Transmission and Persistence of *Ceratomyxa shasta* in Chinook Salmon

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SALMON DISEASE LAB

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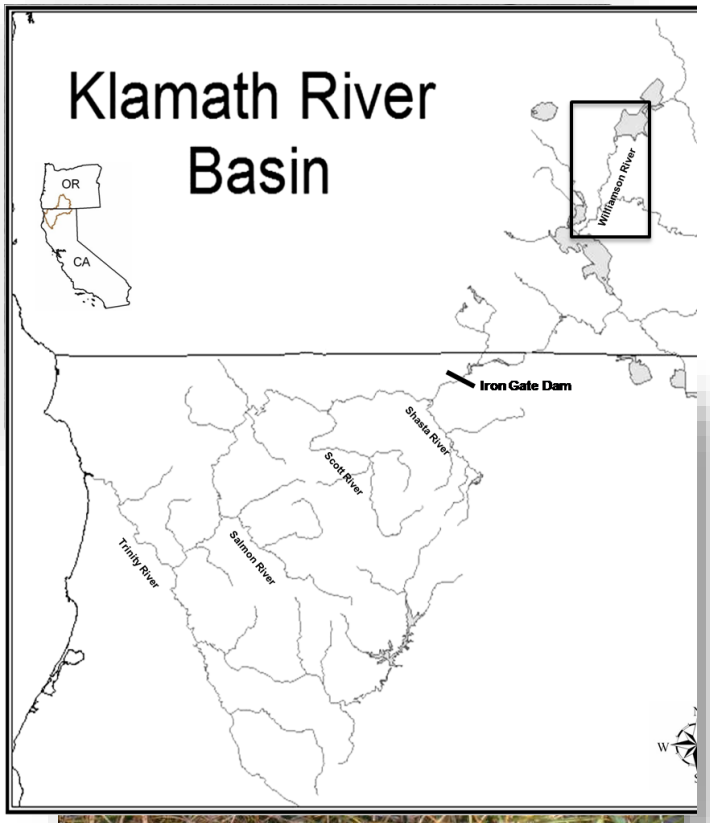
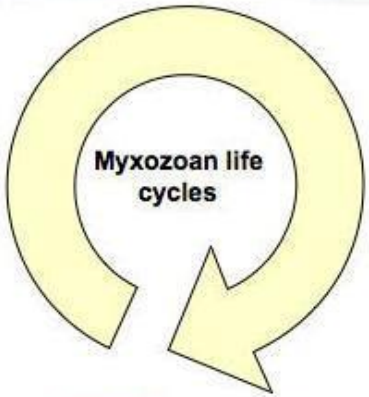
What is *Ceratomyxa shasta*?

- A parasite commonly found in the Pacific Northwest.
- Significant factor in the declining salmon population in the Klamath river.
- Can be fatal to salmon due to the severe hemorrhaging and internal bleeding of the intestine.
- 2 major parasite genotypes (aka strains)
  - Type I
  - Type II



C. shasta life cycle

Infects via the gills and travels to the intestine where replicates



Upon death, salmon releases myxospores in to the water

Actinospores are released into the water once the myxospores have matured in the worms

Myxospores infect polychaete worms

Research Questions

Are mixed genotypes infections more frequent than single infection in Chinook salmon exposed in the river?

- Hypothesis: Mixed infections will be more frequent

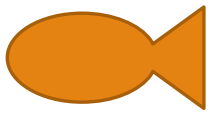
Is there a difference in infection between age 0 and age 1 Chinook?

- Hypothesis: Older fish are more susceptible to infection due to smoltification

Do infected Chinook salmon release parasites before death?

- Hypothesis: No. They only release parasites upon death.

Field Methods



X 70

Age 1

Neg. Control x 20
Rep 1 x 25
Rep 2 x 25

Exposed Rep groups for 22 hours at the Klamath River. Taking three 1L water samples on the first and second day.

Neg. Control 1 x 15
Rep 1 x 15 (8 alive)
Rep 2 x 15 (5 alive)

[monitored for 60 days at the SDL]

X 140

Age 0

Neg. Control 1 x 25
Neg. Control 2 x 25
Rep 1 x 30
Rep 2 x 30
Rep 3 x 30

Then sampled 10 gills per rep group and 5 for each control group

Neg. Control 1 x 20
Neg. Control 2 x 20
Rep 1 x 20
Rep 2 x 20
Rep 3 x 20

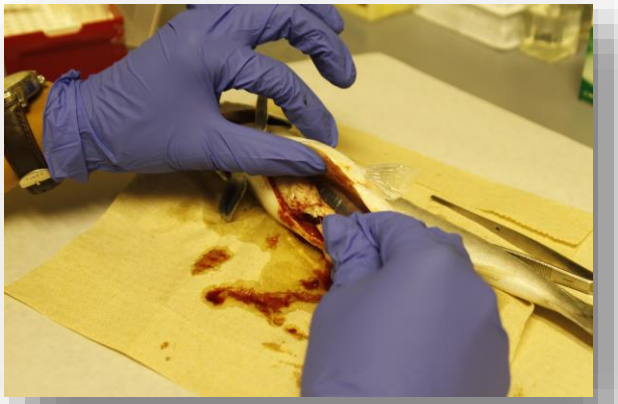
Lab Methods

First two weeks:

- Treated with medicated feed and gills were removed in any fish mortalities

After Two weeks

- Intestine were removed instead of gills in any fish mortalities
- Began looking for spore stages in age 0 tank water
  - Turned off flow from each tank
  - Put the fish on air for 24 hours
  - Took three 1L water samples from each tank for qPCR
  - Filtered for 2 hours using a 243 micron mesh with 5 micron filter for each tank to look for spores stages



Techniques

Fish Necropsy
- To extract gill and intestinal samples

Water sample collection
- From the river and tank

DNA extraction and purification of both the water and tissue samples

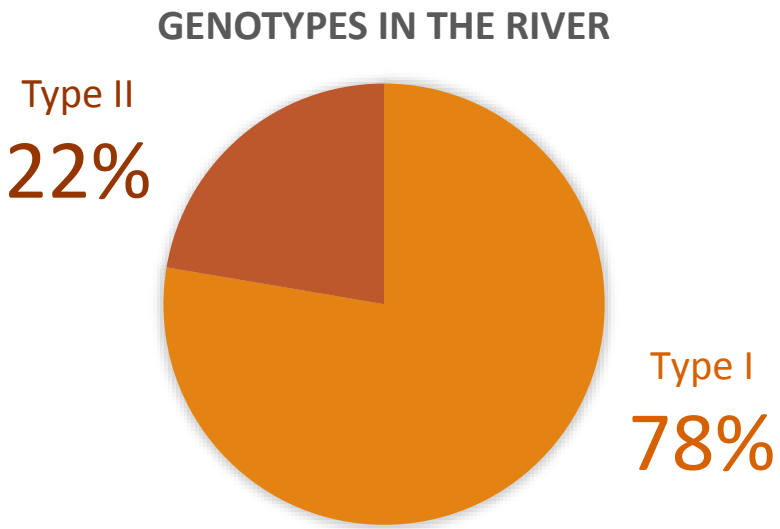
qPCR
- Real time PCR to determine the concentration of spores per liter in both the water and tissue samples

Sequencing
- To determine the genotype of the parasite

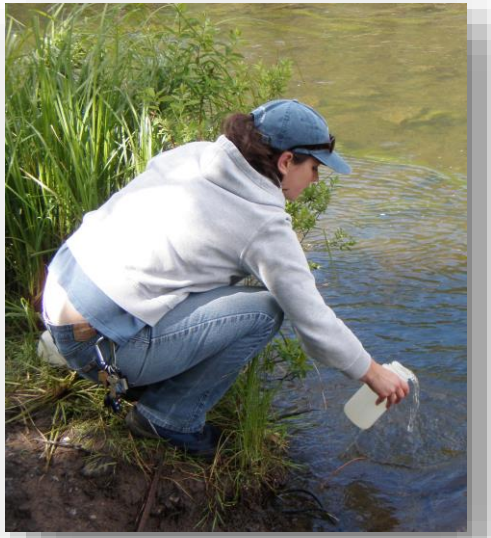
Parasite counts using hemacytometer

Results (genotypes in the river water)

Genotypes found in the Klamath river.

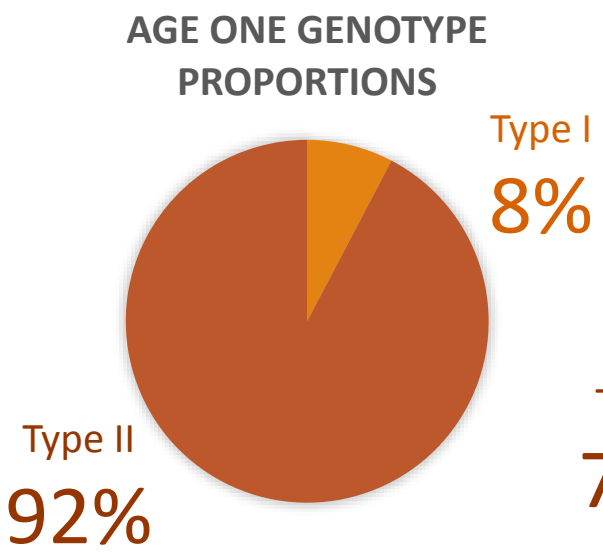


Spores per liter – 1

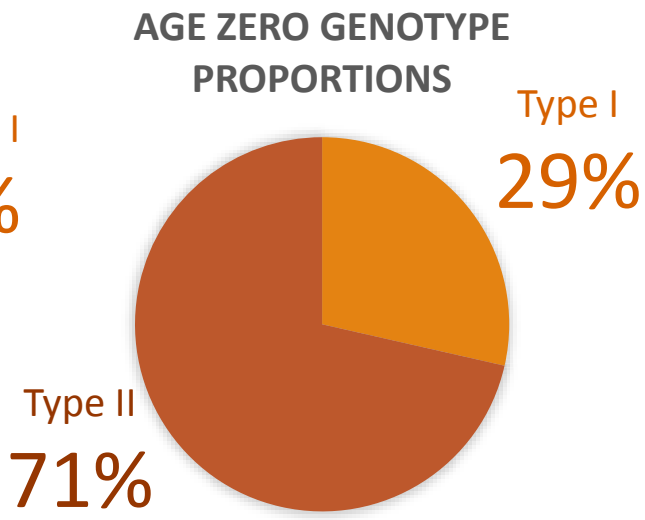


Results (genotype proportions in gills)

The mixed vs. single proportion in the sampled gills



3.58 average actinospores per fish



3.22 average actinospores per fish

Age 0 infection rate: 100%

Age 1 infection rate: 100%

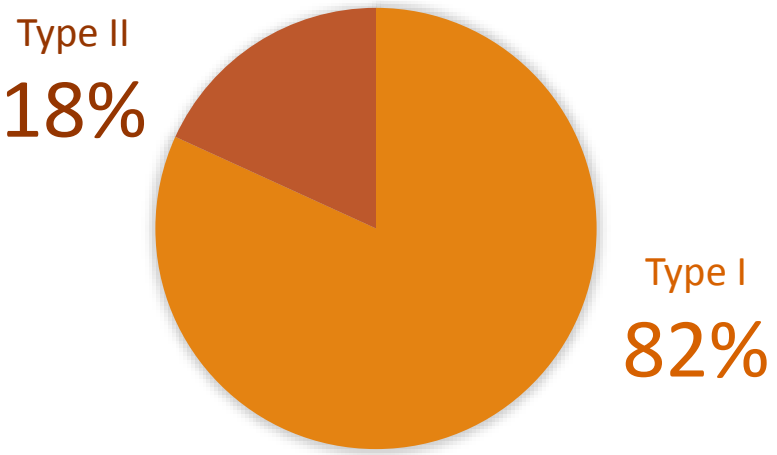
Total Single Infection (Type I): 11.111%

Total Mixed (I&II) Infection: 88.889%

Results (genotype proportions in intestine)

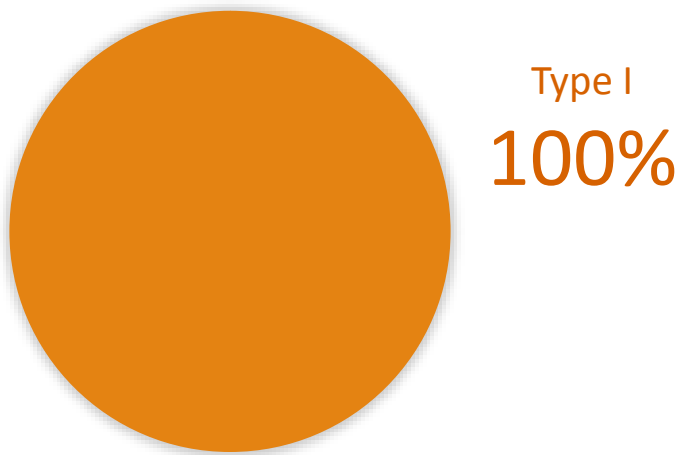
The mixed vs. single proportion in the intestine

AGE 1 GENOTYPE PROPORTIONS



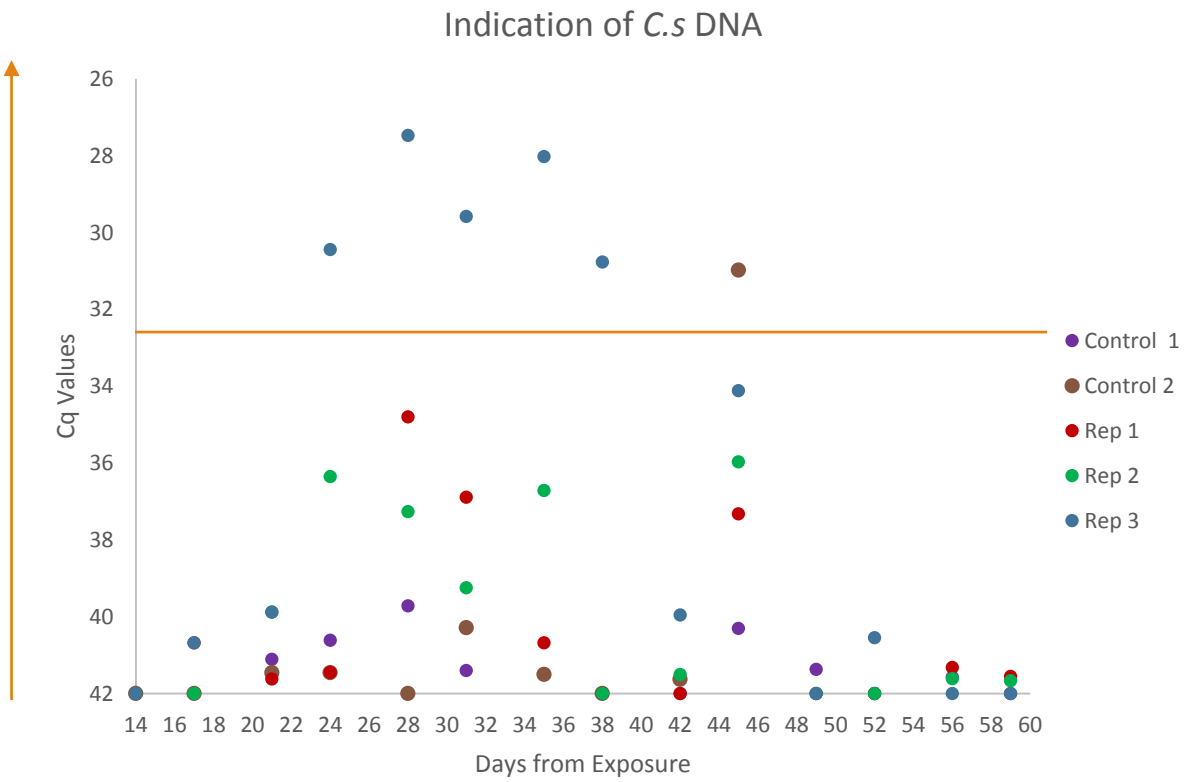
Single Infection (Type I): 82%
Single Infection (Type II): 0%
Mixed Infection: 18%

AGE 0 GENOTYPE PROPORTIONS



Single Infection (Type I): 91.3%
Single Infection (Type II): 8.7%
Mixed Infection: 0

Results (# of pre-spores in tank water)



Observed:
Only pre-spores, no myxospores.

Decreasing Cq value means higher spores per liter.

32.5 Cq = 1 spore/liter

Conclusions

Are mixed genotypes infections more frequent than single infection in Chinook salmon exposed in the river?

- Yes. The gill data shows that 88.9% percent of the infection where mixed.

Is there a difference in infection between age 0 and age 1 Chinook?

- No. 100% of all my fishes were infected regardless of age.

Do infected fish release parasites before death?

- Yes. Pre-spores were observed, but no myxospores suggests that salmon didn't receive a high enough infection dose.
- No myxospores released means no polycheate worms infected.
- Lack of spores could also mean that death is necessary or the methods may be flawed.

Discussion (the big picture)

Results would…

- Better inform the disease model of *C.shasta* for Chinook salmon population.
- Show that Chinook salmon have the potential to release type II parasites which would infect coho salmon, an endangered species.
- Demonstrates that competition between parasite genotypes could occur.
  - If there is competition, then it could lead to higher mortality.

Future question:

- Determine if pre-spores can mature into myxospores in an environment outside of the intestine and infect polychaete worms.

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HHMI Program

URISC: Start