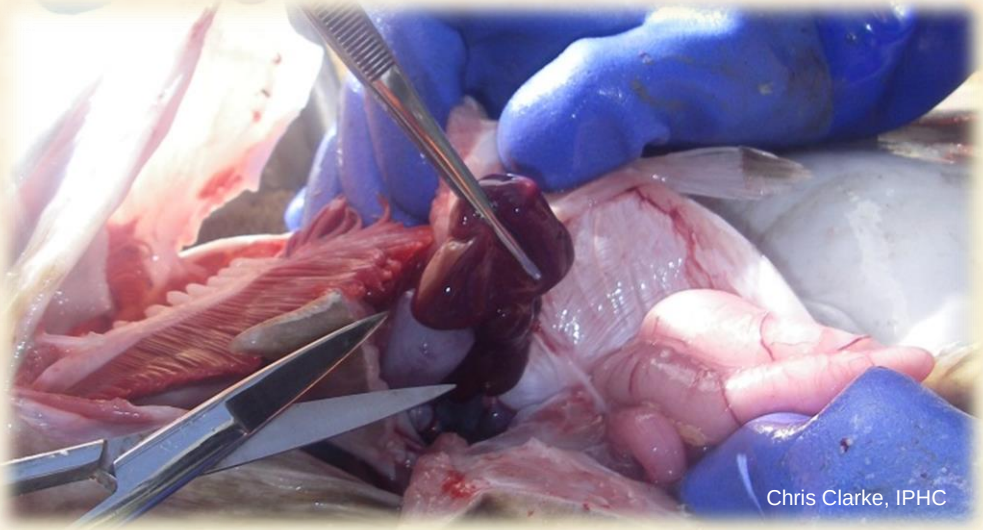
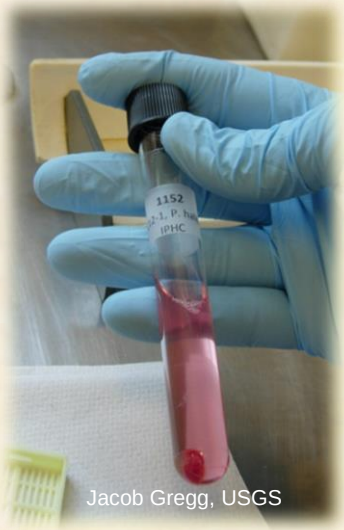
Epizootiology of *Ichthyophonus* sp. in Pacific halibut (*Hippoglossus stenolepis*) in the Northeast Pacific Ocean and Bering Sea





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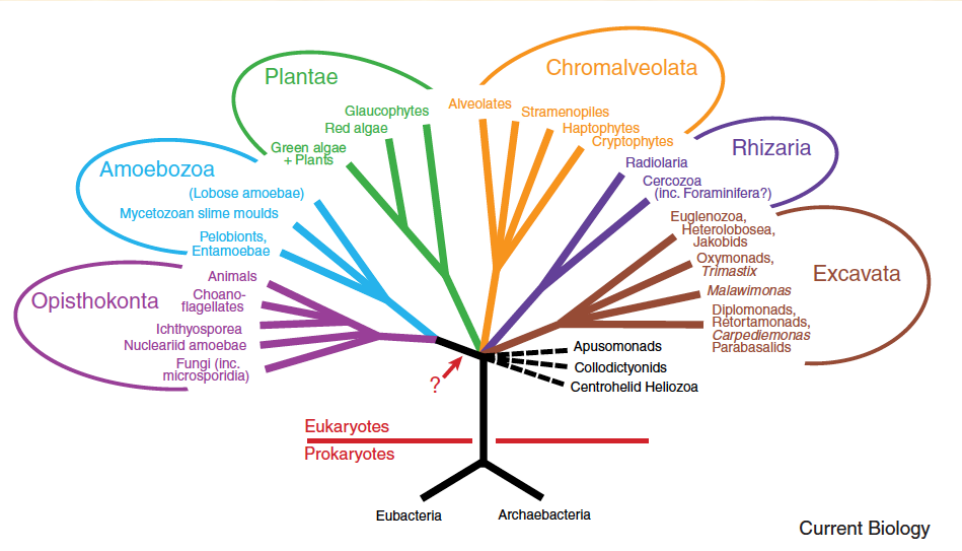
Outline

- What is *Ichthyophonus*?
- Why do we care?
- Study objectives.
- Study methods.
- Results with regard to spatial, temporal, and population composition (sex, size, age).
- General discussion.
- What's next?

What is *Ichthyophonus sp.*?

- Cosmopolitan parasite of the class Mesomycetozoea



Simpson and Roger 2004

- Low host specificity
- Internal histozoic parasite
  - Found in all visceral organs and musculature of infected hosts

Potential effects of *Ichthyophonus*

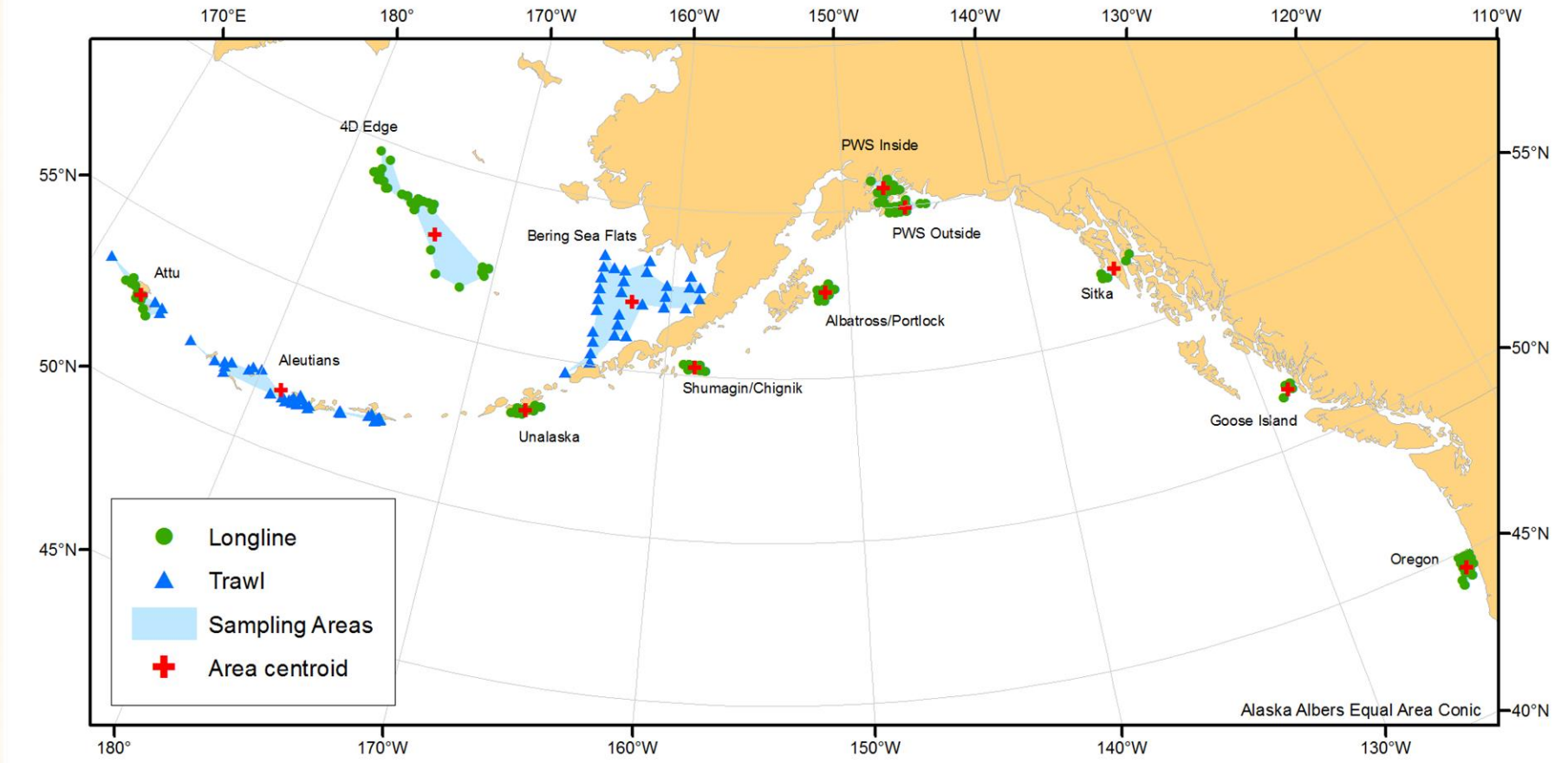
- Population level
  - Epizootics causing large mortality events
- Individual level
  - Reduced growth rate
  - Decreased swimming speed
  - Increased morbidity and mortality
- Food quality
  - Salmon drying
  - Pollock texture/taste

Chinook Heart: Stan Zuray – Rapids Research Center

Study Objectives

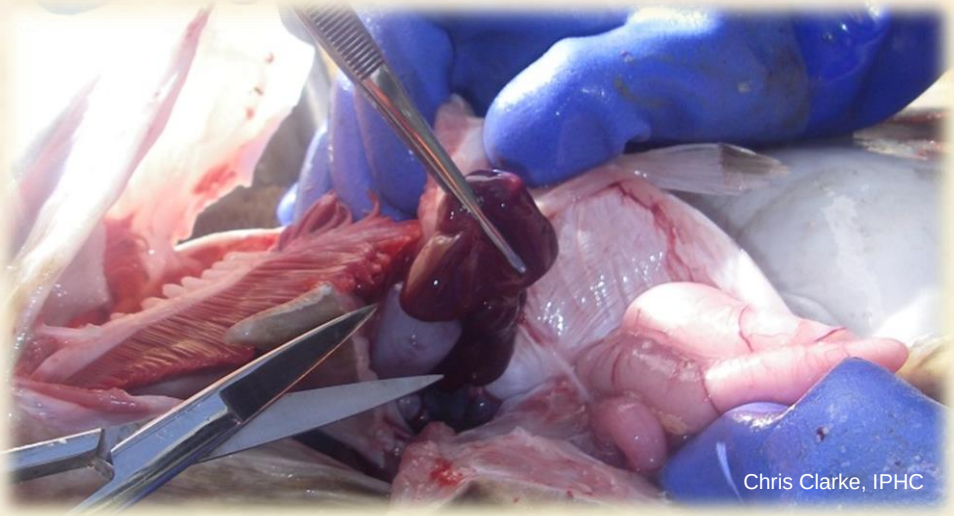
- Determine:
  - Infection prevalence of *Ichthyophonus sp.* in Pacific halibut throughout the NE Pacific and Bering Sea.
    - Spatial
    - Temporal
    - Correlation to host characteristics
      - Sex
      - Size
      - Age
  - Infection intensity of the organism within halibut
  - Any evidence of population impacts

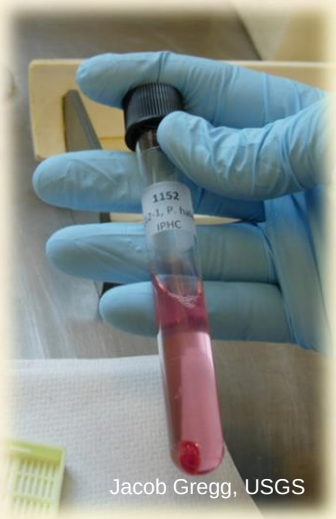
Methods


170°E
180°
170°W
160°W
150°W
140°W
130°W
120°W
110°W
55°N
50°N
45°N
40°N
4D Edge
PWS Inside
PWS Outside
Bering Sea Flats
Attu
Aleutians
Albatross/Portlock
Sitka
Shumagin/Chignik
Unalaska
Goose Island
Oregon
Longline
Trawl
Sampling Areas
Area centroid
Alaska Albers Equal Area Conic

Methods – Parasite culture (prevalence)

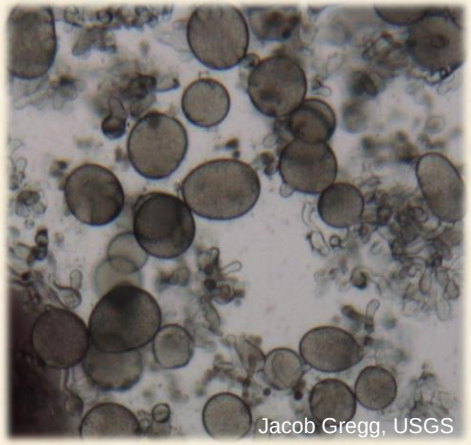
- Field Technique
  - Sterile resection of heart tissue (0.5-1.5 cm^3) for parasite culture
    - Aseptically placed in 15-ml tube containing 7 ml of growth medium (MEM) containing antibiotics and antifungals





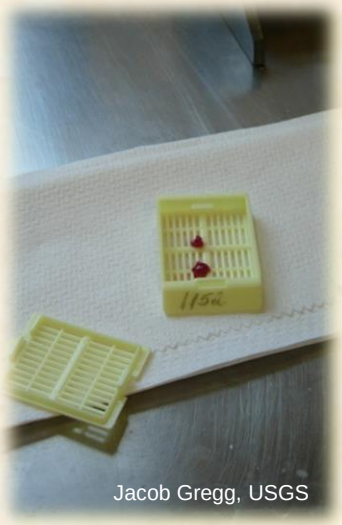
Methods – Parasite culture (prevalence)

- Lab Technique
  - Heart tissue cultured at 15°C
  - Examined microscopically (40X magnification) for presence of *Ichthyophonus* schizonts and/or hyphae
  - Examined twice, after 7d and 14d incubation
  - Media was exchanged in tubes that became turbid due to host tissue autolysis



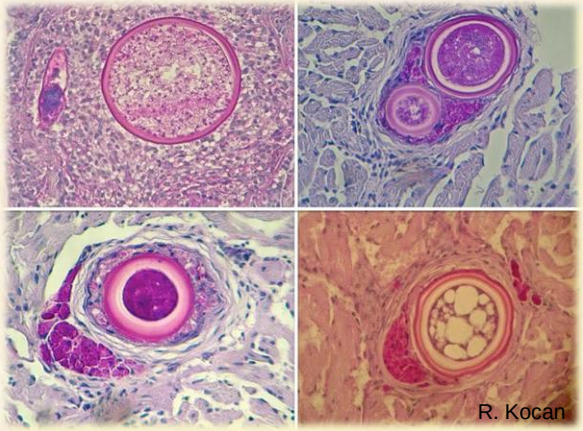
Methods – Tissue Histology (intensity)

- Field Technique
  - Non-sterile resection of heart and liver tissue (0.5 cm^3)
  - Fixed in 5% neutral buffered formalin

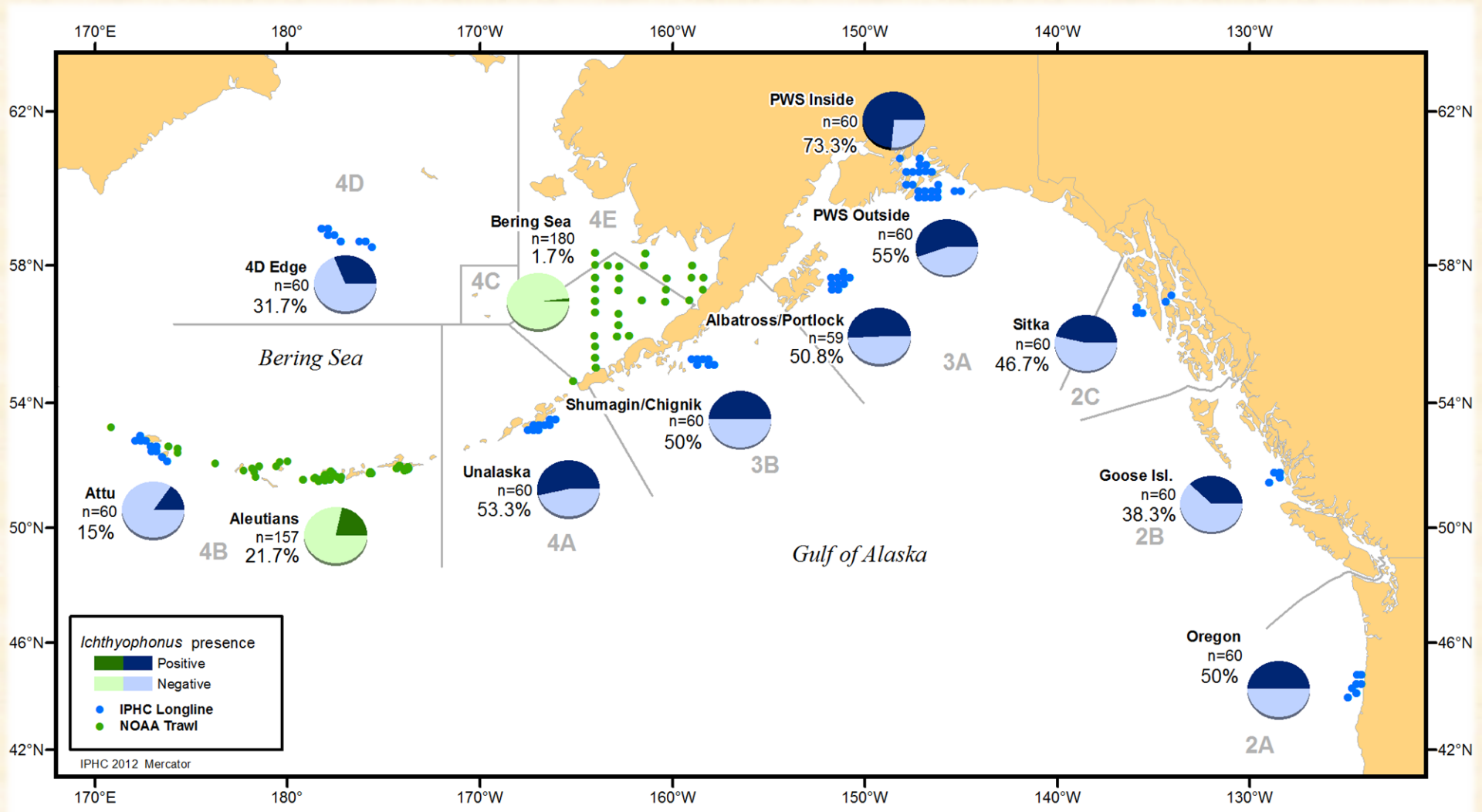


Methods – Tissue Histology (intensity)

- Lab Technique
  - Fixed tissue thin sectioned
  - Mounted and stained with hematoxylin-eosin and PAS
  - Examined under compound microscope and all *Ichthyophonus* schizonts present in a single 100x field of view were counted.
  - If no schizonts detected, entire section was examined.



Results – Prevalence – Spatial



Results – Prevalence – Temporal

- Significant differences for repeated locations
  - 2011 (χ^2_2=36.94, p<0.001), 2012 (χ^2_2=20.97, p<0.001) and 2013 (χ^2_2=17.61, p<0.001)
- Within locations, relatively stable inter-annually

Table 1. Prevalence of *Ichthyophonus* sp. infections in Pacific halibut from 2011 to 2103 at three locations. Numbers in brackets are infected fish/total sample size.

	Prevalence (%)			
Location	2011	2012	2013	Combined
4D Edge	26.1 [17/65]	31.7 [19/60]	29.6 [19/64]	29.1 [55/189]
PWS-Inside	76.7 [46/60]	73.3 [44/60]	58.3 [35/60]	69.4 [125/180]
Oregon*	33.8 [22/65]	50.0 [30/60]	23.7% [14/59]	35.9 [66/184]

*Significant heterogeneity between years at this location (χ^2_2=9.10, p<0.01).

Results – Prevalence – Temporal (cont'd)

- Some evidence of within season variance
  - ($\chi^2_1 = 9.03$, $p<0.003$) early June vs. late August

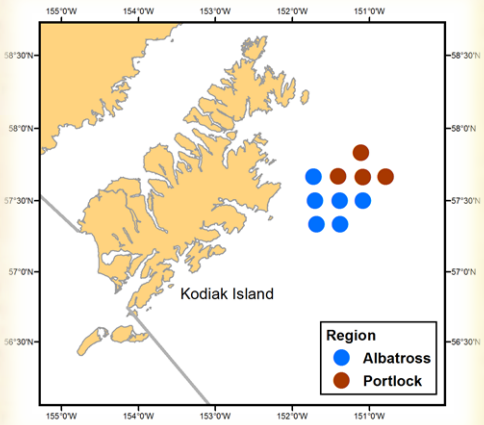


Table 2. Temporal Sampling Differences for the Albatross-Portlock site.

Site	Data Range	Samples	% Positive	Ave. Length (cm)	Ave. Age (yr)
Albatross	June 2-10, 2012	28	71.4%	77.4	13.1
Portlock	Aug. 26-27, 2012	32	32.3%	75.9	11.1

Results – Prevalence – Sex

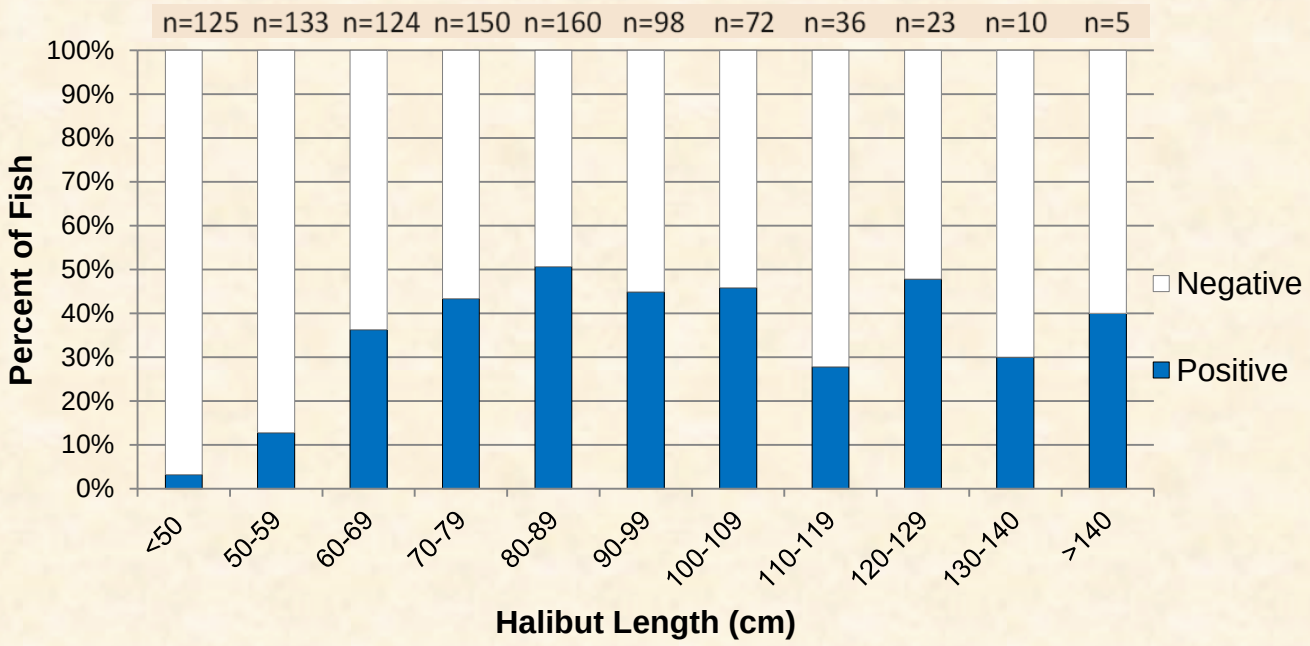
- Infection prevalence was higher in females (39.0%) than males (31.5%) (χ^2_1=7.73, p<0.005)

Table 3. Prevalence of *Ichthyophonus* infections in Pacific halibut by sex and year.

	Year			
Sex	**2011**	**2012**	**2013**	**Average**
Female	**48.2%**	**37.1%**	**36.4%**	**39.0%**
Male	**35.4%**	**30.1%**	**38.9%**	**31.5%**
Average	**44.7%**	**33.7%**	**37.2%**	**36.0%**

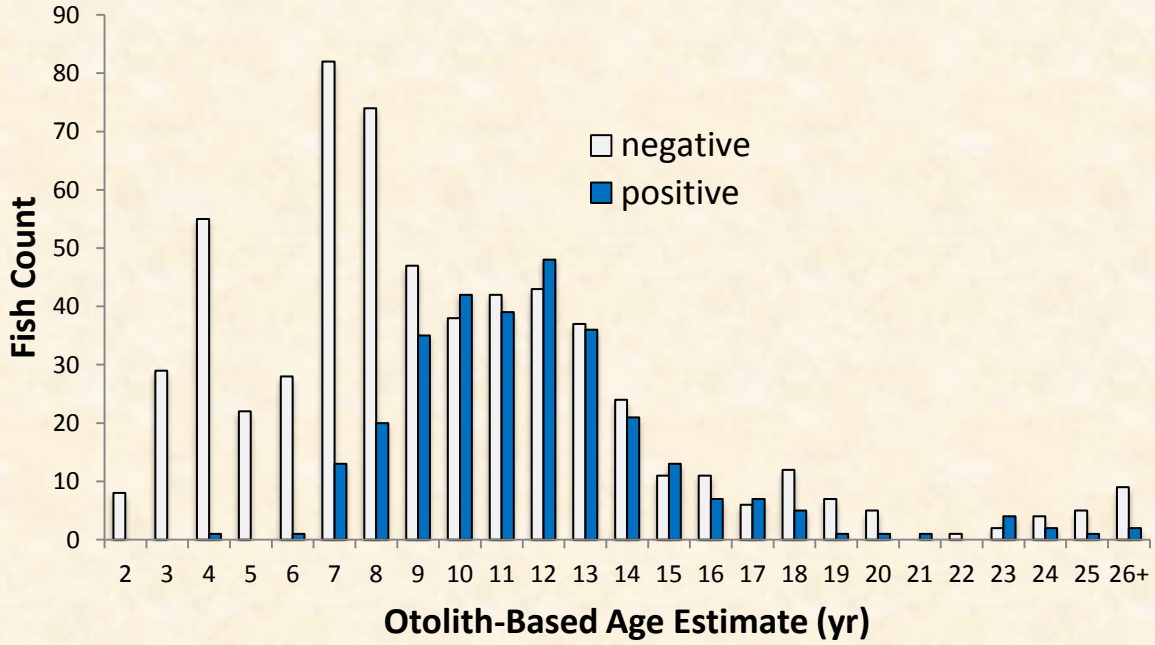
Results – Prevalence – Size

- Prevalence varies significantly with size (χ^2_{10} = 118, p<0.001)

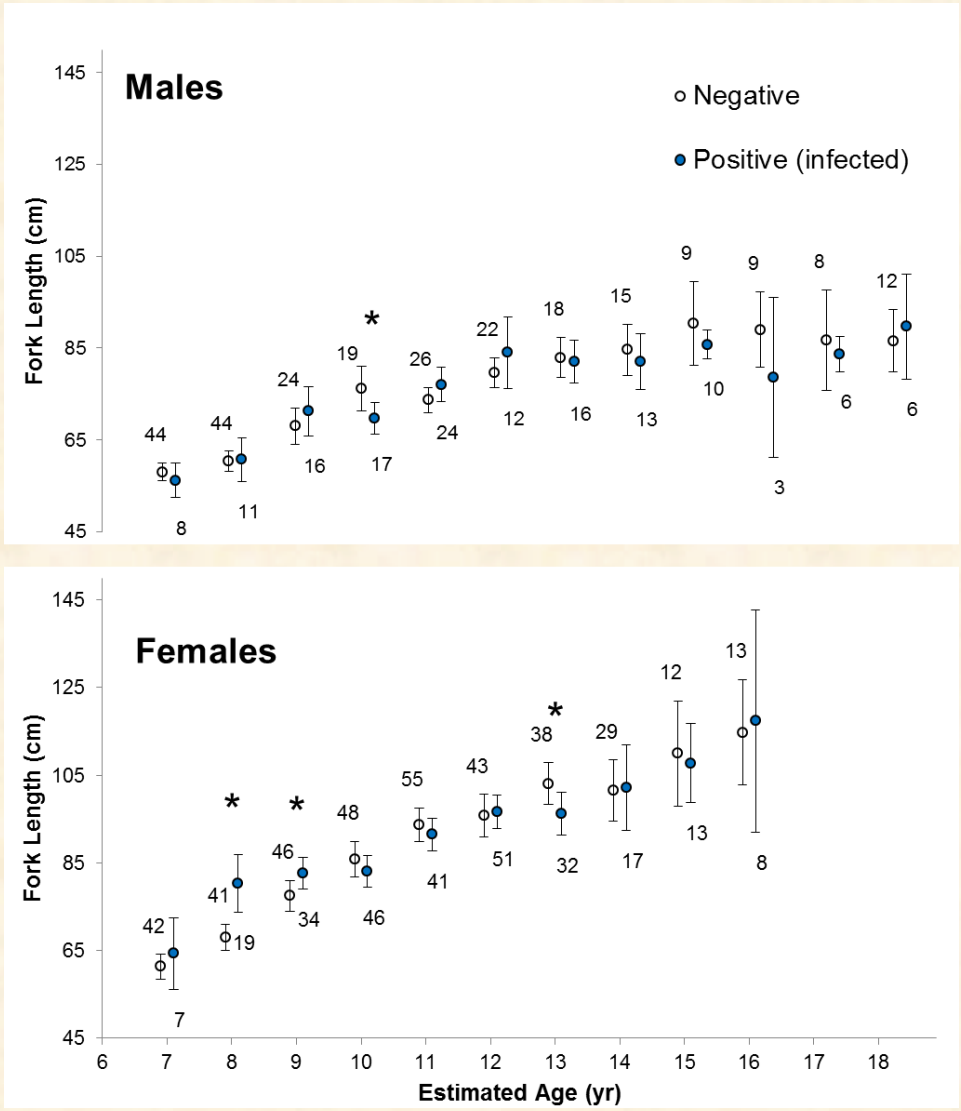


Results – Prevalence – Age

- Prevalence varies significantly with age (χ^2_5=98.34, p<0.001)

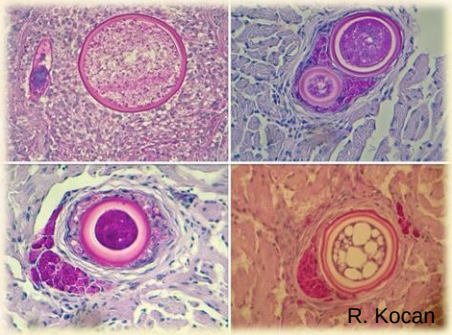


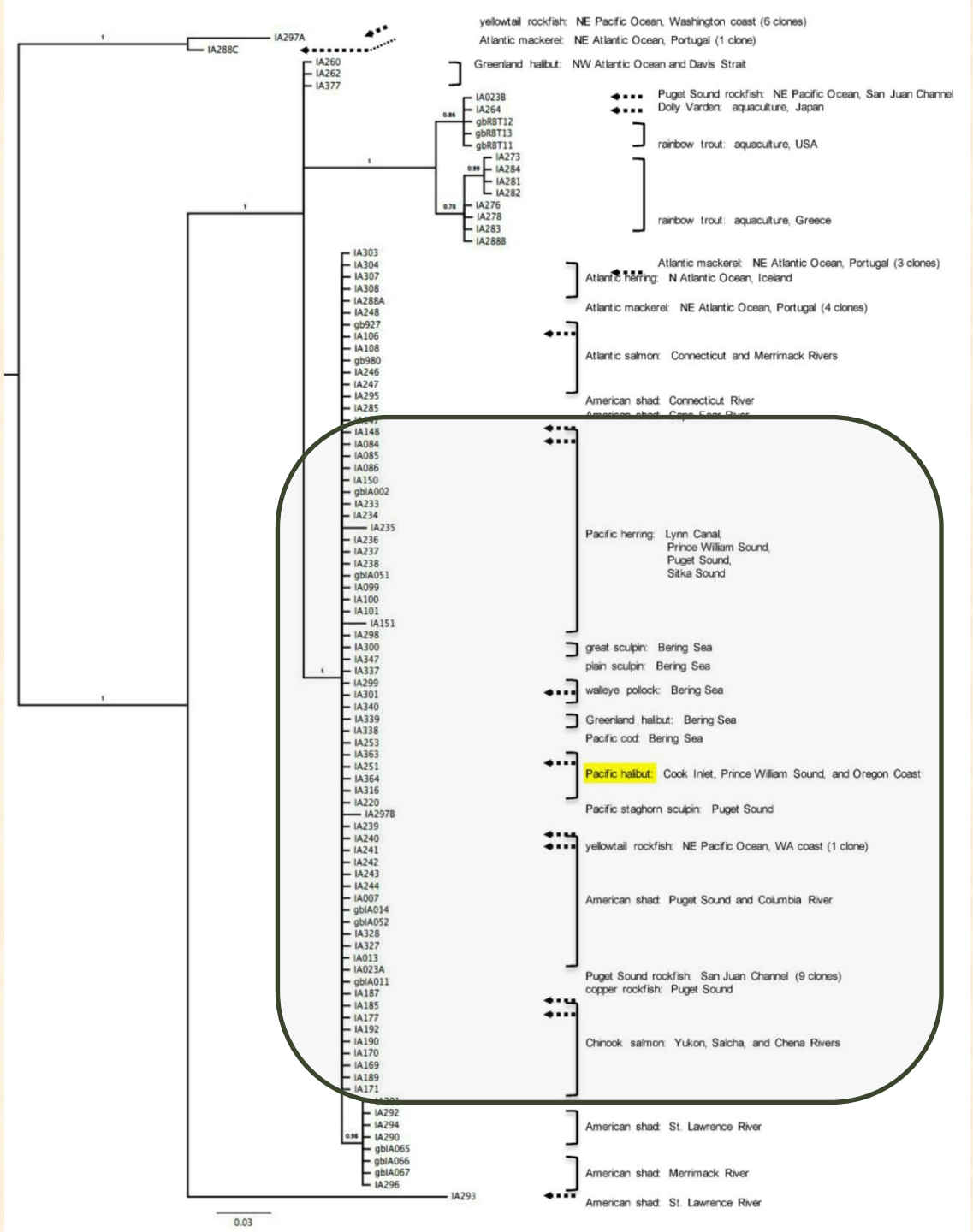
Results – Prevalence – Size at Age



Results – Intensity

- Intensity work (2012) provided interesting results
  - Of 278 culture positive halibut:
    - Schizonts positively detected in 7 (2.5%) heart tissue sections
    - None detected in liver tissue.
  - Schizonts were not dense, 1 or 2 present per section
  - None detected from culture negative halibut (n=52)




yellowtail rockfish: NE Pacific Ocean, Washington coast (6 clones)
Atlantic mackerel: NE Atlantic Ocean, Portugal (1 clone)
Greenland halibut: NW Atlantic Ocean and Davis Strait
Puget Sound rockfish: NE Pacific Ocean, San Juan Channel
Dolly Varden: aquaculture, Japan
rainbow trout: aquaculture, USA
rainbow trout: aquaculture, Greece
Atlantic mackerel: NE Atlantic Ocean, Portugal (3 clones)
Atlantic herring: N Atlantic Ocean, Iceland
Atlantic mackerel: NE Atlantic Ocean, Portugal (4 clones)
Atlantic salmon: Connecticut and Merrimack Rivers
American shad: Connecticut River
Pacific herring: Lynn Canal, Prince William Sound, Puget Sound, Sitka Sound
great sculpin: Bering Sea
plain sculpin: Bering Sea
walleye pollock: Bering Sea
Greenland halibut: Bering Sea
Pacific cod: Bering Sea
Pacific halibut: Cook Inlet, Prince William Sound, and Oregon Coast
Pacific staghorn sculpin: Puget Sound
yellowtail rockfish: NE Pacific Ocean, WA coast (1 clone)
American shad: Puget Sound and Columbia River
Puget Sound rockfish: San Juan Channel (9 clones)
copper rockfish: Puget Sound
Chinook salmon: Yukon, Saicha, and Chena Rivers
American shad: St. Lawrence River
American shad: Merrimack River
American shad: St. Lawrence River
0.03

General Discussion

- Relatively high prevalence across the range
- Higher prevalence in older, larger fish and females
  - Ontogenetic shift in diet
- Very low intensity
- Spatial and temporal stability
- Genetically similar organism throughout range
- No historical data on *Ichthyophonus* in P. halibut.
  - Unknown if new or long term commensal with halibut
  - Unknown effect (if any) on health of individual, growth dynamics, or mortality (population)

Next Steps

- Continued monitoring at the three sites
  - Both prevalence and intensity
  - Sudden change in either could be indicative of a mortality effect
- Such changes could prompt a growth/energetics study
- Further investigation intensity with genetic probe

Acknowledgements

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