Attachment 5

Presentation by Ralph Lampman on Development of Artificial Propagation Methods for Production of Juvenile Pacific Lamprey

Outline

1. Background
2. Larval Rearing Experiment (2015)
3. Larval Rearing Experiment (2016)
4. Larval Rearing Experiment (2017)
5. Future Needs

Art. Prop. & Rearing Protocols

2016 AFS Book "Jawless Fishes of the World"

CHAPTER 21

DEVELOPING TECHNIQUES FOR ARTIFICIAL PROPAGATION AND EARLY REARING OF PACIFIC LAMPREY (*ENTOSPHENUS TRIDENTATUS*) FOR SPECIES RECOVERY AND RESTORATION

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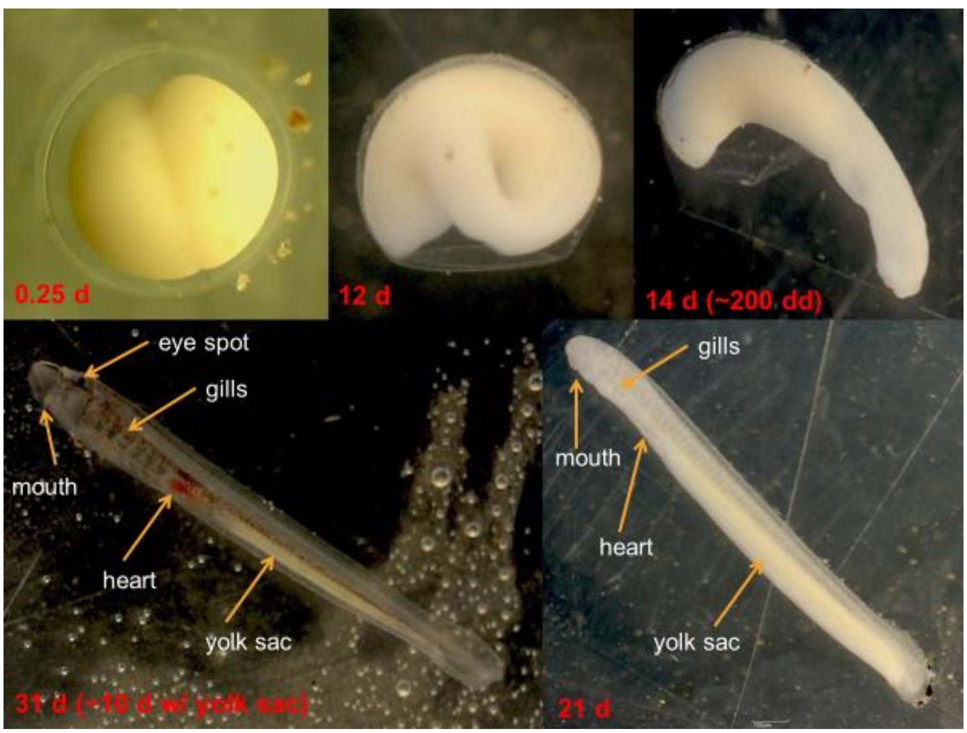
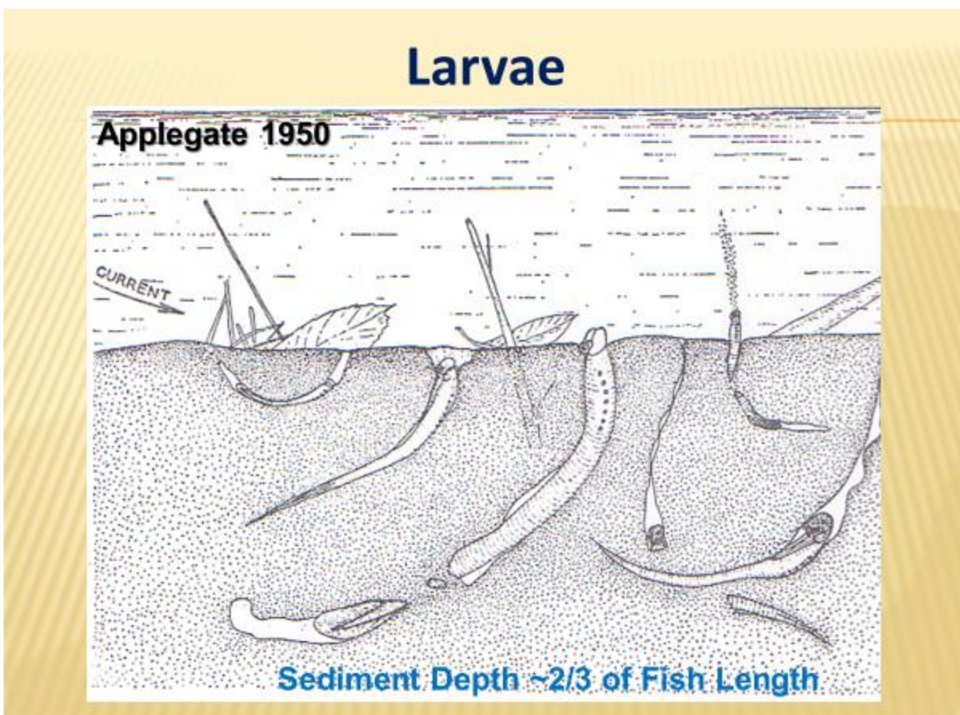
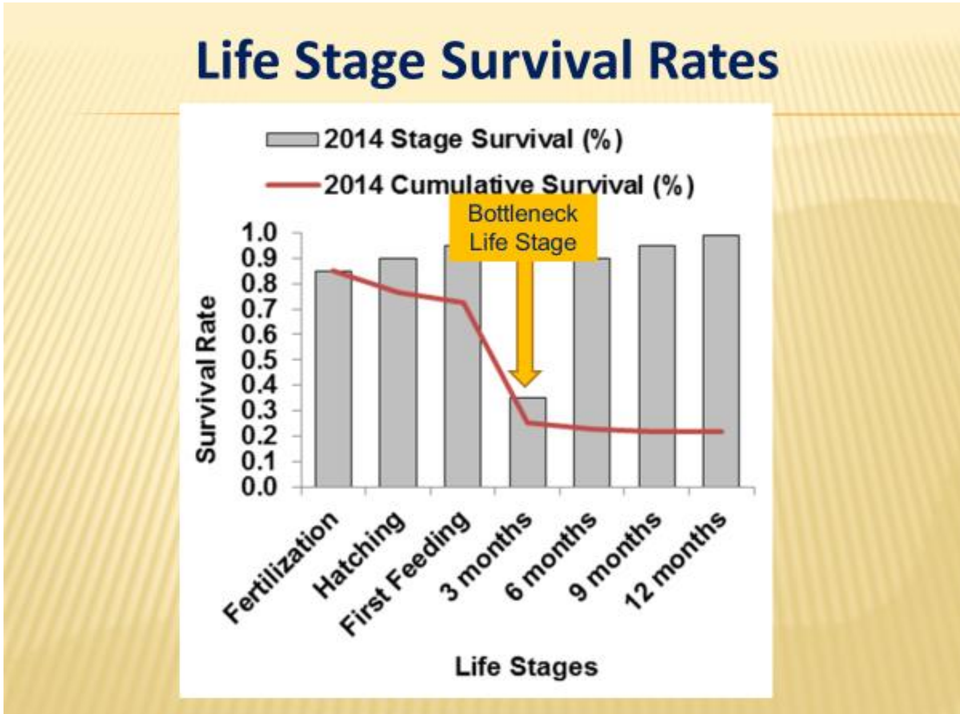
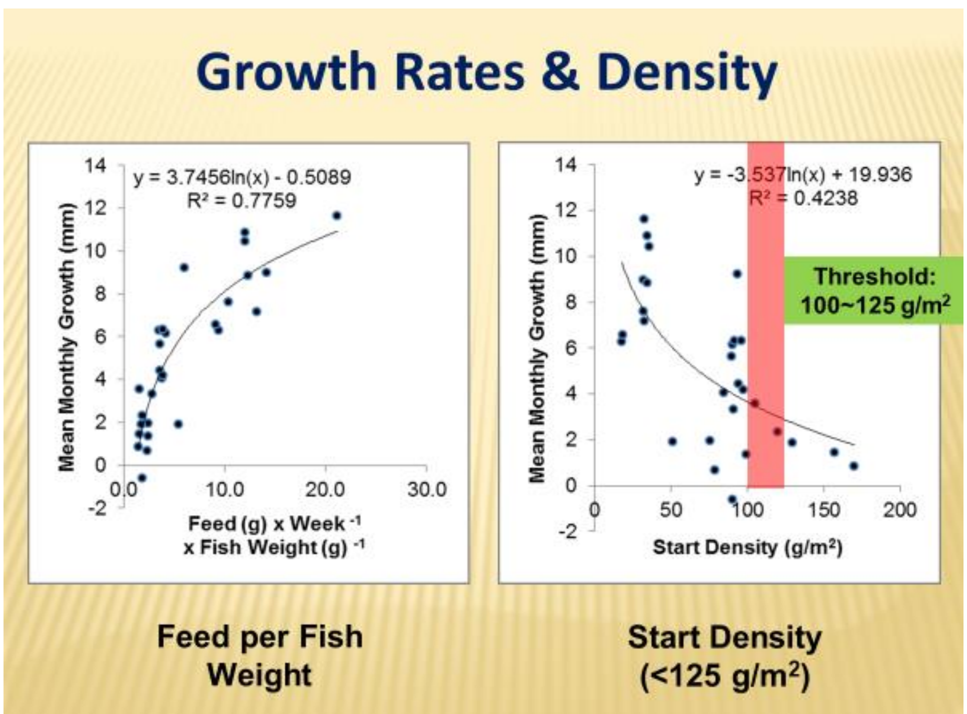
A
B
Prolonged spawning season (April – July)
Fecund species (>100,000 eggs)
Eggs are sticky (stick to substrate)


0.25 d
12 d
14 d (~200 dd)
eye spot
gills
mouth
heart
yolk sac
31 d (~10 d w/ yolk sac)
gills
mouth
heart
yolk sac
21 d


Prolarva->Larva
XperCount
Accurate
2. Press COUNT


Larvae
Applegate 1950
CURRENT
Sediment Depth ~2/3 of Fish Length


Life Stage Survival Rates
2014 Stage Survival (%)
2014 Cumulative Survival (%)
Bottleneck
Life Stage
Survival Rate
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fertilization
Hatching
First Feeding
3 months
6 months
9 months
12 months
Life Stages

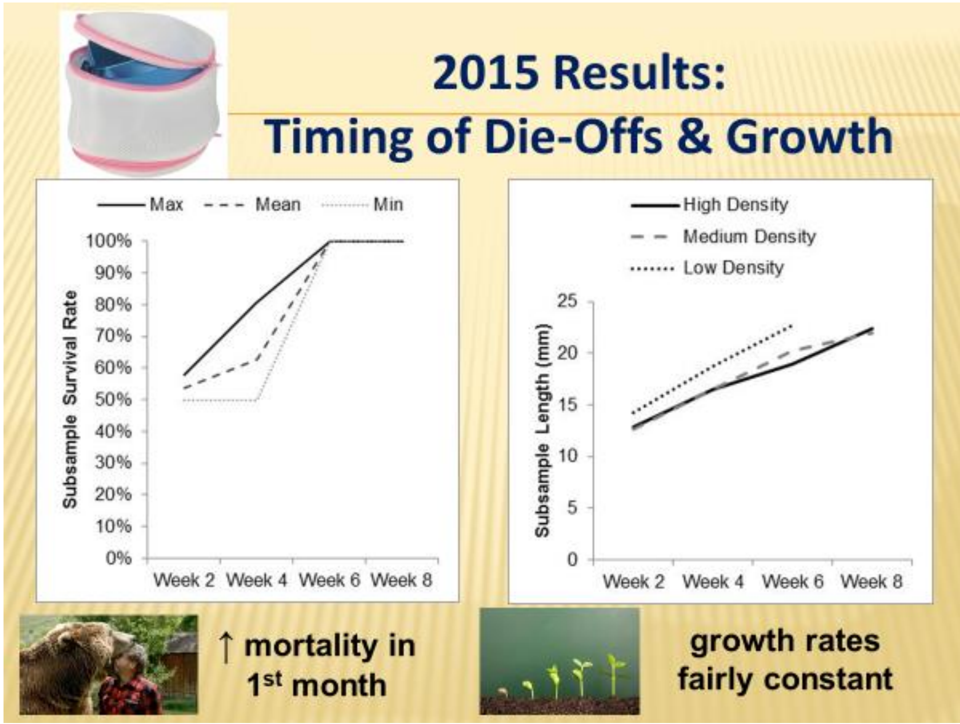

Growth Rates & Density
y = 3.7456ln(x) - 0.5089
R² = 0.7759
Mean Monthly Growth (mm)
Feed (g) x Week -1
x Fish Weight (g) -1
y = -3.537ln(x) + 19.936
R² = 0.4238
Threshold:
100~125 g/m²
Start Density (g/m²)
Feed per Fish
Weight
Start Density
(<125 g/m²)

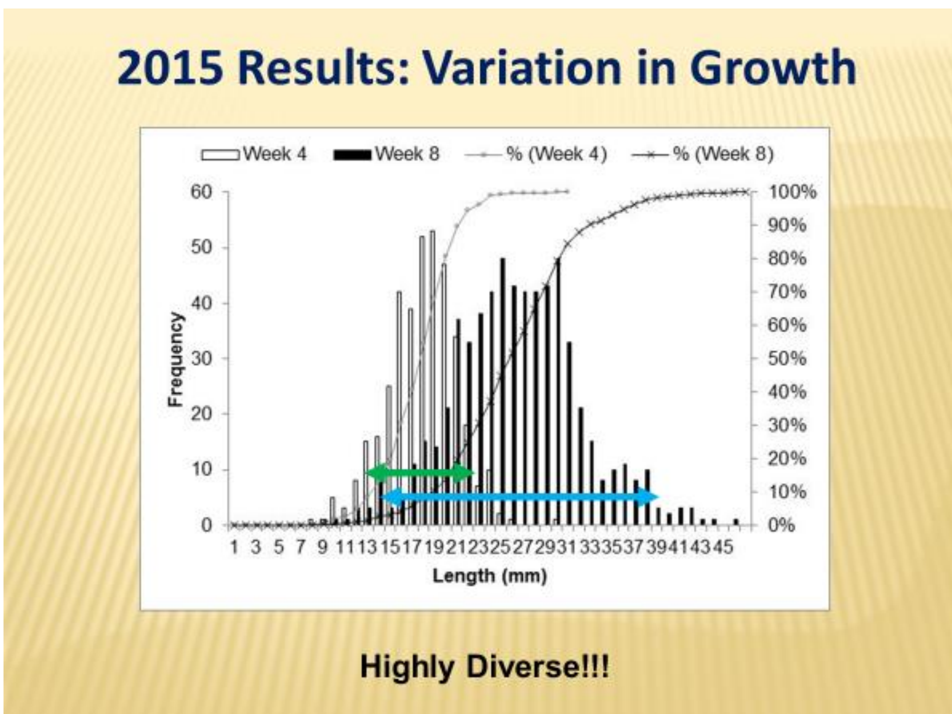
Collaborative Experiment on Bottleneck Life Stage (YN, CTUIR, USFWS)

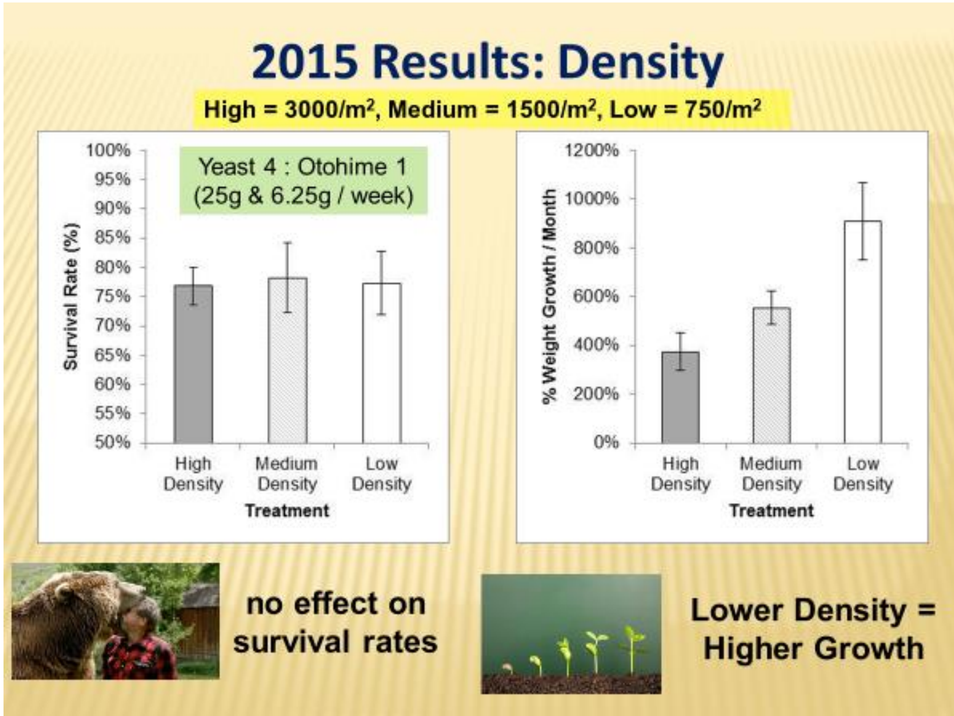
- **Timing of Initial Feeding (USFWS)**
  (25, 30, 35 days post fertilization)
- **Feed Particle Size / Amount (CTUIR/NOAA)**
  (150, 100, 50, <50 micron)
- **Density of Fish (YN)**
  (300, 150, 75 g/m^2)

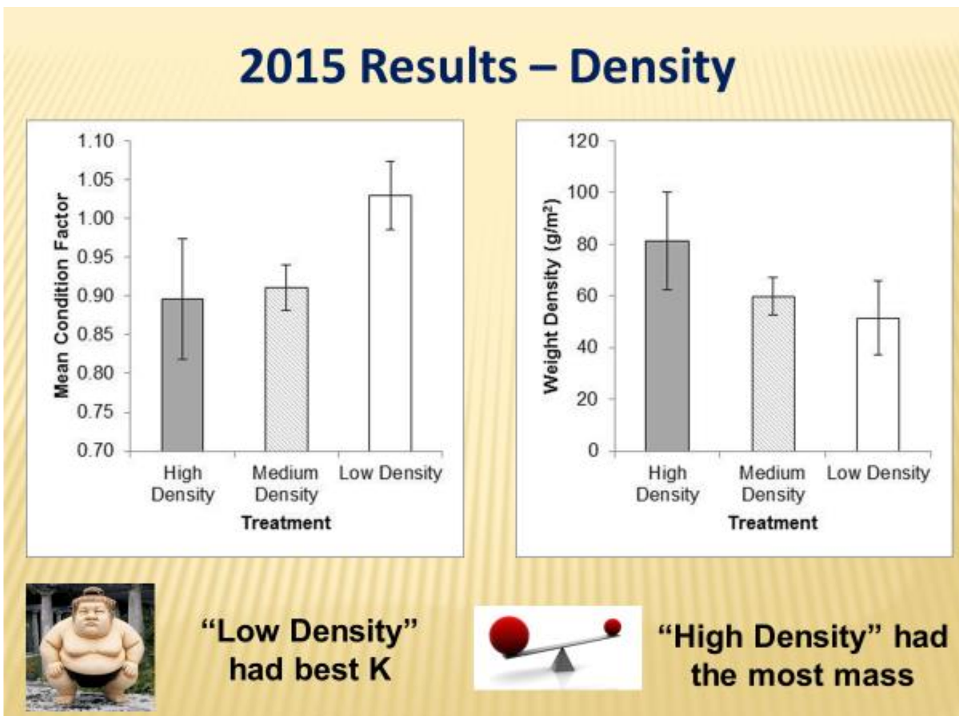
Early Larva Feeding Study (2015)

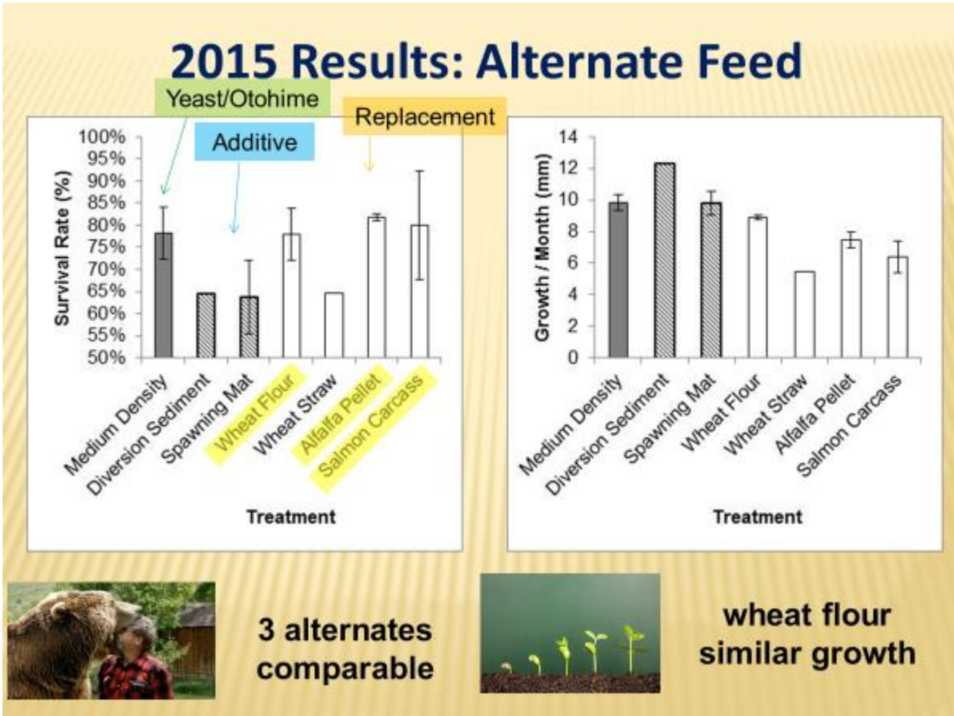
- **20 aquariums (25 L, 0.125 m^2, 1 L/min)**
- **<u>2015 study questions</u>**
  - ✓ **Timing of die offs**
  - ✓ **Effects of density**
  - ✓ **Effects of larval & alternative feeds**


2015 Results:
Timing of Die-Offs & Growth
Max
Mean
Min
Subsample Survival Rate
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Week 2 Week 4 Week 6 Week 8
High Density
Medium Density
Low Density
Subsample Length (mm)
25
20
15
10
5
0
Week 2 Week 4 Week 6 Week 8
↑ mortality in 1st month
growth rates fairly constant

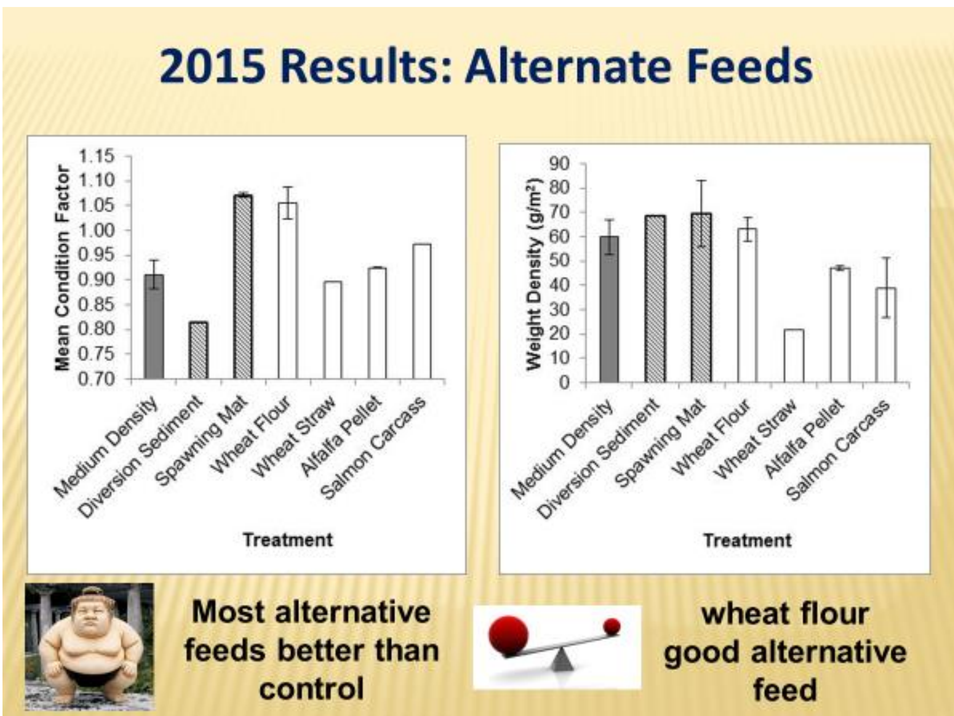

2015 Results: Variation in Growth
Week 4
Week 8
% (Week 4)
% (Week 8)
Frequency
60
50
40
30
20
10
0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45
Length (mm)
Highly Diverse!!!


2015 Results: Density
High = 3000/m², Medium = 1500/m², Low = 750/m²
Yeast 4 : Otohime 1
(25g & 6.25g / week)
Survival Rate (%)
100%
95%
90%
85%
80%
75%
70%
65%
60%
55%
50%
High Density
Medium Density
Low Density
Treatment
% Weight Growth / Month
1200%
1000%
800%
600%
400%
200%
0%
High Density
Medium Density
Low Density
Treatment
no effect on survival rates
Lower Density = Higher Growth

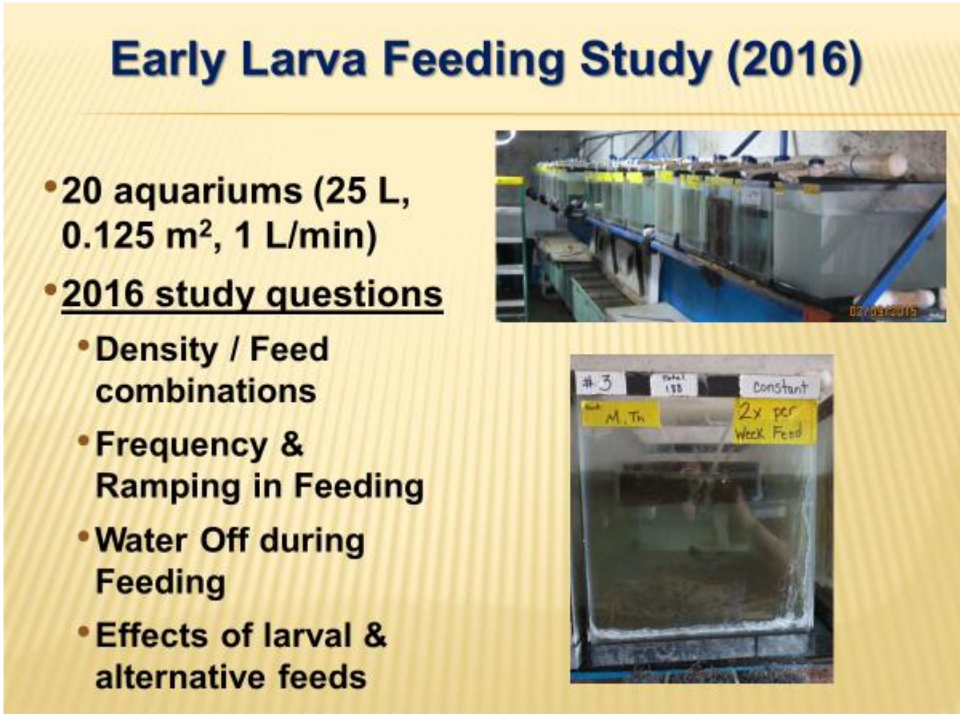

2015 Results – Density
Mean Condition Factor
1.10
1.05
1.00
0.95
0.90
0.85
0.80
0.75
0.70
High Density
Medium Density
Low Density
Treatment
Weight Density (g/m²)
120
100
80
60
40
20
0
High Density
Medium Density
Low Density
Treatment
"Low Density" had best K
"High Density" had the most mass

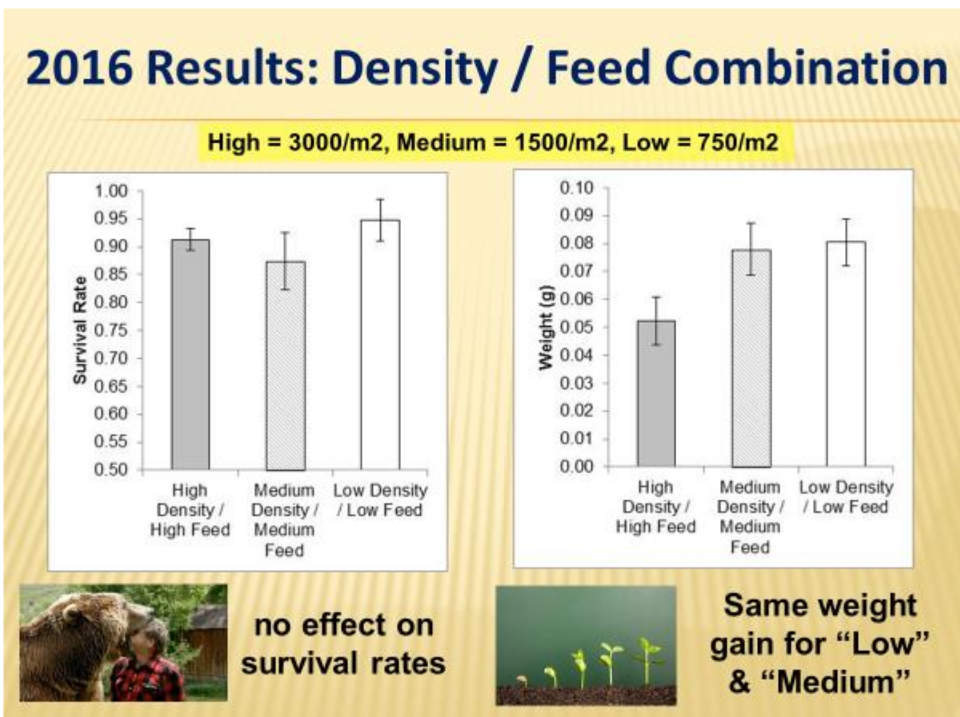


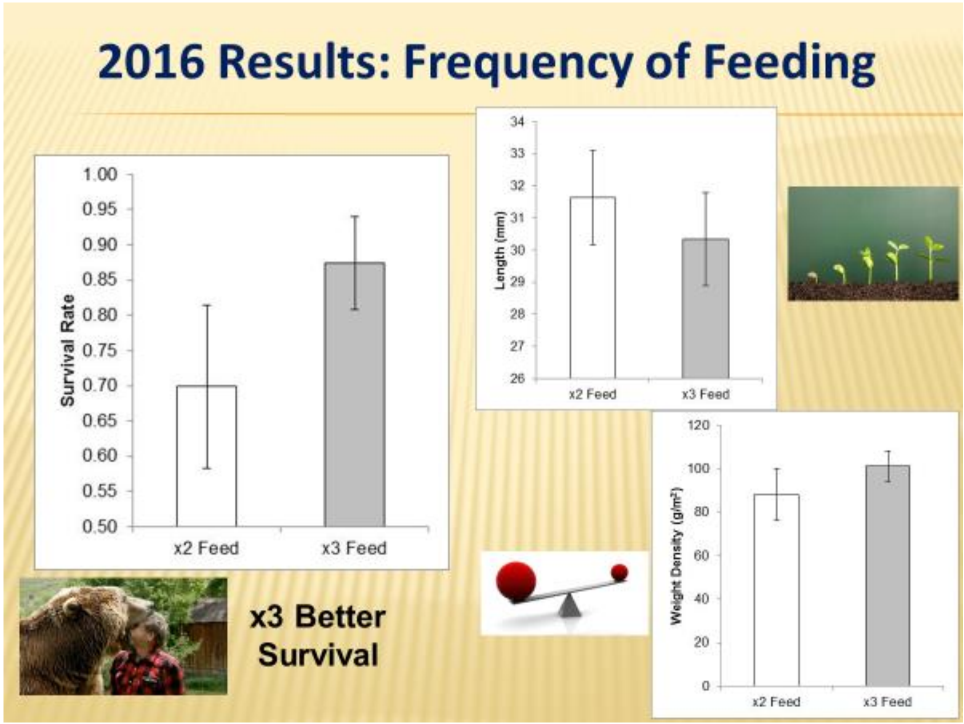
2015 Results: Alternate Feed
Yeast/Otohime
Additive
Replacement
Survival Rate (%)
Growth / Month (mm)
Medium Density
Diversion Sediment
Spawning Mat
Wheat Flour
Wheat Straw
Alfalfa Pellet
Salmon Carcass
Treatment
3 alternates comparable
wheat flour similar growth

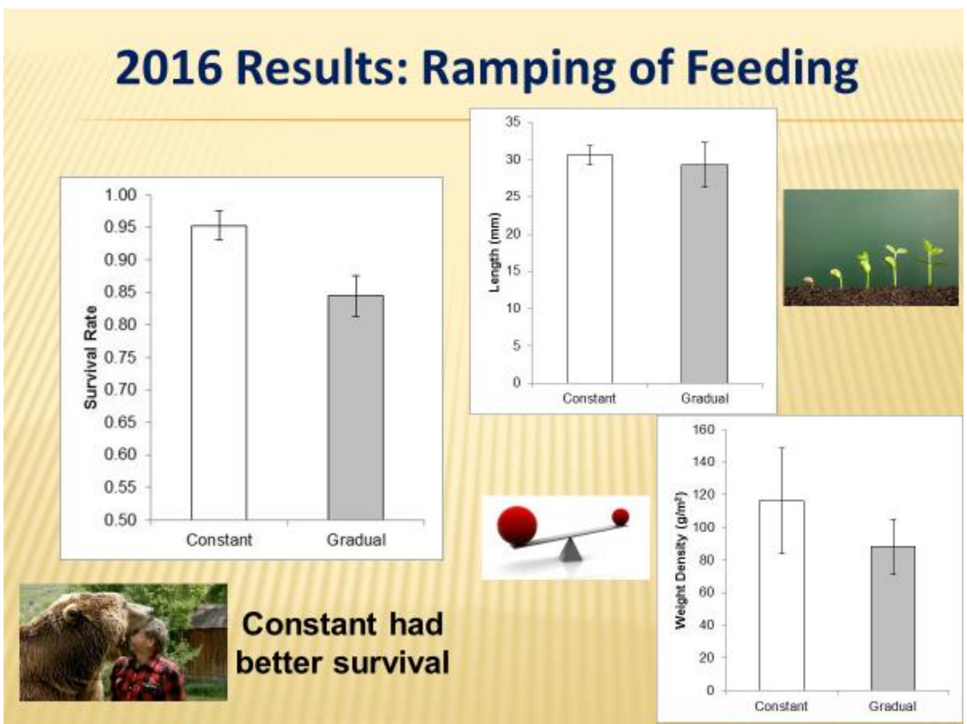


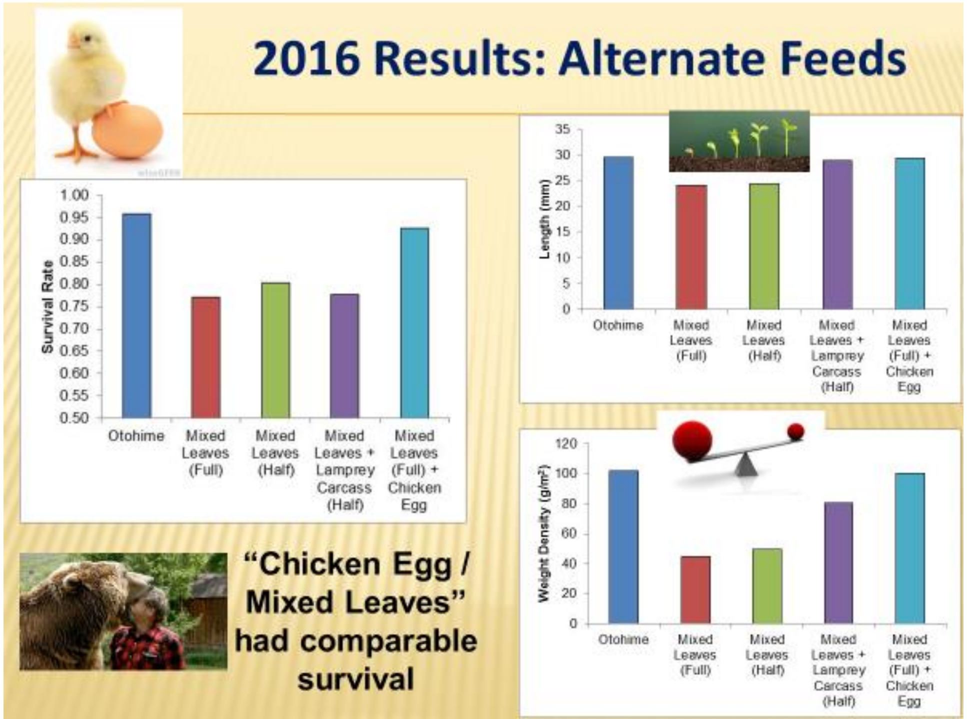
2015 Results: Alternate Feeds
Mean Condition Factor
Weight Density (g/m²)
Medium Density
Diversion Sediment
Spawning Mat
Wheat Flour
Wheat Straw
Alfalfa Pellet
Salmon Carcass
Treatment
Most alternative feeds better than control
wheat flour good alternative feed

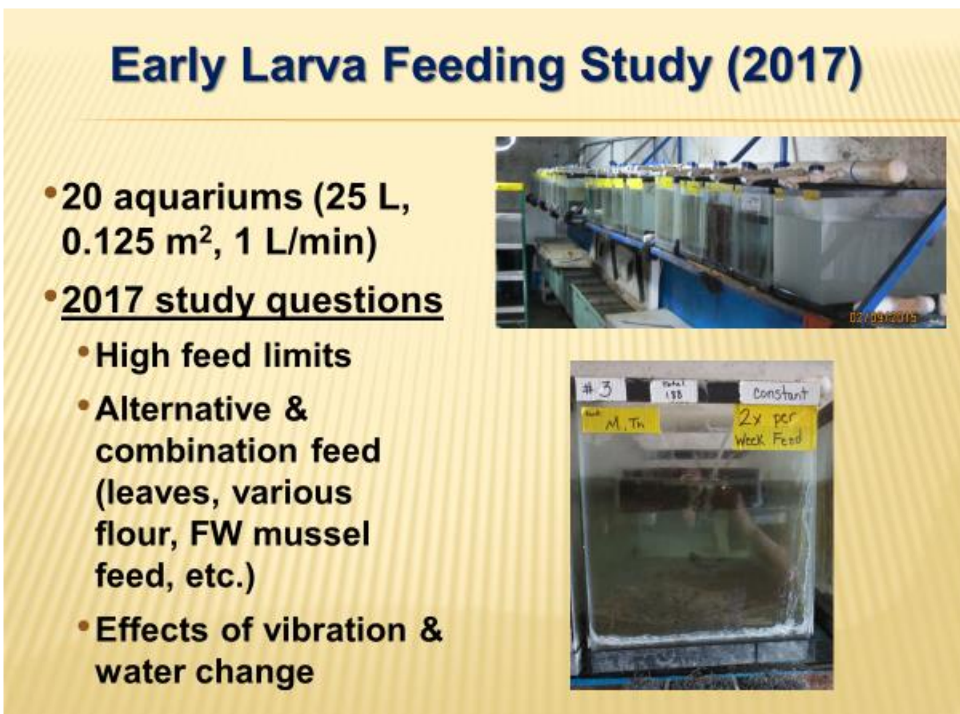

Early Larva Feeding Study (2016)
20 aquariums (25 L, 0.125 m², 1 L/min)
2016 study questions
Density / Feed combinations
Frequency & Ramping in Feeding
Water Off during Feeding
Effects of larval & alternative feeds
#3
M, Th
Constant
2x per Week Feed

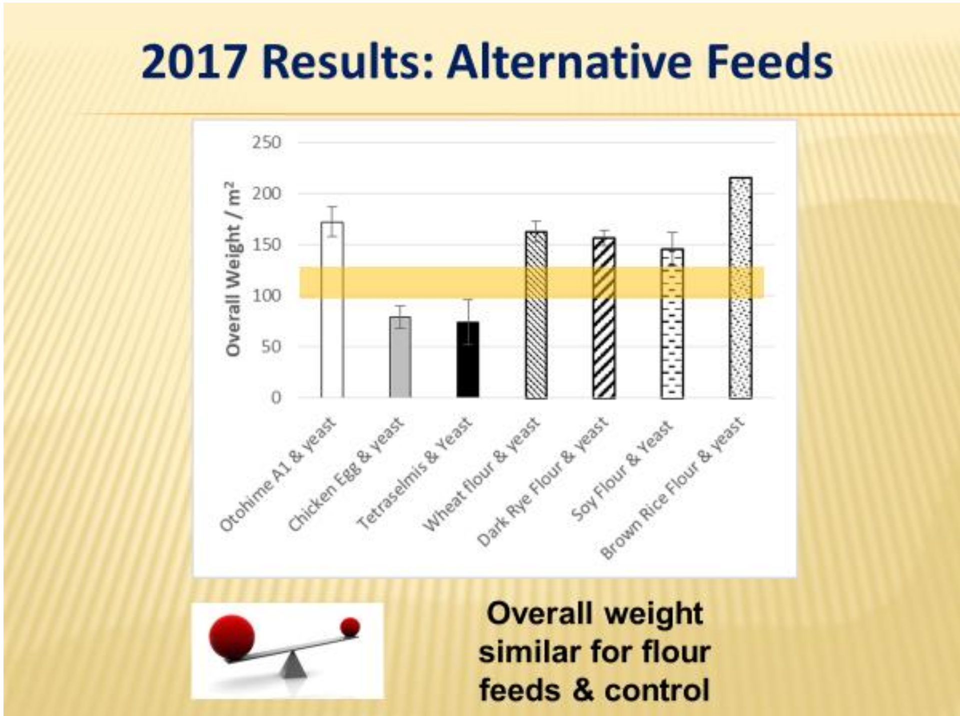

2016 Results: Density / Feed Combination
High = 3000/m2, Medium = 1500/m2, Low = 750/m2
Survival Rate
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
High Density / High Feed
Medium Density / Medium Feed
Low Density / Low Feed
Weight (g)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
High Density / High Feed
Medium Density / Medium Feed
Low Density / Low Feed
no effect on survival rates
Same weight gain for "Low" & "Medium"

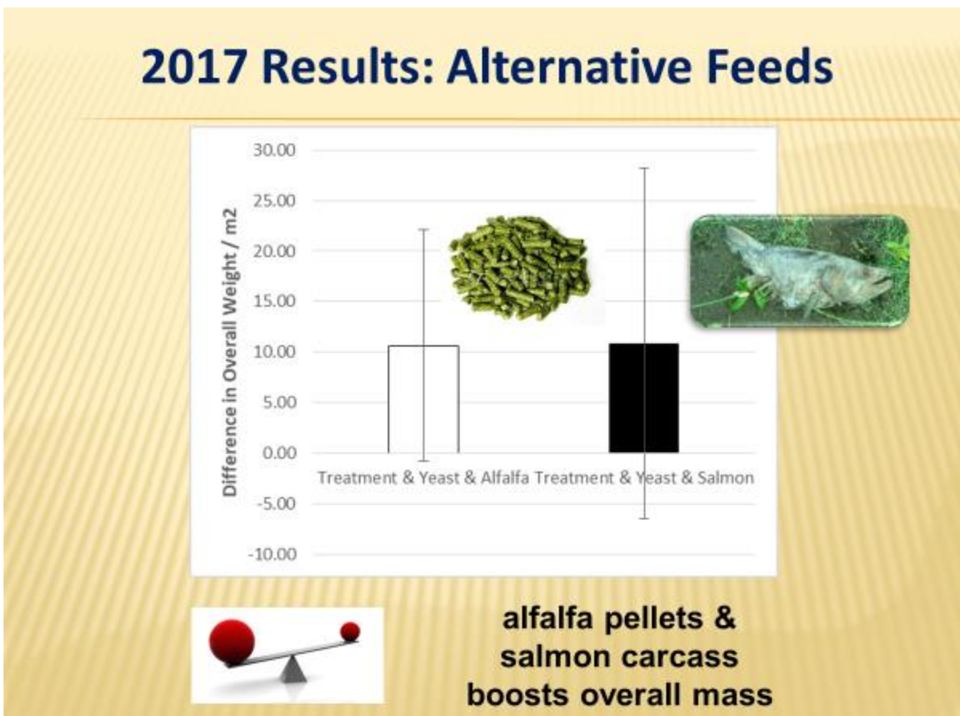

2016 Results: Frequency of Feeding
Survival Rate
x2 Feed
x3 Feed
Length (mm)
Weight Density (g/m²)
x3 Better Survival

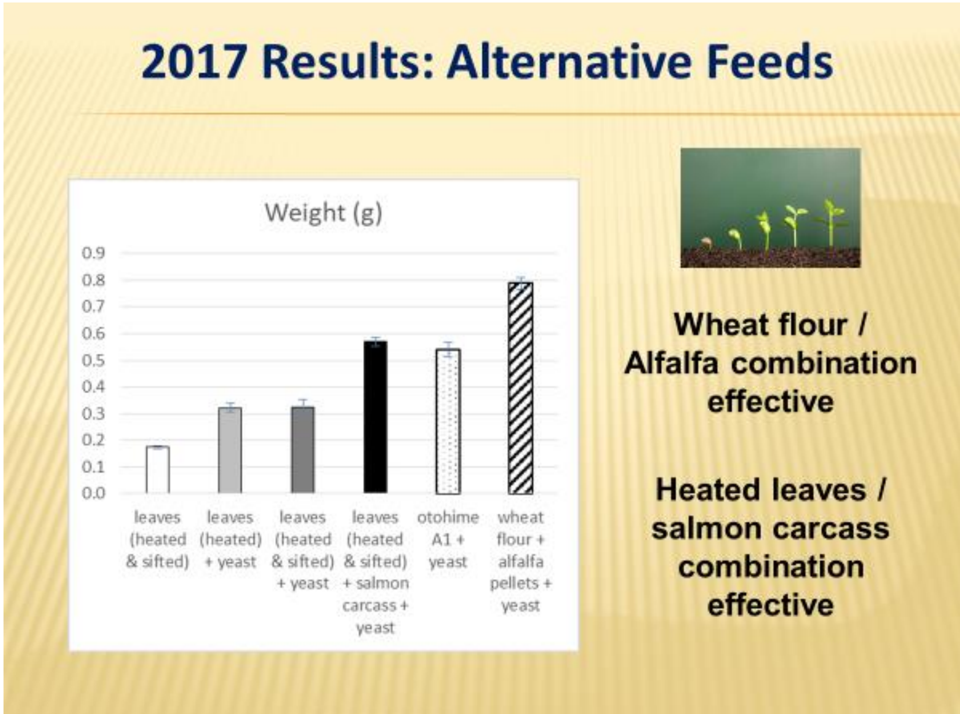

2016 Results: Ramping of Feeding
Survival Rate
Constant
Gradual
Length (mm)
Weight Density (g/m²)
Constant had better survival

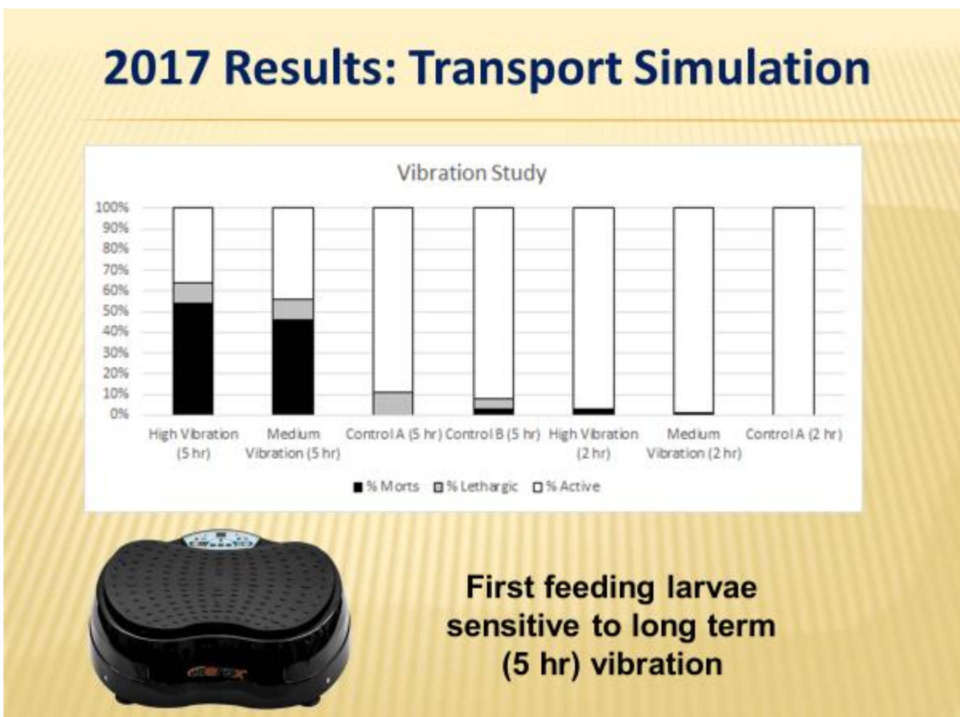

2016 Results: Alternate Feeds
Survival Rate
Length (mm)
Weight Density (g/m²)
Otohime
Mixed Leaves (Full)
Mixed Leaves (Half)
Mixed Leaves + Lamprey Carcass (Half)
Mixed Leaves (Full) + Chicken Egg
"Chicken Egg / Mixed Leaves" had comparable survival

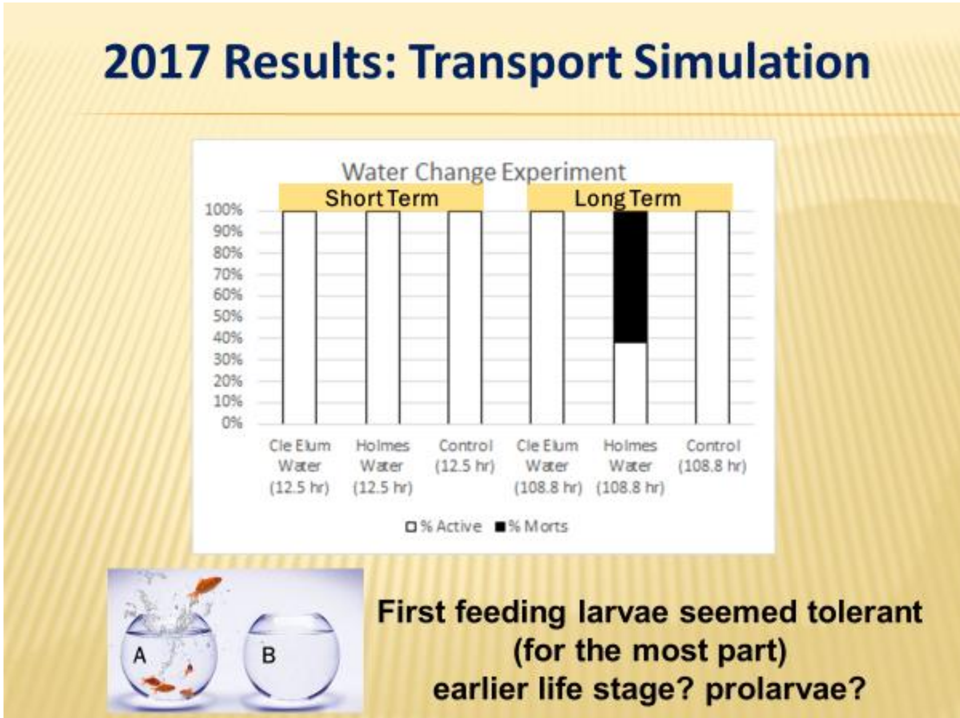

Early Larva Feeding Study (2017)
•20 aquariums (25 L, 0.125 m², 1 L/min)
•2017 study questions
•High feed limits
•Alternative & combination feed (leaves, various flour, FW mussel feed, etc.)
•Effects of vibration & water change
#3
Constant
M, Th
2x per Week Feed

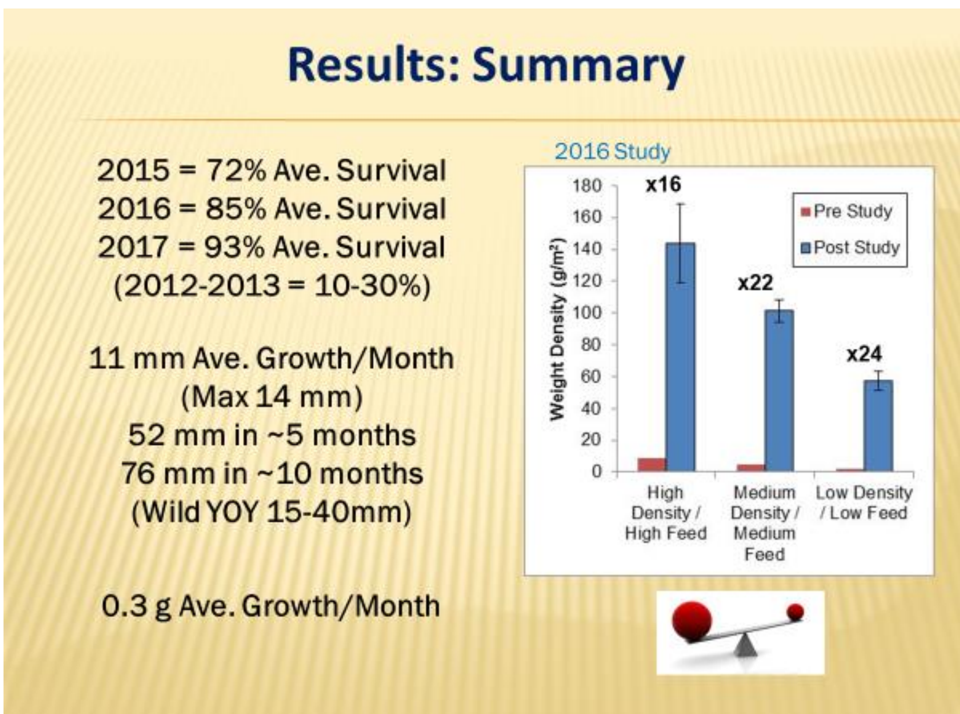

2017 Results: Alternative Feeds
250
200
150
100
50
0
Overall Weight / m²
Otohime A1 & yeast
Chicken Egg & yeast
Tetraselmis & Yeast
Wheat flour & yeast
Dark Rye Flour & yeast
Soy Flour & Yeast
Brown Rice Flour & yeast
Overall weight similar for flour feeds & control


2017 Results: Alternative Feeds
30.00
25.00
20.00
15.00
10.00
5.00
0.00
-5.00
-10.00
Difference in Overall Weight / m2
Treatment & Yeast & Alfalfa
Treatment & Yeast & Salmon
alfalfa pellets & salmon carcass boosts overall mass


2017 Results: Alternative Feeds
Weight (g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
leaves (heated & sifted)
leaves (heated) + yeast
leaves (heated & sifted) + yeast
leaves (heated & sifted) + salmon carcass + yeast
otohime A1 + yeast
wheat flour + alfalfa pellets + yeast
Wheat flour / Alfalfa combination effective
Heated leaves / salmon carcass combination effective


2017 Results: Transport Simulation
Vibration Study
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
High Vibration (5 hr)
Medium Vibration (5 hr)
Control A (5 hr)
Control B (5 hr)
High Vibration (2 hr)
Medium Vibration (2 hr)
Control A (2 hr)
% Morts
% Lethargic
% Active
First feeding larvae sensitive to long term (5 hr) vibration

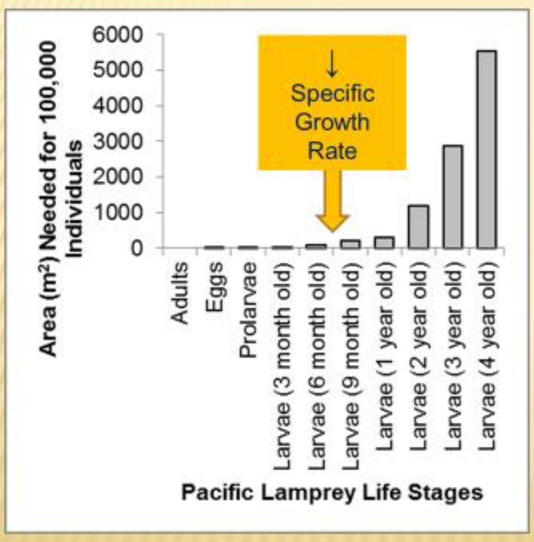

2017 Results: Transport Simulation
Water Change Experiment
Short Term
Long Term
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cle Elum Water (12.5 hr)
Holmes Water (12.5 hr)
Control (12.5 hr)
Cle Elum Water (108.8 hr)
Holmes Water (108.8 hr)
Control (108.8 hr)
% Active
% Morts
A
B
First feeding larvae seemed tolerant
(for the most part)
earlier life stage? prolarvae?


Results: Summary
2015 = 72% Ave. Survival
2016 = 85% Ave. Survival
2017 = 93% Ave. Survival
(2012-2013 = 10-30%)
11 mm Ave. Growth/Month
(Max 14 mm)
52 mm in ~5 months
76 mm in ~10 months
(Wild YOY 15-40mm)
0.3 g Ave. Growth/Month
2016 Study
Weight Density (g/m²)
180
160
140
120
100
80
60
40
20
0
x16
x22
x24
Pre Study
Post Study
High Density / High Feed
Medium Density / Medium Feed
Low Density / Low Feed

2018 YN Plans

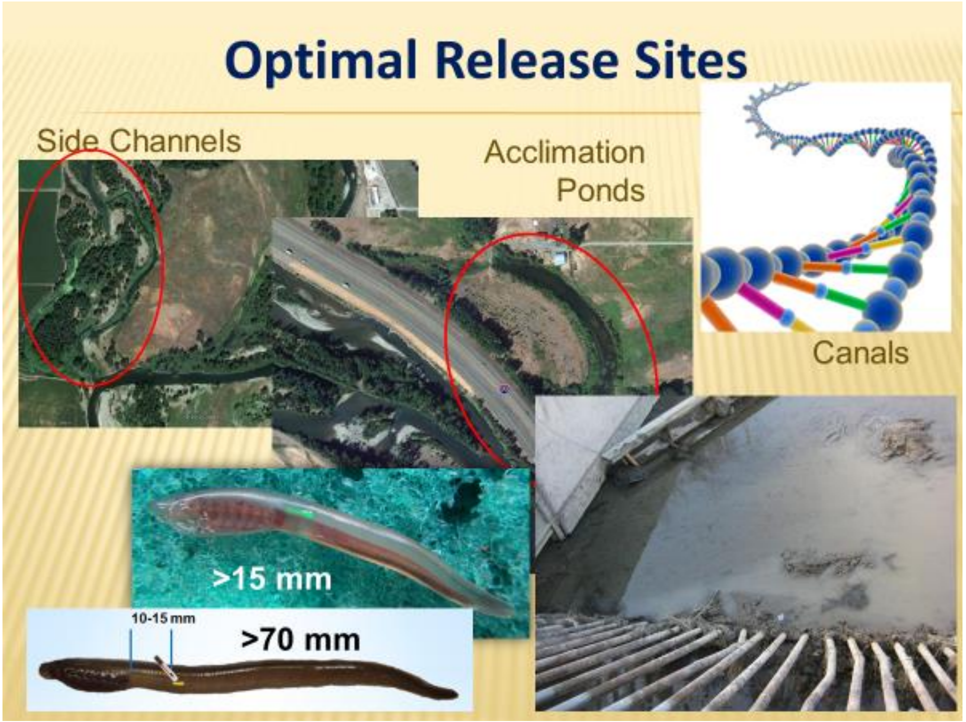
- Adult Sexual Maturation Study (January – March)
  - 90% River / 10% Well
  - 50% River / 50% Well
  - 10% River / 90% Well
- Combined effects of sediment depth & density
  - YOY larvae
- Optimum feeding frequency
  - x1 / week vs. x3 / week
- Alternative Feeds
  - Flour (wheat vs. brown rice vs. 10 grain)
  - Synergistic combinations of alternative feeds
- Sensitivity to transportation (egg to larvae stage)
- Continue to rear older larvae to reach macrophthalmia stage

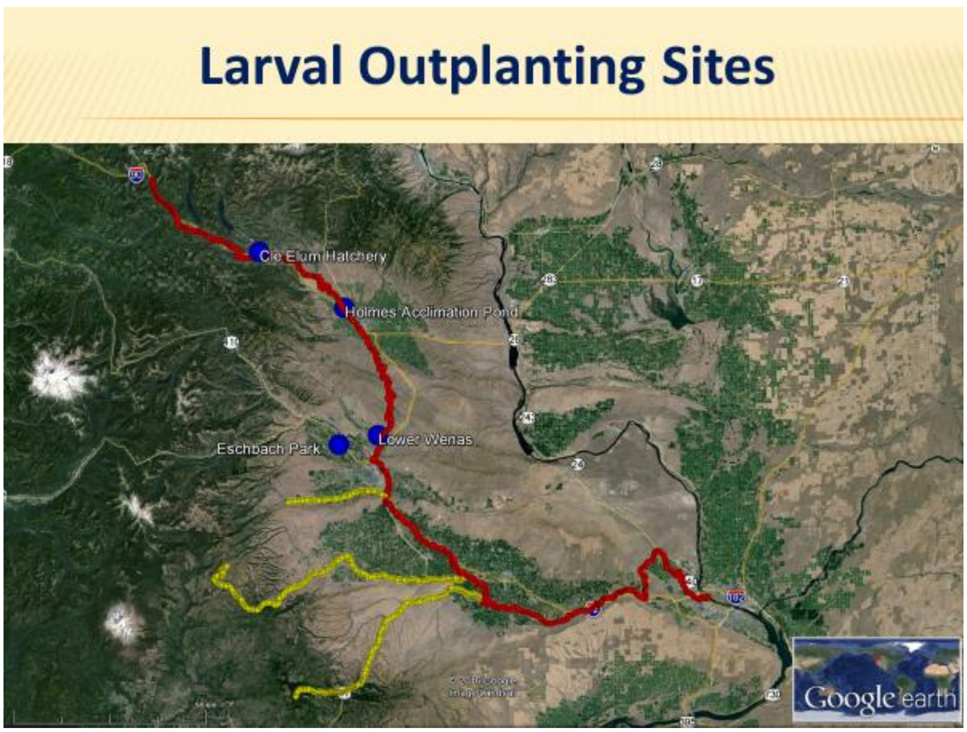
Space Requirement



Space (m^2) Needed for 100,000 individuals

- Eggs = 0.13
- Prolarvae = 2.0
- 3 month old = 14
- 6 month old = 97
- 9 month old = 199
- 1 year old = 319
- 2 year old = 1,205
- 3 year old = 2,889
- 4 year old = 5,553


Optimal Release Sites
Side Channels
Acclimation
Ponds
Canals
>15 mm
10-15 mm
>70 mm


Larval Outplanting Sites
Cle Elum Hatchery
Holmes Acclimation Pond
Lower Wenas
Eschbach Park
Google earth

In Summary

- ❑ We have made <u>huge strides</u> in advancing the techniques for art prop & rearing of larvae to macro (only <u>one</u> other group from Japan in 1980s have ever succeeded!)
- ❑ The <u>unique assets</u> of the 3 partners made it possible:
  - YN – broodstock holding capacity, larger culture facility for multiple year classes, river & well water access, access to wild / translocated fish (macro/larvae), etc.
  - CTUIR/NOAA – broodstock holding, small recirculating well water facility, minimal water footprint, high biosecurity, access to wild / translocated fish, etc.
  - USFWS – medium sized facility, flow-through creek & well water, analysis lab for fish & feed analysis, temperature control capability, many small tanks for replicated experiments

Future Needs

- ❑ Seeking funding for <u>USFWS (2018)</u>
- ❑ Seeking funding for <u>2019-2021</u> (YN, CTUIR/NOAA, USFWS)
  - Why 3 more yrs?
    - ✓ Macrophthalmia production takes 4-7 yrs
    - ✓ 6 yrs is a min. to succeed in the macro production & associated tests (hence, 3 more years)
    - ✓ Continuous & annual propagation & rearing needed to have sustained supply of study fish
  - Why 3 entities?
    - ✓ Best not to "put all the eggs in the same basket" (spreading across 3 partners for "source" fish)
    - ✓ Insures biosecurity of older larvae & more space, infrastructure, and expertise available

Future Needs

- ❑ Seeking funding for 2019-2021 (YN, CTUIR/NOAA, USFWS)
  - Why not use wild macros?
    - ✓ Wild macro migration timing sporadic (typically all at once) & hard to predict
    - ✓ Even predictable, it may not coincide with study timing
    - ✓ Dryden Diversion has many macro, but uncertainty about # (year to year) & capture season in the fall (not part of migratory season)
  - End results?
    - ✓ Ability to produce a few hundred to thousands of macro at the end of the extended contract (2021) & road map for "how to" produce them effectively

Future Needs

- ❑ Seeking funding for 2019-2021 (YN, CTUIR/NOAA, USFWS)
  - Macro certainly useful for survival studies, but also a benefit in pursuing this for a <u>restoration / mitigation tool (supplementation)</u> as well as for <u>conservation hatchery (biosecurity)</u>
  - Lots of cost share / matching funds from the 3 partners – high "bang for the buck"
  - Next 3 years may be the <u>most critical time</u> to advance our knowledge on macro production & future management directions
  - Chelan funding critical for continuing the <u>unique collaboration</u> and focused research for <u>macro production</u>


Future Needs
Maximize Survival and Growth
- optimization of feed & ration
- optimization of food delivery
- optimization of culture density


Future Needs
Identify env. or physiological cues that trigger metamorphosis
- assess metamorphosis of larvae in various physiological conditions (e.g., lipid levels, size at age, etc.)
- assess metamorphosis of larvae held under different env. conditions (e.g., simulated winter cooling)
- assess metamorphosis rates of late-stage cultured larvae held in lab & field (mesocosm)

Future Needs

Assess "wild-like" characteristics to insure that cultured fish are good surrogates for wild ones

- compare morphology of wild & cultured macrophthalmia
- compare swimming performance of wild & cultured macrophthalmia
- compare behavior of wild & cultured macrophthalmia (e.g., depth preference, light responses, etc.).

Future Needs

Increase efficiency & economy of hatchery operations

- investigate options for automatic feeding
- investigate options for high density culture & vertical stacking
- investigate options for polyculture & use of enclosed ponds, raceways, or canals

Capsule Hotel in Tokyo, Japan

"the art of packing people in comfortably"