A potential vaccine to control bacterial coldwater disease (CWD)

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Background

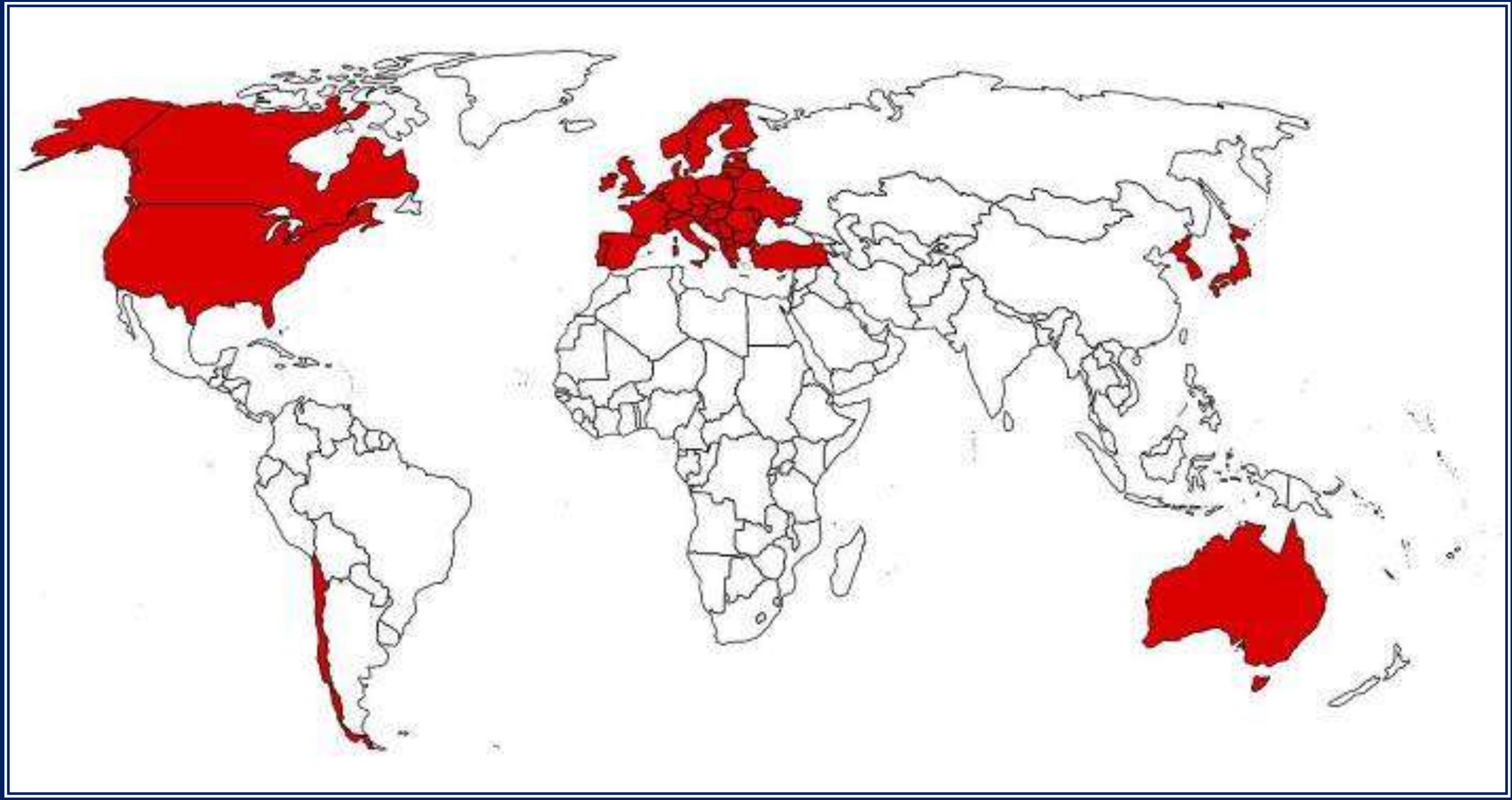
- Etiological agent is *Flavobacterium psychrophilum*
- Originally isolated in 1948 in Washington state from coho salmon at 10°C
  - Was referred to as “low temperature disease”, “coldwater disease”, and “peduncle disease”
- Taxonomic status of this bacterium has changed due to DNA technology
  - Formerly known as *Cytophaga psychrophila* and *Flexibacter psychrophilus*

Host Susceptibility

- **Salmonid Species**
  - Coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*) are particularly susceptible to *F. psychrophilum* infection
  - Atlantic salmon and others affected
- **Non-salmonid species**
  - Eel (*Anguilla anguilla*)
  - Tench (*Tinca tinca*)
  - Carp (*Cyprinus carpio*)
  - Crucian carp (*Carassius carassius*)
  - Ayu (*Plecoglossus altivelis*)
  - Sea Lamprey (*Petromyzon marinus*)

F. Psychrophilum distribution

- Found throughout North America, Europe, Korea, and Japan
- Identified in Atlantic salmon in Chile and Australia



Clinical Signs of CWD

- Fry and fingerlings
  - Lesions of the caudal peduncle
  - Erosion and fraying of fins
  - Dark coloration
  - Loss of appetite


Coho salmon


Coho salmon


Coho salmon

Significance

CWD considered number one bacterial disease in the Pacific NW

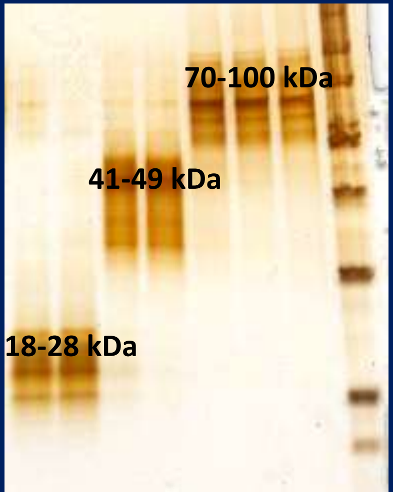
- Commercial aquaculture losses in Idaho alone are estimated at $9.6 million dollars per year
  - Does not include treatment, egg replacement, and market devaluation for deformities of survivors
- Public aquaculture (WA state facilities) losses estimated at $4 million dollars per year
  - Does not include treatment, fish and egg replacement

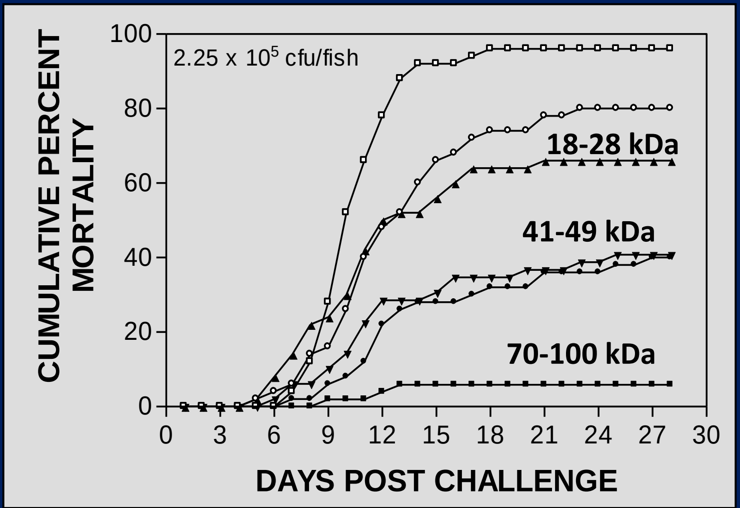
Control options for CWD?

1. Good management/culture practices!
2. Antibiotic treatments
   – Aquaflor approved under VFD
3. Egg disinfection (vertical transmission)
   – Reduces surface bacteria but can't eliminate intra-ovum bacteria
4. Culling program? (ongoing research)
5. Probiotics? (promising)
6. Vaccination
   – There are no commercially available vaccines for CWD
   – A standard bacterin does not work well – not a "silver bullet" as in the case of ERM

CWD Vaccine Research UI:2000-2011

1. Immune response - Antibody development important for disease protection
2. Tested vaccine formulations - Whole-cell “killed” bacteria (standard bacterin) not effective
3. Identified many bacterial genes/proteins associated with immunity – Developed recombinant and DNA vaccines and immunized fish: Limited protection





Other options??

- Alternative: Can we develop an efficacious live attenuated vaccine?
  - Numerous studies on different fish pathogens
  - Stimulate both innate and specific immune responses
- Three live fish vaccines approved by USDA-APHIS-CVB
  - RENIGEN (*Arthrobacter* live culture) – *Renibacterium salmoninarum*: Bacterial Kidney Disease
  - AQUAVAC-ESC™ - *Edwardsiella ictaluri*: Enteric Septicemia of Catfish
  - AQUAVAC-COL™ – *Flavobacterium columnare*: Columnaris

Attenuated Vaccine Development

AQUAVAC-ESC™ and AQUAVAC-COL™ were developed using a rifampicin-resistant strategy

- Originally used for the development of a live attenuated *Brucella abortus* vaccine for cattle in the US

- Bacteria are passed in presence of increasing concentrations of the antibiotic rifampicin
    - Results in attenuation of the pathogenic bacteria

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Isolation of rifampicin resistant *Flavobacterium psychrophilum* strains and their potential as live attenuated vaccine candidates

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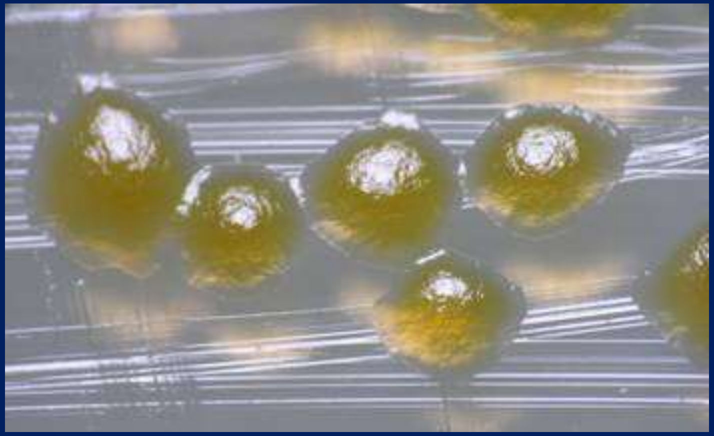
Attenuation of *F. psychrophilum*

- Generation of rifampicin-resistant strains
  - FP 259-93 (virulent) used as parent isolate
  - A single colony was passed to TYES agar containing 5 µg ml^{-1} rifampicin
  - Two colonies were selected and independently passed on increasing concentrations of rifampicin
    - 259-93A.16: passed 16 times to 200 µg ml^{-1} RIF
    - 259-93B.17: passed 17 times to 250 µg ml^{-1} RIF

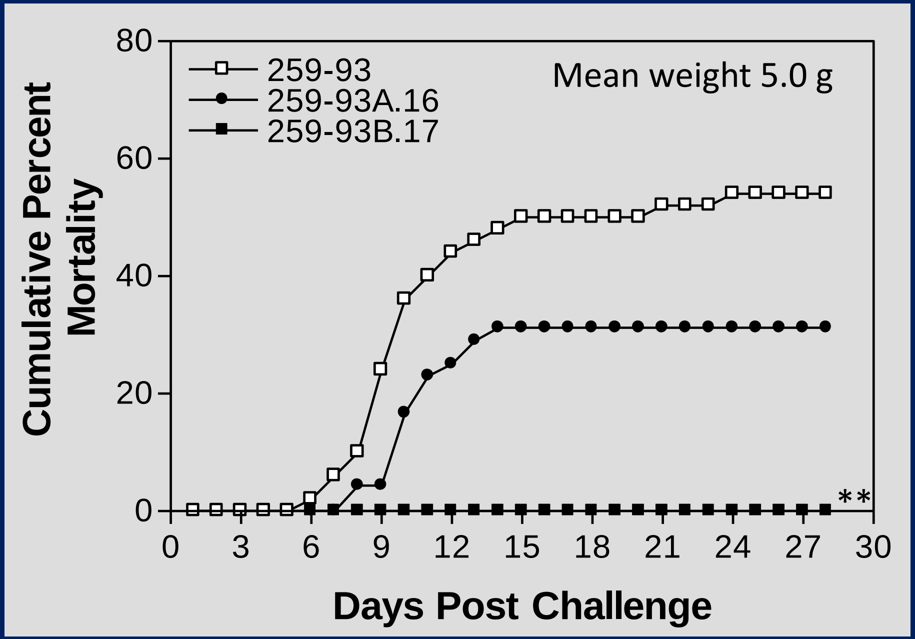


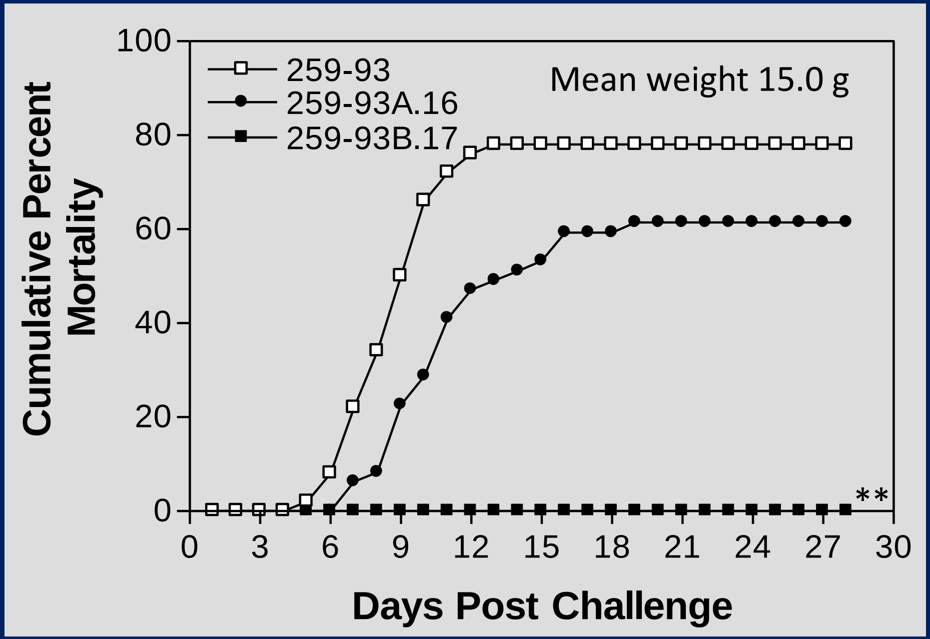
Methods

- Assessment of attenuation
  - Rainbow trout (mean weights of 5.0 and 15.0 g)
  - Subcutaneously injected 2 doses of FP 259-93, 259-93A.16, and 259-93B.17
  - Mortality monitored for 28 d and CPM determined

Results

- Experimental CWD challenges demonstrated attenuation of both resistant strains
  - Complete attenuation of the 259-93B.17 strain



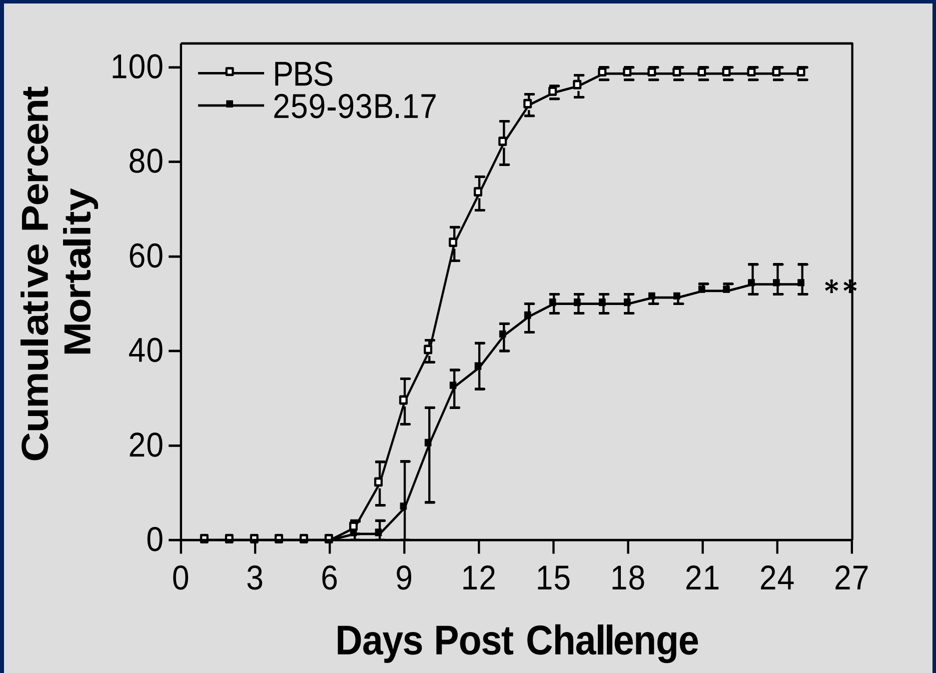


** Indicates a significant difference in CPM compared to 259-93 control ($P < 0.05$)

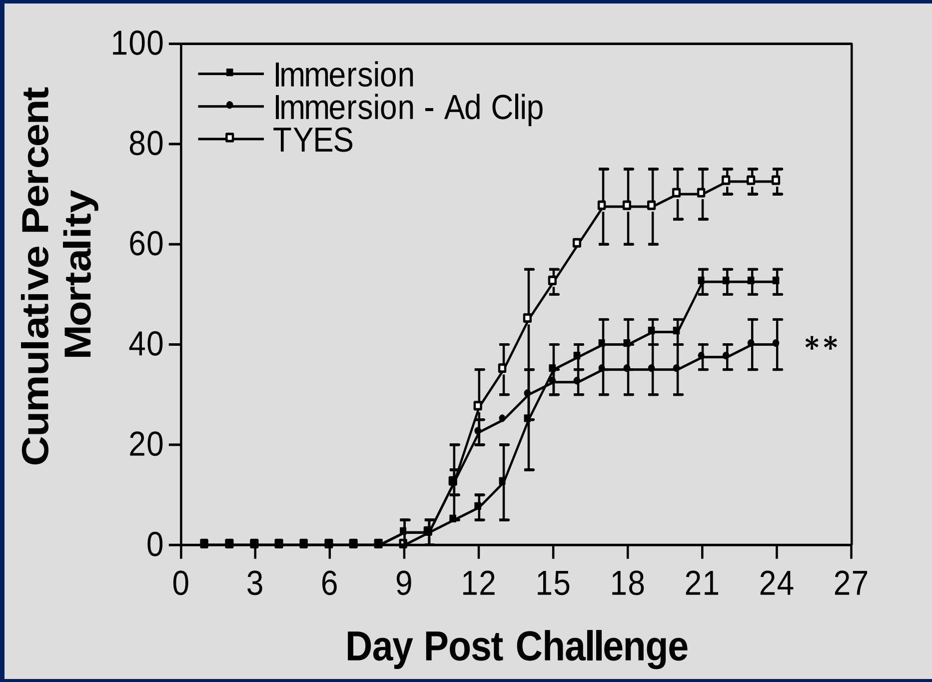
Methods

- Immunization study
  - Injection: Two groups of 350 rainbow trout (mean weight, 2.4 g) were injected intraperitoneally with:
    - PBS and 259-93B.17 (8.3×10^6 cfu fish^{-1})
    - Boosted at 5 weeks
    - Challenged at 8 weeks
  - "Pilot" immersion: Three groups of 100 rainbow trout (mean weight, 3.4 g) were vaccinated by immersion (1 h):
    - 259-93B.17 diluted into water
      - With adipose fin removal
      - Without adipose fin removal
      - Booster at 4 weeks
      - Challenged at 10 weeks

Vaccination Results



Injection: RPS = 45%



Immersion: RPS = 28 and 45%

** Indicates a significant difference in CPM compared to controls ($P < 0.05$)

Coho salmon vaccine trial results

Delivery method	Treatment	Ab Titer 4 weeks	Ab Titer 6 weeks	Ab Titer 12 weeks	CPM	RPS
Injection	PBS	40 ± 7	40 ± 7	200 ± 55	65	
	259-93 B.17	800 ± 278*	2720 ± 697*	8960 ± 1568*	7^	90
	259-93 B.17 w/ DPD	490 ± 90*	1640 ± 374*	14720 ± 4703*	1^	98
Immersion	TYES	< 50	< 50	140 ± 24	54	
	259-93 B.17	1480 ± 315#	1760 ± 261#	4480 ± 784*	29	47
	259-93 B.17 w/ DPD	1680 ± 278#	880 ± 80#	5440 ± 1998*	15§	73

Summary

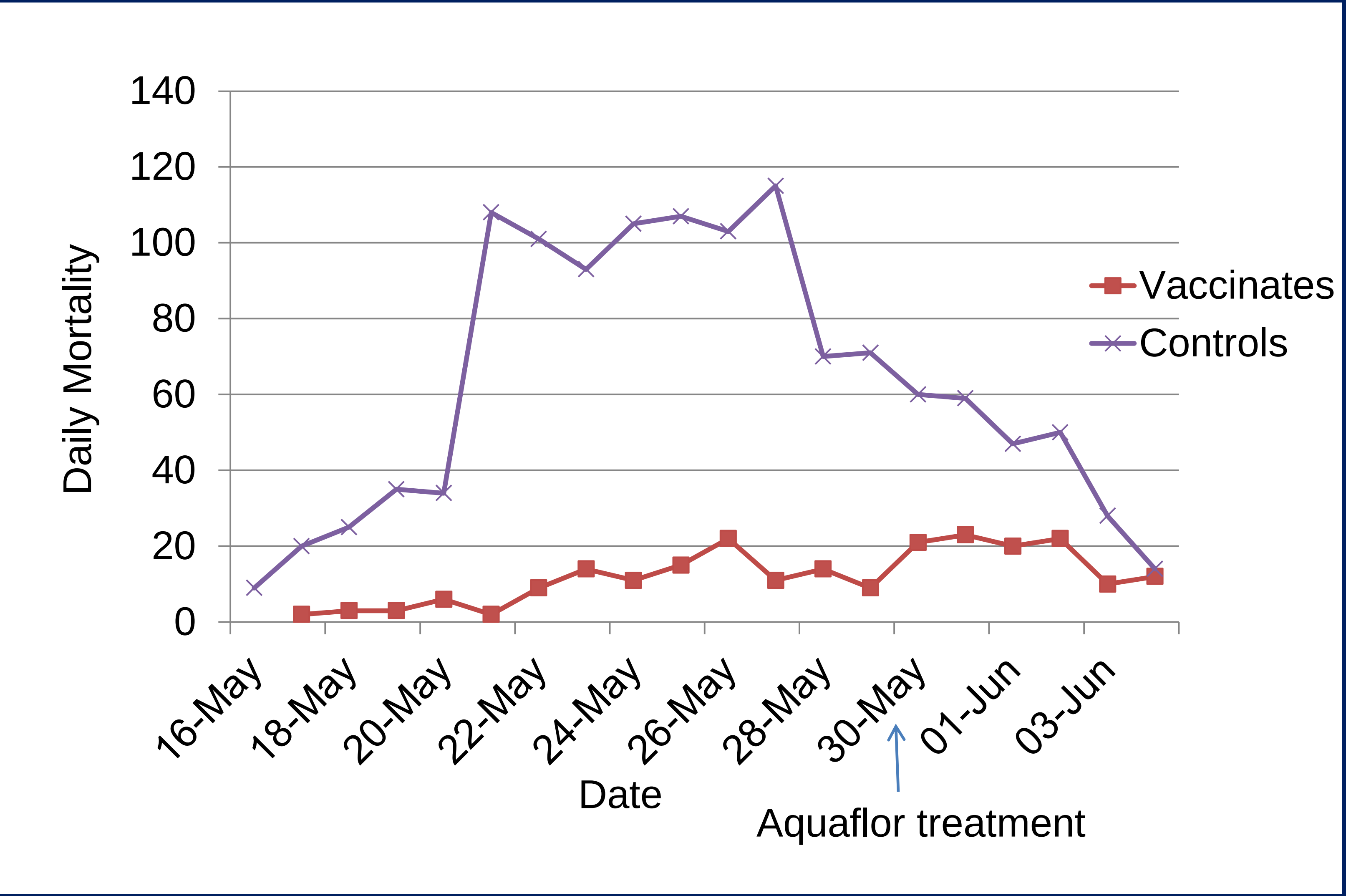
- Rifampicin-resistant strategy resulted in complete attenuation of the 259-93B.17 strain – potential vaccine candidate
- Immunization with the live attenuated 259-93B.17 strain resulted in protective immunity (RBT and Coho)
  - Injection delivery
  - Immersion delivery
- Alternative growth conditions for B.17 may enhance efficacy
- **Speculation** – Protection should be enhanced during a natural outbreak compared to laboratory injection challenge

Field trials
(Glenwood State Fish Hatchery)

Methods:

- 140,000 rainbow trout (0.93 g initial wt) designated as test fish
- Group split and vaccinated 30 days post feeding
- Primary and booster vaccination (at 14 days) – 1.5 min dip
- Vaccinates = 69,984; Controls = 69,984
- Two additional unhandled groups from same lot were also monitored.

Results



140
120
100
80
60
40
20
0
Daily Mortality
16-May
18-May
20-May
22-May
24-May
26-May
28-May
30-May
01-Jun
03-Jun
Date
Vaccinates
Controls
Aquaflor treatment

Mortality from 5/16 – 6/13

	Vaccinates	Controls	Others (2 ponds)
Total	284	1316	3727
Percent	0.41%	1.88%	1.86%
%per day	avg .02%/day	0.08%/day	.08%/day
RPS	0.784194529	-	-1.832066869

Summary/Observations

- CWD outbreak confirmed
  - Colony growth for controls = very concentrated with enlarged spleens
  - Colony growth for vaccinates = limited
- RPS in vaccinated fish = 78%
  - Mortality never exceeded expected natural mortality rates (0.02%/d)
- Two additional ponds (110,000 fish) broke and mortality mirrored controls
- Note: medicated feed administered due to stocking needs
- Trial demonstrates both safety and efficacy of the vaccine
- Replicate trials at other locations are necessary

Current status

- Established partnership with private company (Aquatic Life Sciences, Inc.) for field evaluation at hatcheries in Utah, ID, Co, etc.
- ALS will have first option to license patent rights from UI
- Initial field trials appear promising
- Alternative growth conditions for B.17 may enhance efficacy

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Questions?