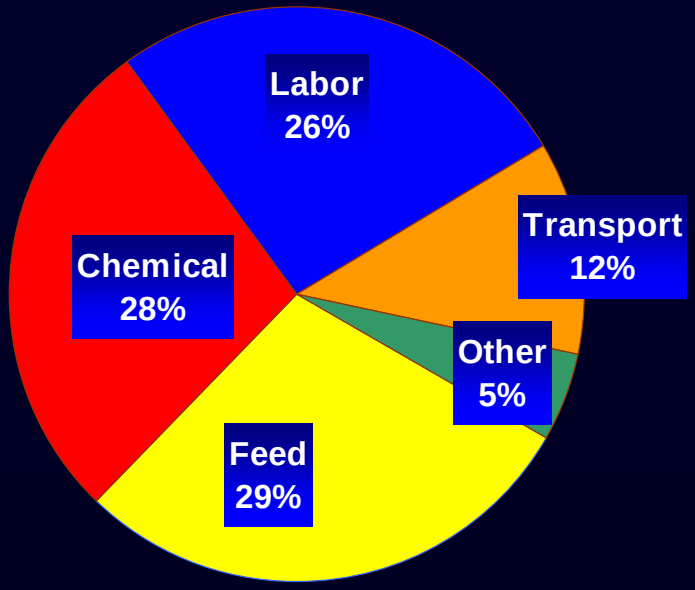
Cannibalism loss – 9% (4% - 15%).

Feed Conversion – 1.9 (1.6 – 2.4)

Production Costs

Production costs have more than doubled in the past 6 years.

- **2003: $0.51/fish**
- **2008: $1.08/fish**



Why the increase?

Input cost increases 55%

Larger fish are produced

- **2007- 2x heavier than in 2000.**
- **More feed and stocking trips!**

Rathbun Survival (%) by Culture Phase

Year	I	II	III	Fry to fall fingerling
2001	71.5	28.9	88.7	46.6
2002	89.6	26.0	90.8	59.7
2003	84.6	33.1	97.4	60.5
2004	81.7	46.9	92.7	35.5
2005	99.6	52.9	83.0	60.7
2006	87.7	67.3	82.8	48.8
2007	95.0	91.8	85.5	74.4
2008	85.0	89.1	88.7	67.2
2009	100.9	87.5	85.6	74.9
2010	92.9	84.0	94.1	73.4
2011	90.7	85.1	82.8	63.9
2012	89.0	61.0	82.9	45.0
2013	89.8	71.4	80.2	51.4
2014	89.6	71.0	80.1	50.9

Disease and therapeutants

Rathbun Fish Hatchery

Annual production:

- 200,000 walleye fingerlings (200 mm).
- 250,000 catfish fingerlings (175 mm)

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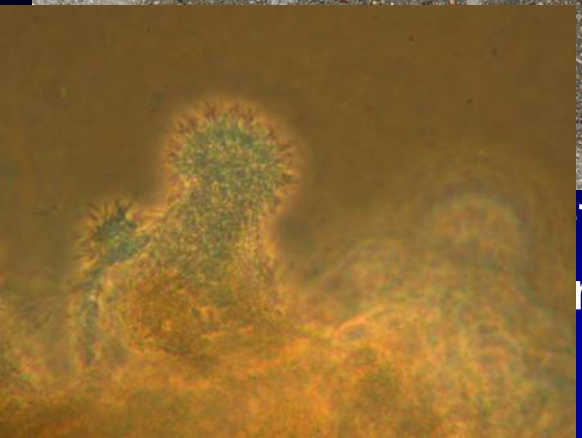
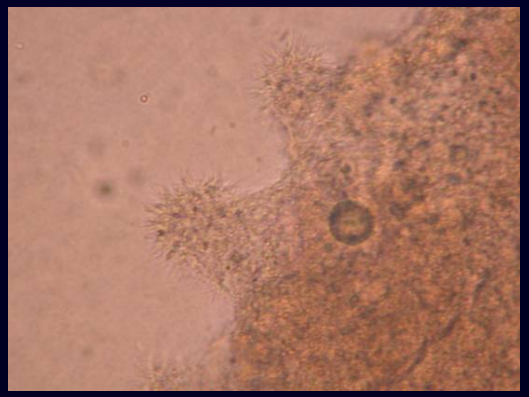


Overview

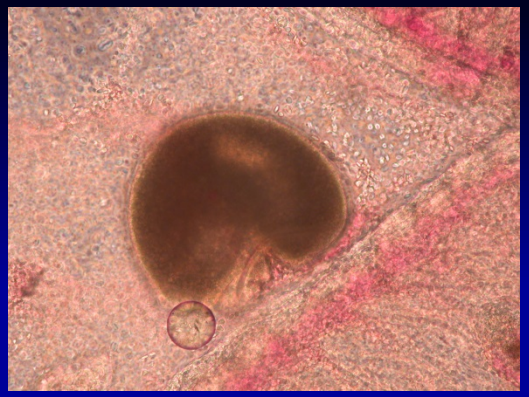
Efficacy trials 2006

- Catfish – Columnaris – Aquaflor™
- Walleye – Columnaris – Chloramine-T



Theraputant comparison:

- Walleye columnaris
- Diquat, Chloramine-T, Hydrogen Peroxide



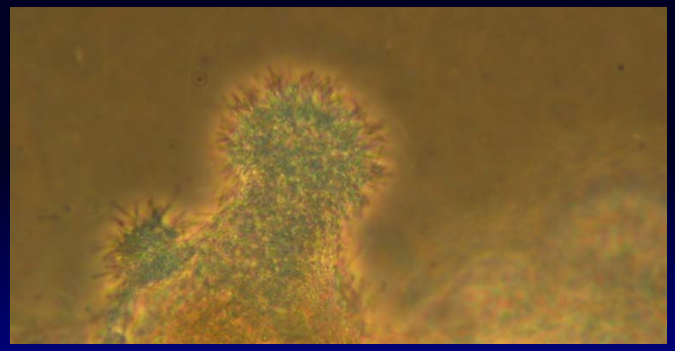
Theraputant regime modifications:

- Walleye *Ich* infections
- Compared formalin treatment regimes

Columnaris infections

Net handling – skin abrasions

Saddlebacks

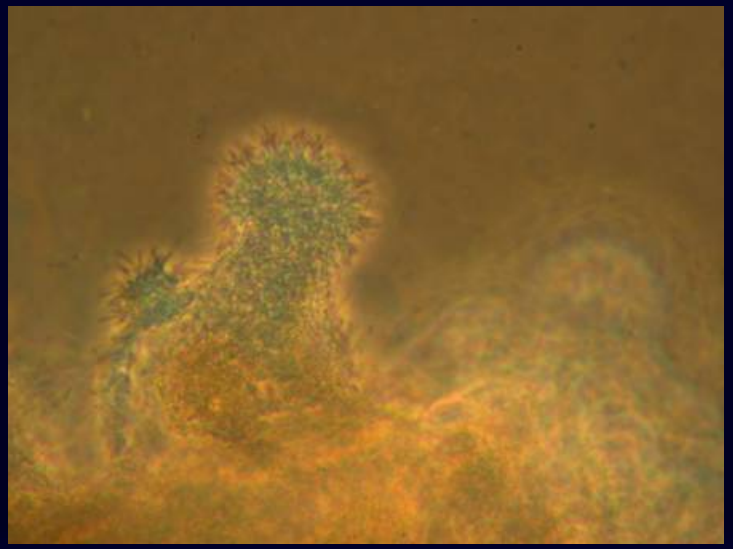


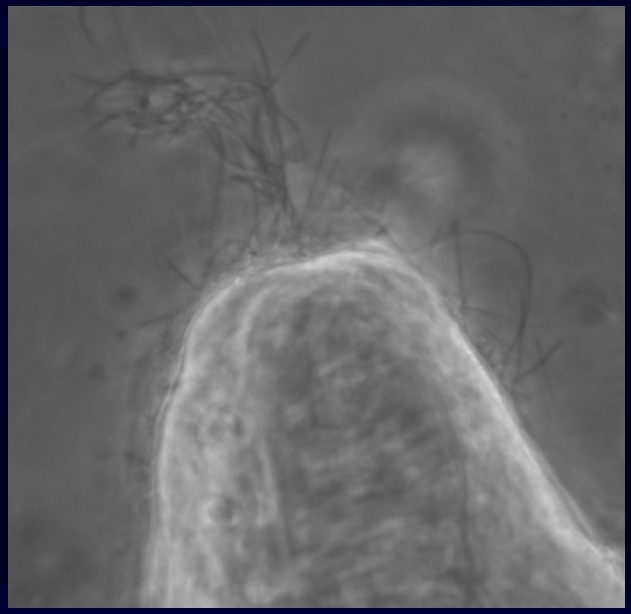
Diagnosis:
Flavobacter columnare

Phase II Diseases

Common diseases:

- **Columnaris**
- **Bacterial gill disease**





Disease management:

1. **Monitor**
2. **Identify**
3. **Treat – Diquat or Chloramine-T**

Columnaris theraputant comparisons

Objective: Compare bath theraputants for columnaris disease during habituation.

- **Diquat**
- **Hydrogen Peroxide**
- **Chloramine-T**

Theraputant use :

1. **Diagnose columnaris.**
2. **Apply theraputant.**
3. **Monitor post treatment mortality, re-infection.**

Columnaris therapeutant comparisons

Fish – Walleye fingerlings.

- Pond reared fingerlings.
- Initial size: 45 mm, 0.65 g
- 3,500 fish/raceway, triplicate raceways.

Standard habituation practices

- Study duration: 27 days.

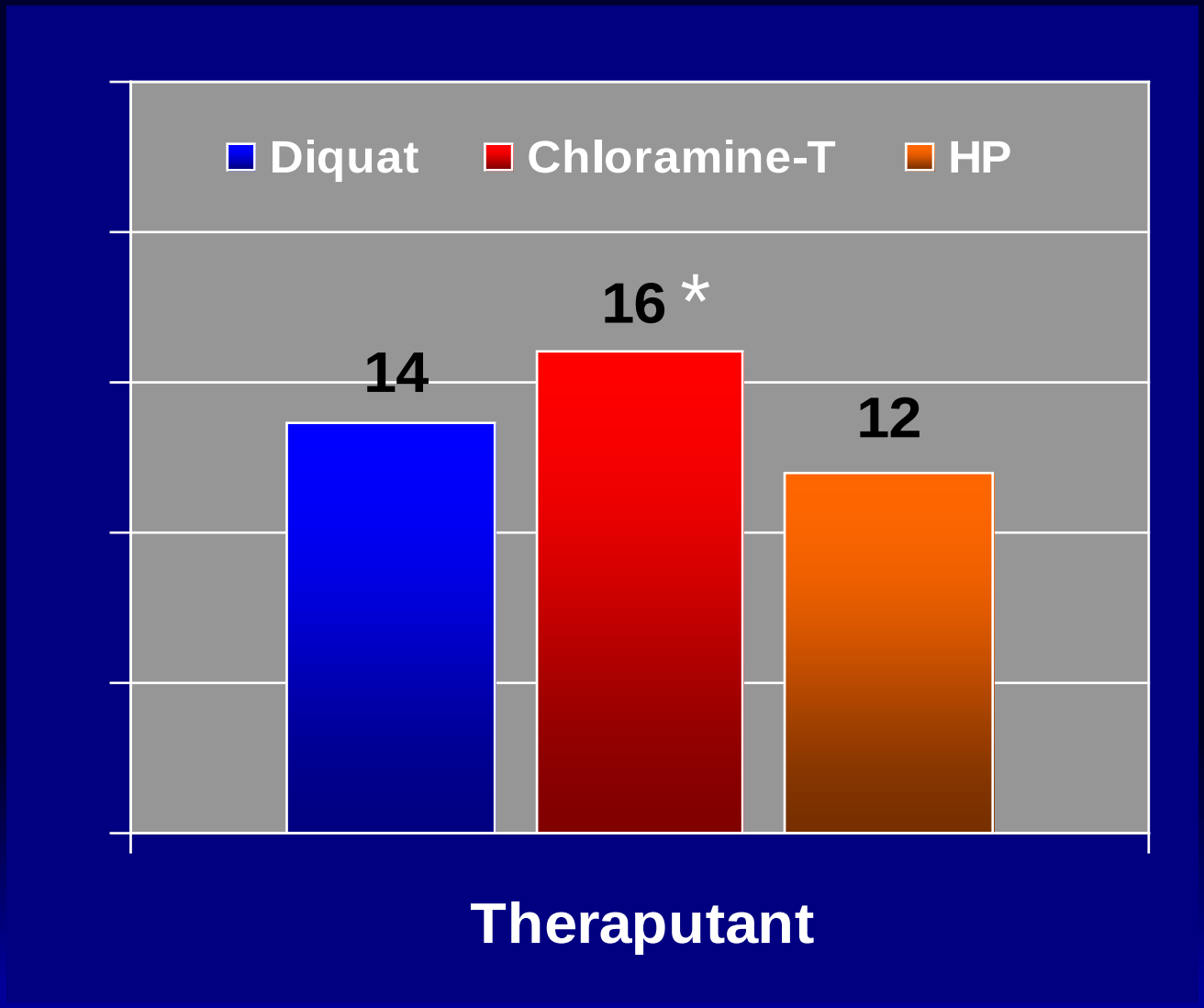
Theraputant application –

- Started on day two.
- Day one: 0.5% salt for all tanks.

Columnaris theraputant comparisons

Theraputants	Treatment Rate	Duration
Diquat	12 to 18 mg/l	2 h
Perox-aide	First treatment: 25 mg/L Thereafter: 50 mg/L	30 m up to 1 h
Chloramine-T	15 to 20 mg/L	1 hr
Control (1 tank)	None	-

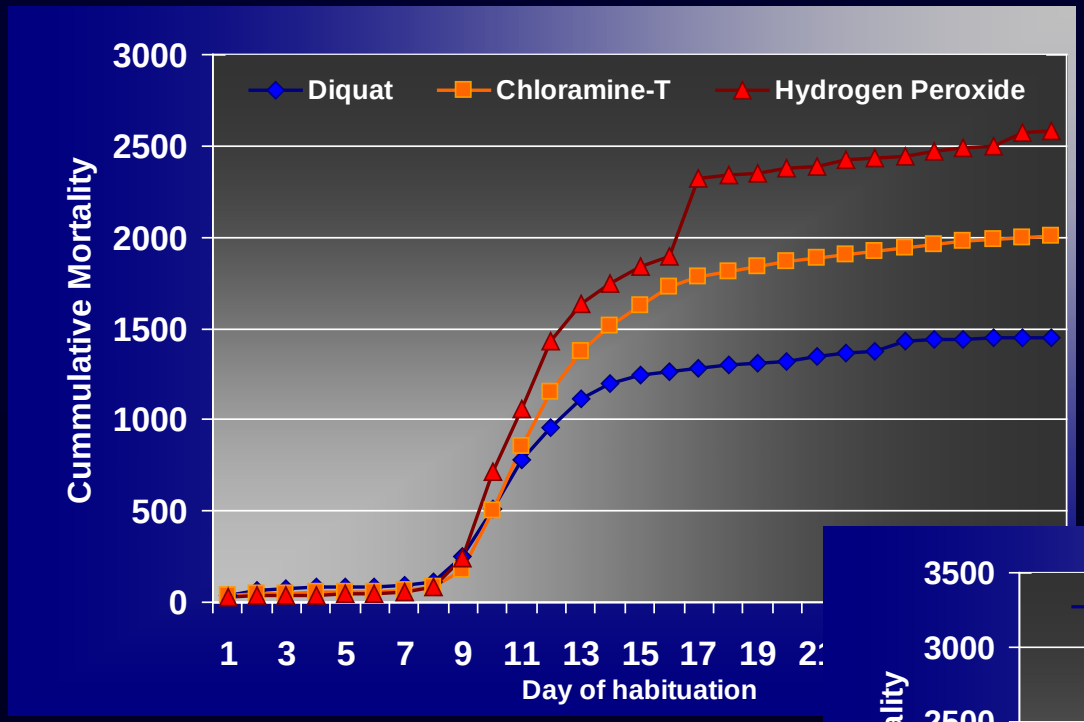
Number of Applications



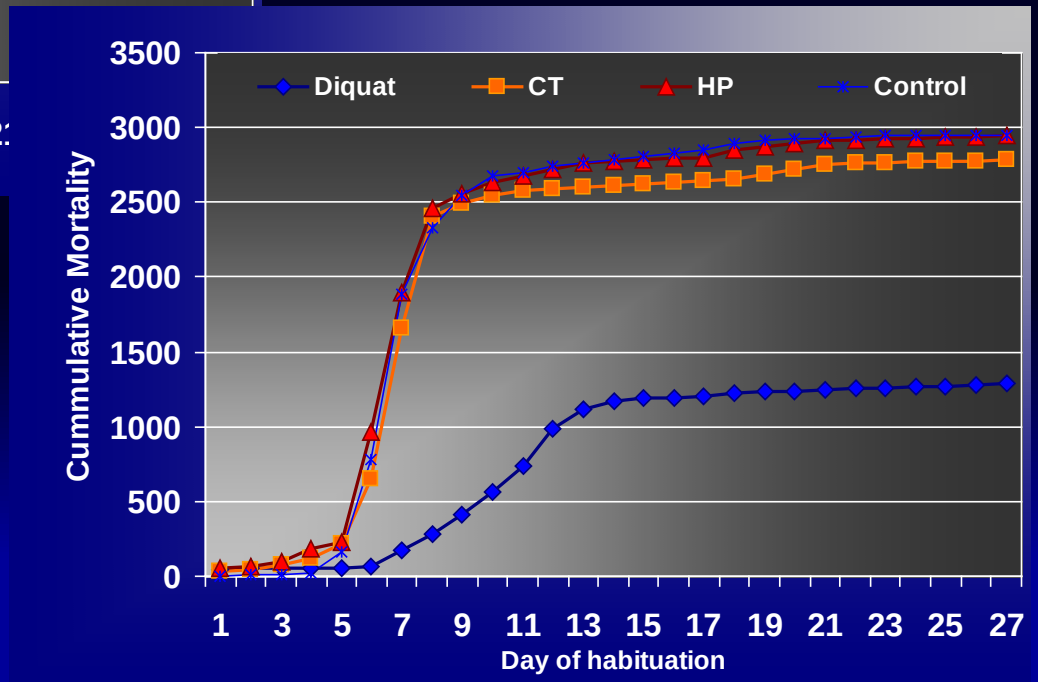
*Chloramine –T was applied more often than others (P=0.004)

Year comparison

2005 Cumulative mortality curves



2006 Cumulative mortality curves

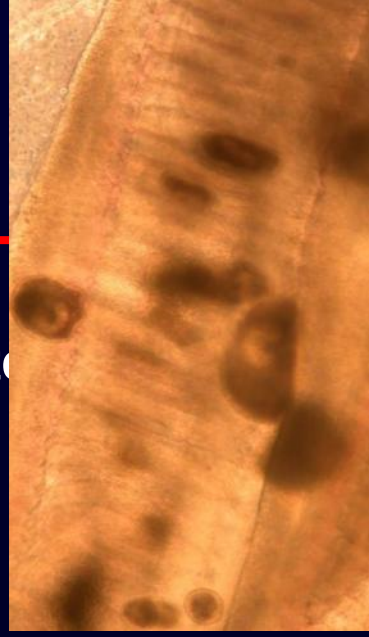


Challenge 3. Ich Treatment

Parasite-S (formalin) applied July – October

- Prior to 2008 prophylactic treatment regime:
  - Daily 9 hour flowing water treatment
  - 45 mg/l formalin via metering pump
- $30,000 – $35,000 annually

2009 Ich Research



Treatment - Daily 24-h formalin treatment until eradicat

- **"Action levels"**
  - **If Ich > 15 cells/gill arch – begin daily treatments**

Results

- **1. Threshold count of 15 cells/gill arch was adequate**
- **2. Infestations can be controlled with consecutive daily treatments.**
- **3. Seven days of continuous treatment required to eradicate Ich if tempetures are above 21˚ C**