

Transmission of Apicomplexan Infection in Gray Meat Atlantic Sea Scallops

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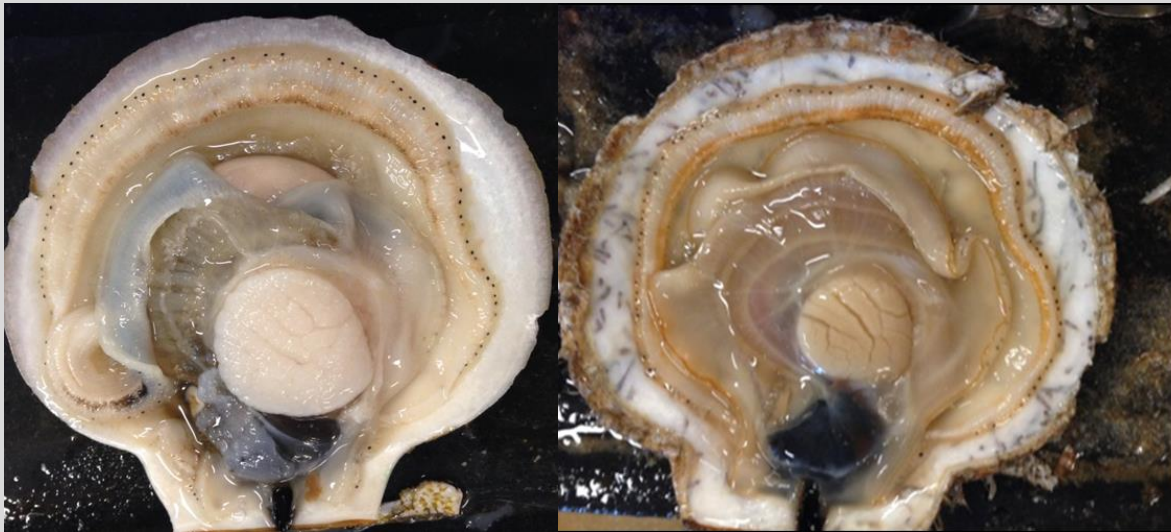
Background

What are “gray meat” scallops?

Since 1936: Episodic reports of gray meat in Atlantic sea scallops, *Placopecten magellanicus*

Clinical Signs:

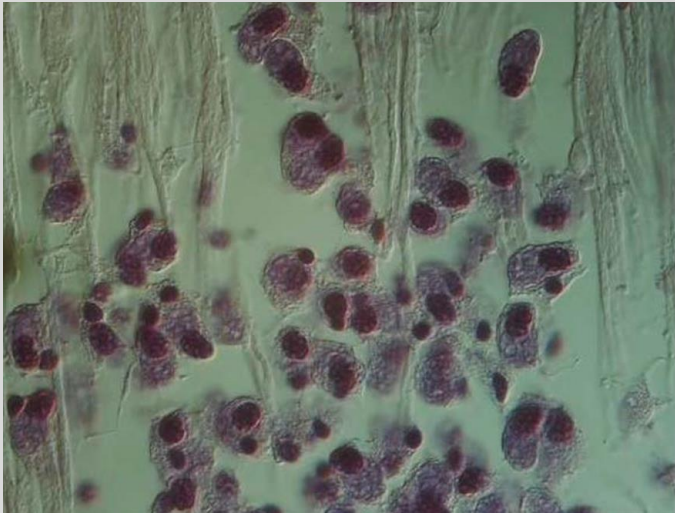
- discoloration of meat from white to brown/gray, loosely bound adductor muscle



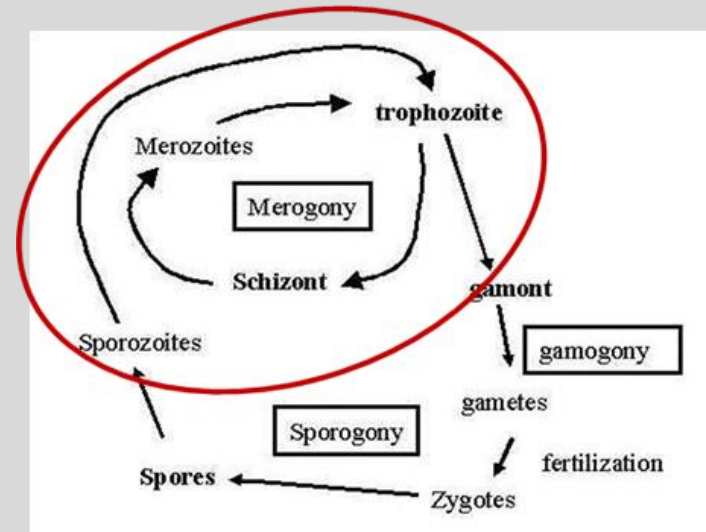
From Inglis et al. 2016

Background

Linked to highly pathogenetic apicomplexan parasite that targets muscle tissue



Inglis et al 2016



NOT ZONOTIC

Geographical Range

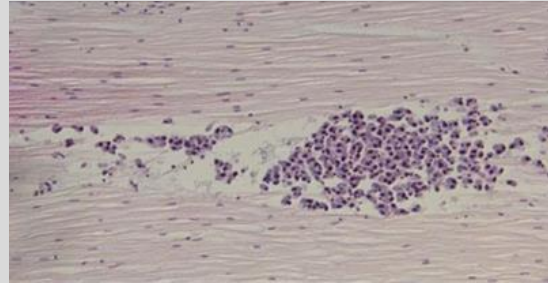
Impacts several scallop fisheries globally:

- **Atlantic sea scallop**, *Placopecten magellanicus*
Canada, Gulf of Maine, Georges Bank, Mid-Atlantic
- **Iceland scallop**, *Chlamys islandica* in Icelandic waters
- **Queen scallop**, *Aequipecten opercularis*, Faroe Islands
- **King scallop**, *Pecten maximus*, West Coast of Scotland
- **Weathervane scallop**, *Patinopecten caurinus*, Alaska
(Histology, PCR confirmation, but not sequenced yet)

Histopathology

Apicomplexan found in all muscular tissues

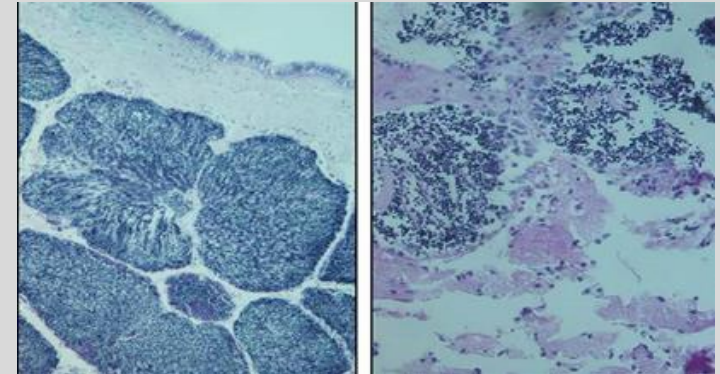
- Intracellular in muscular tissue
- Free in extracellular space



- Adductor muscle heavily infected
- “Gray scallops” highest intensity
- Some white scallops lightly infected

Effect of Parasite on Muscle Tissue:

- Causes severe histopathological changes in adductor muscle as well as other muscular tissues (heart, gonad)
- Focal or disseminated NECROSIS
- **Lethal** in severely infected scallops (Levesque et al 2016)



NORMAL

INFECTED

Gonad (testis) of a gray meat Atlantic sea on left

Adductor muscle

Hyalinization

Muscle fibers become hyalinized into a glossy homogeneous mass



Many apicomplexan life stages found in adductor muscle



Infection Transmission Trials

Saltonstall Kennedy: NA15NMF4270260

1. Can parasite be transmitted by way of dead, infected scallop tissue?

Scallop Research Set Aside (RSA): NA16NMF4540046

2. Can parasite be transmitted by way of live, infected scallops?

Objective 1

Can gray meat tissue transmit the infection to naive scallops?



Test the hypotheses:

1. gray meat tissue can be a vector for further infection
2. treating infected scallop meat in a fresh water bath reduces the virulence of the infection through osmotic disruption of the parasite.

Methods

White meat scallops

- SH ~90-100 mm
- Elephant Trunk (VIMS)

Subsampled to confirm absence of parasite by PCR and histology- Naïve

Gray meat scallops (used for parasite exposure)

- Georges Bank (CFF)

Subsampled to confirm presence of parasite by PCR and histology

Naïve scallops (n=40) randomly assigned to one of three treatment groups:

A. *Infected* – exposure to infected tissue

B. *Control* – sham exposure

C. *Fresh Water Treatment* – exposure to infected tissue treated with freshwater bath

Experimental Period: 8 months



SMAST Salt Water Laboratory

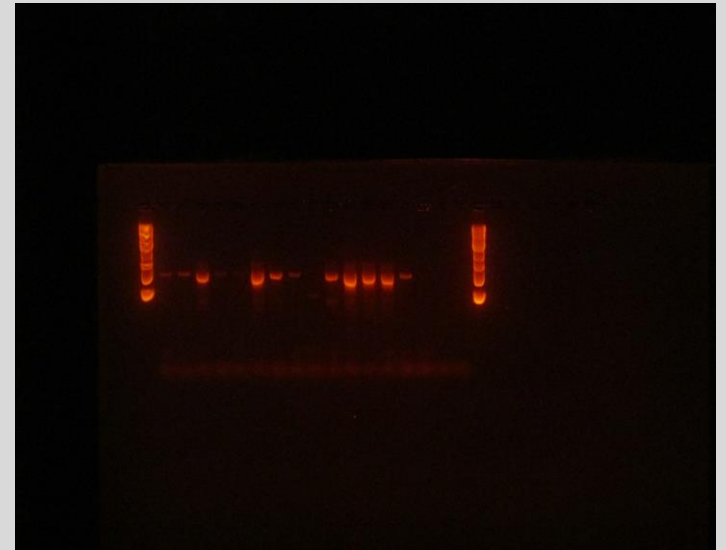
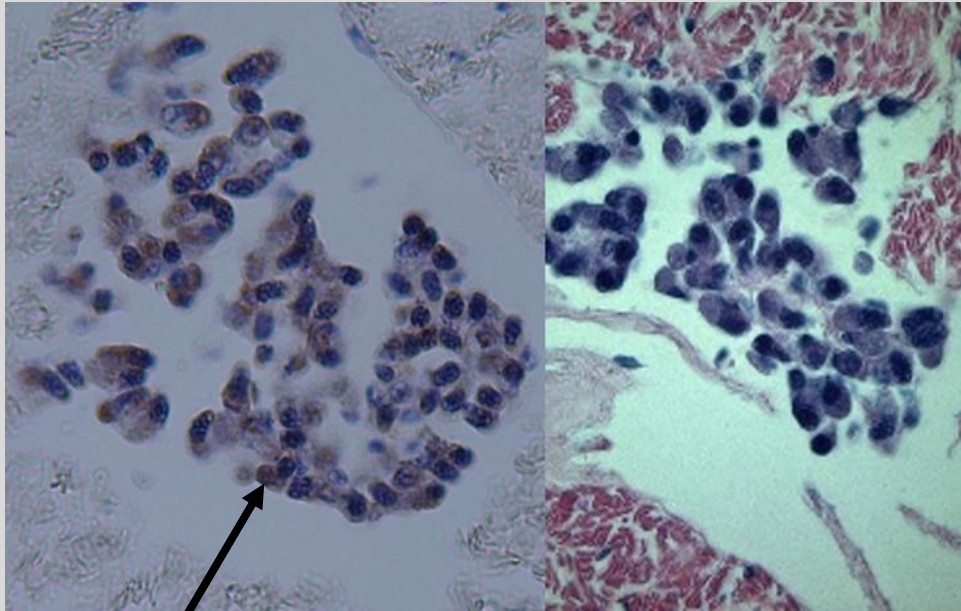


- Sterilization unit ensured all water entering each tank was free of contamination.
- Splash guards were in place between experimental tanks.
- Daily checks of water temp., pH, flow, dissolved oxygen, mortalities
- Fed live algae cultures of *Cheatocecos neogracilis*, *Isochrysis* aff. *galbana* (T-ISO), and *Thalassiosira weissflogii*

Sampling Protocol

- Mortalities
- Assessed for clinical signs of infection (shell height ,meat weight and color, gonad condition)
- Tissue samples collected for histological and molecular analysis

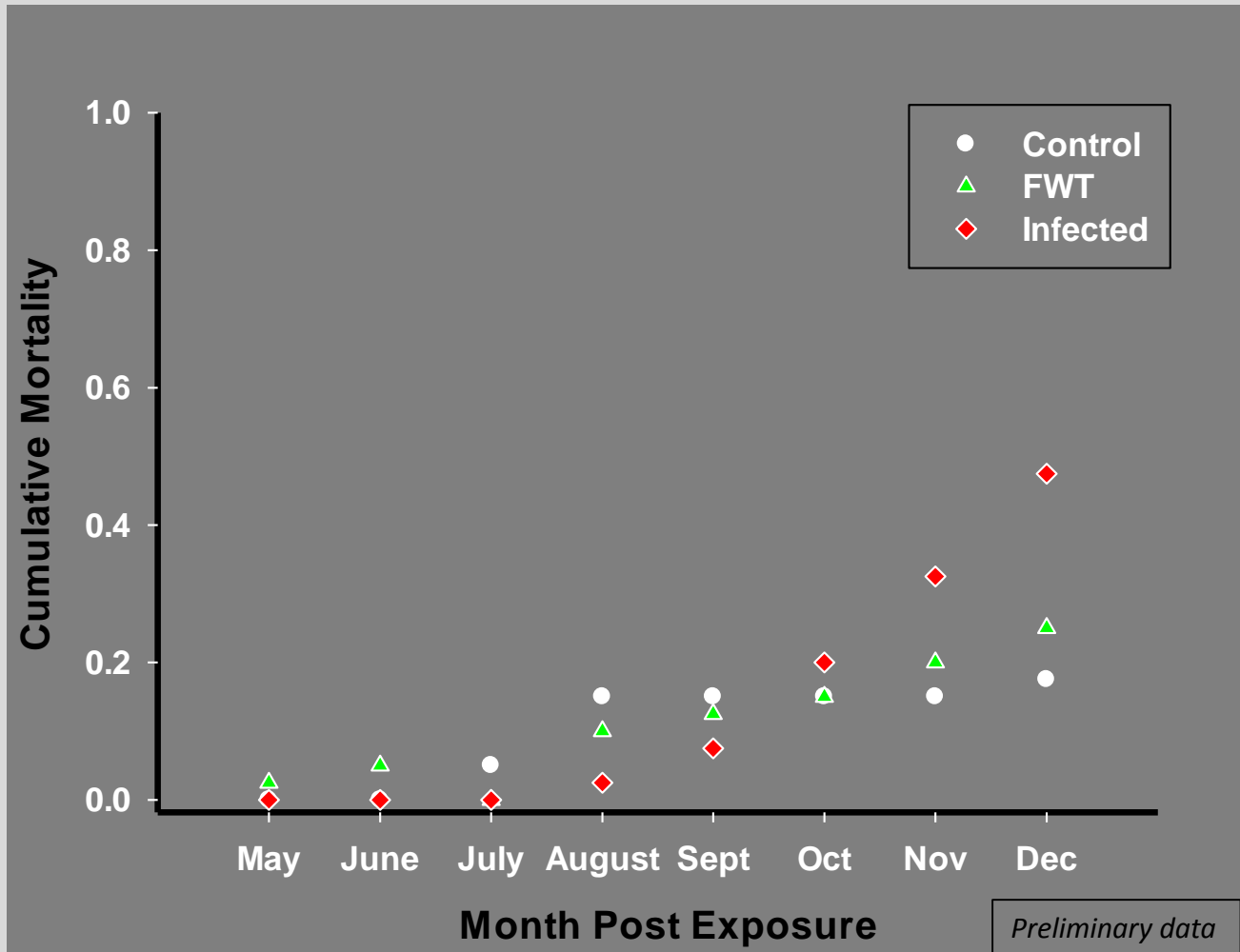
Intensity of the infection: mean number of apicomplexan zoites per 6 fields 250x



Presence of the infection: confirmed through polymerase chain reaction (PCR).

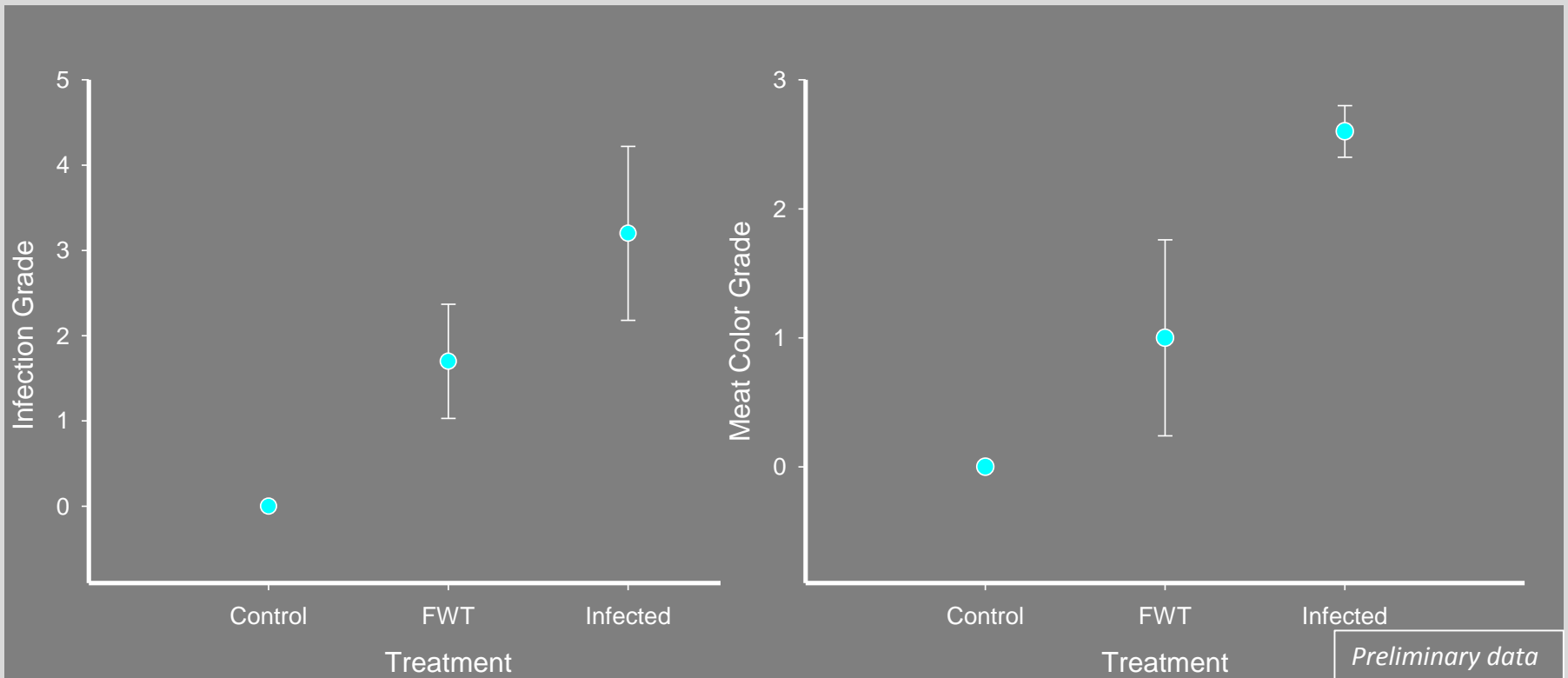
Subsample: using in situ hybridization (left) brown reaction is a positive reaction binding of the probes to messenger RNA of the parasite; one on the (right) is May-Grünwald Giemsa stain (Kistmundsson et al 2015).

Preliminary Results



End of trial: Lost
~50% of
"infected" group

Preliminary data



Infection Grading:

- 0 = none
- 1 = ≤ 20
- 2 = 21-50
- 3 = 51-100
- 4 = 101-200
- 5 = >200

Meat Color Grading:

- 0 = normal white
- 1 = light brown/gray
- 2 = brown
- 3 = dk brown/gray

Summary Preliminary Results

Tank A: *Infected*

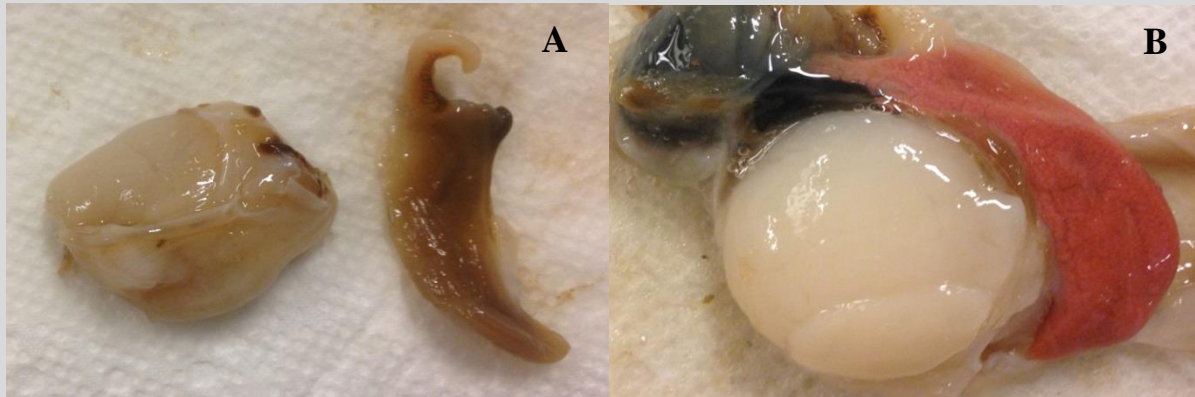
- Severely to moderately infected with parasite with brown/gray meat color and poor gonad condition

Tank B: *Control*

- No clinical (white meat), molecular or histological signs of parasite

Tank C: *Fresh Water Treatment*

- Intermediate response: Did not stop transmission but reduced intensity of infection



Scallops at end of experiment from Infected (A) and Control (B) groups

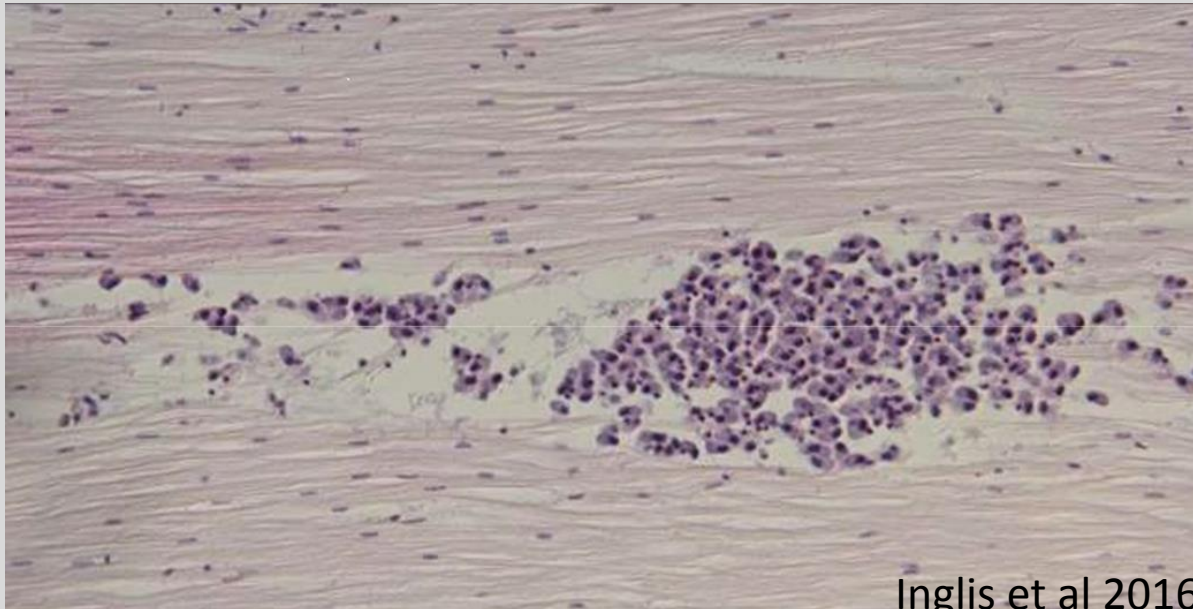
Conclusions

- Scallops severely infected with the parasite that die in a scallop population can transmit the infection to other scallops in the population.

Gray meat tissue contains large numbers of sporozoites

→ Infection stage of the parasite

Thus, not surprising that cut tissue can transmit the parasite



Objective 2

Can parasite be transmitted by way of live, infected scallops?

Methods

White meat scallops

- SH ~100 mm
- Nantucket Lightship

Subsampled to confirm absence of parasite by PCR and histology-Naïve

Gray meat scallops (used for parasite exposure)

- Georges Bank (CFF)

Subsampled to confirm presence of parasite by PCR and histology

Naïve scallops (n=50) randomly assigned to one of two experimental groups:

- Infected** – exposure to live gray meat scallops
- Control** – sham-exposure with live, naïve scallops

Experimental Period: 4 months

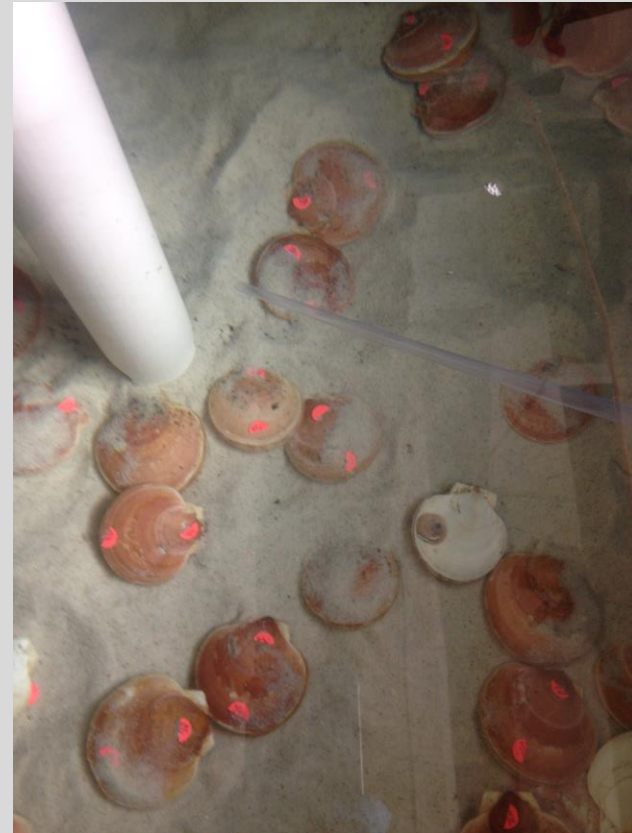


Sampling Protocols

➤ In this experiment water, sediment, feces and different scallop tissues (organs) were serially sampled post inoculation to examine how the parasite moves from one scallop into another scallop, as well as the progression of the infection.

- Same laboratory set up as previous study
- Acclimation period of 3 weeks
- Baseline sample
- Exposure/Sham-exposure
- Sampling at 10, 20, 40, 60, 80, 100, and 120 days

Sampled scallops replaced with tagged naïve scallops to control for density



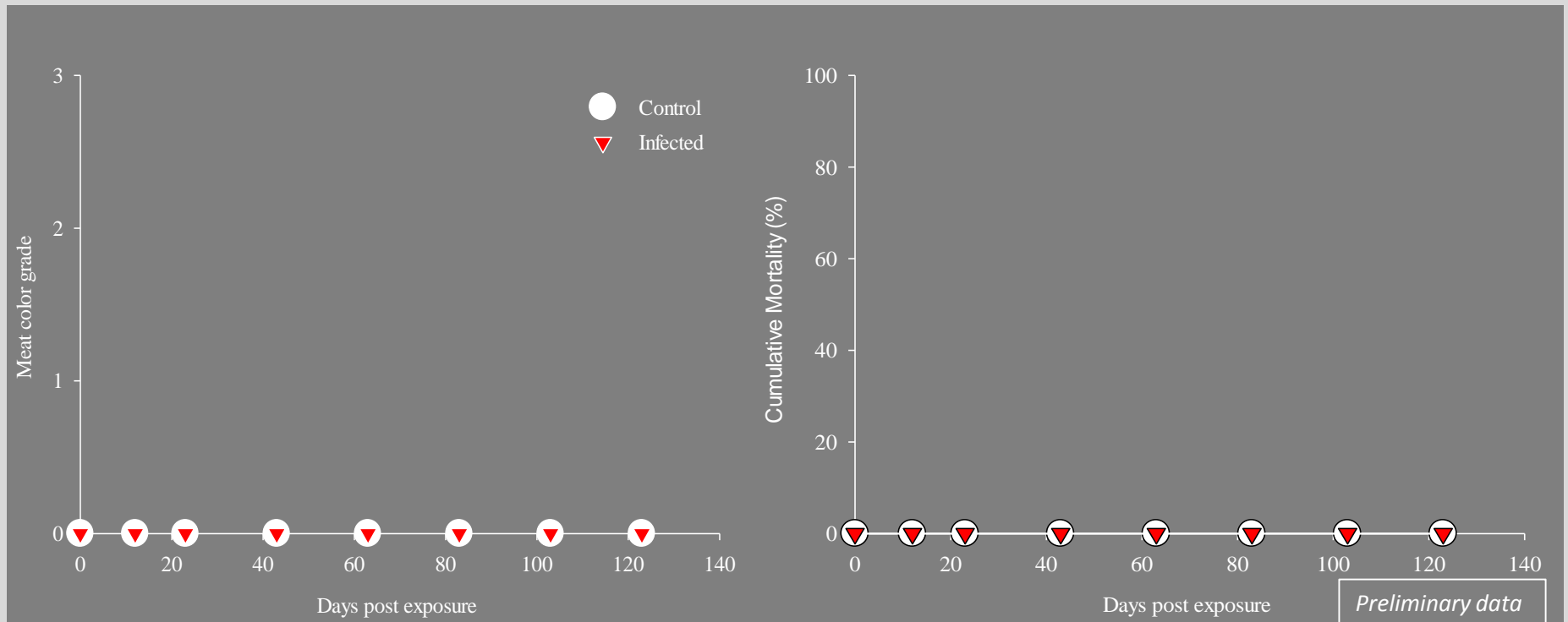
Sampling Protocols *continued*:

- Mortalities
- Assessed for clinical signs of infection (SH, meat color, meat weight, gonad condition)
- Tissue samples collected for histological and molecular analysis, environmental samples collected for molecular analysis

Samples included:

- adductor muscle
- gonad
- digestive tract and gland
- feces (internal and external to digestive tract)
- sediment
- tank water

Preliminary Results



➤ Negative PCR and histological results

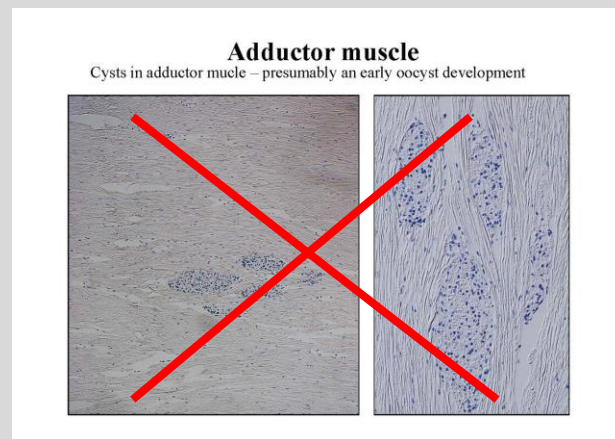
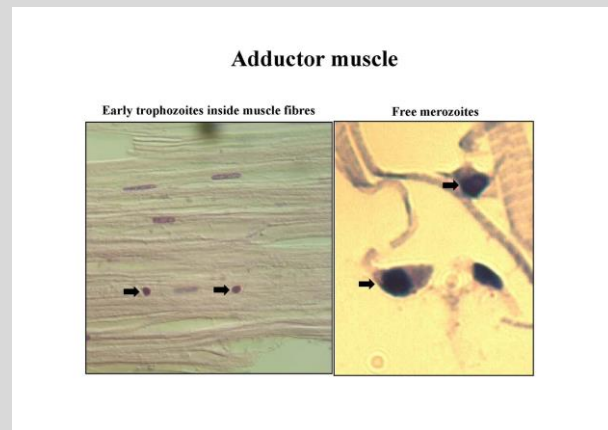
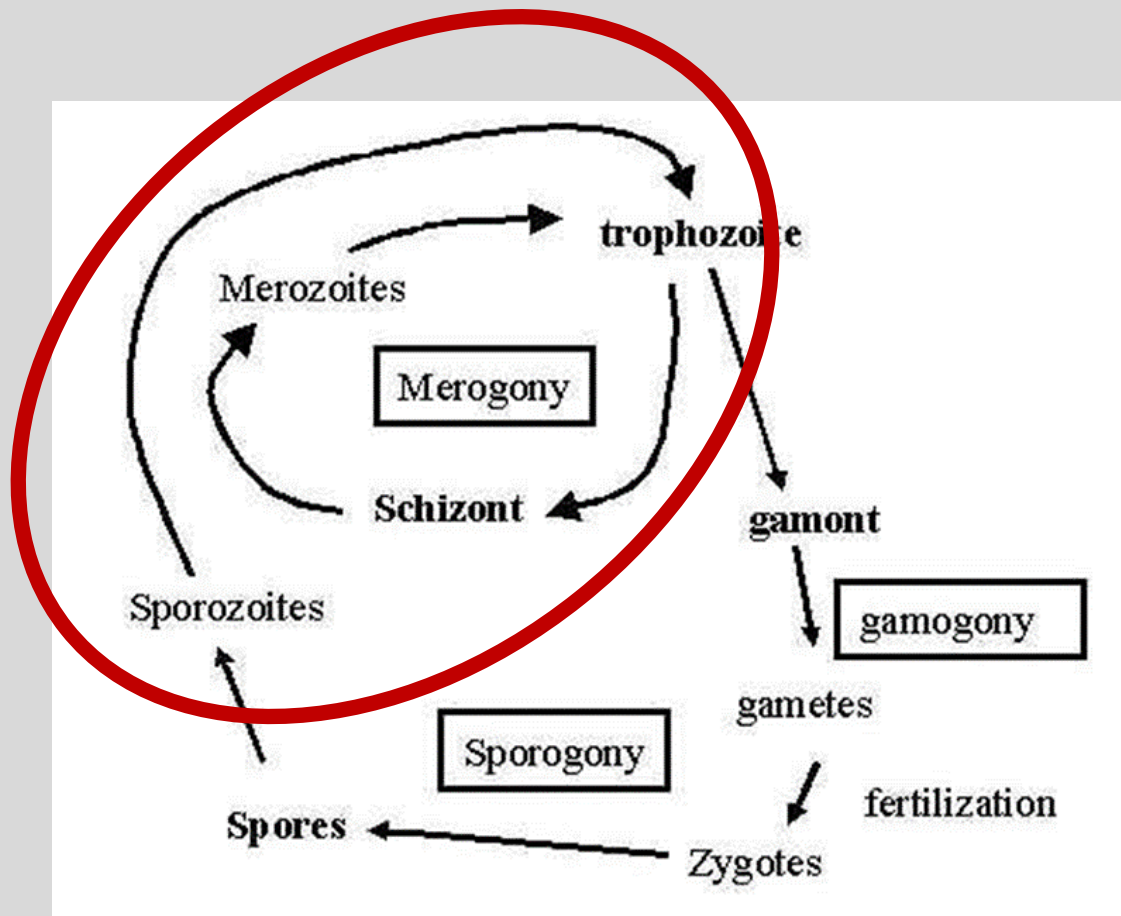
NO SIGN OF INFECTION TRANSMISSION

NOTE: Currently repeating this experiment using gray meat tissue as vector for transmission

Transmission by dead infected tissue but not live infected scallops



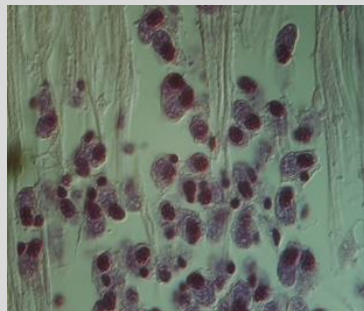
Another Host ?



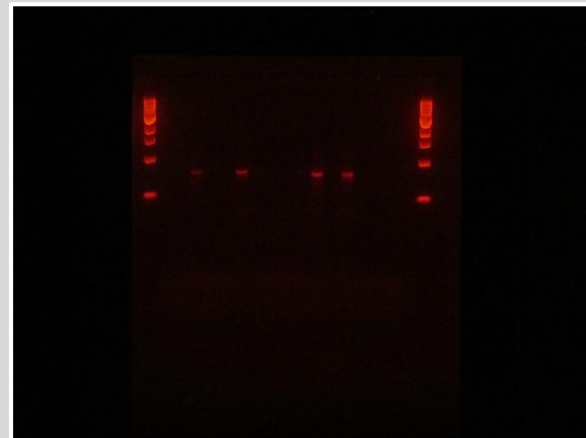
Not oocyst, but meront

Research on Iceland Scallop Infection in Icelandic Waters; Kristmundsson and Freeman

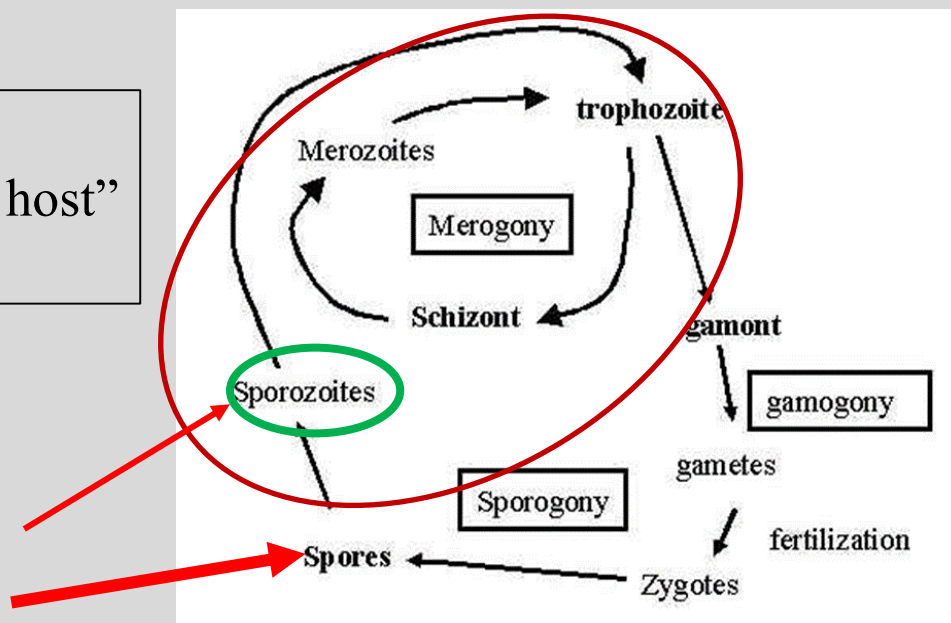
The common (waved) whelk host (*Buccinum undatum*)



Merocystis kathae



Sea scallop
“intermediate host”
pathogenic



Common whelk
“definitive host”
nonpathogenic

Possible Routes of Transmission



Definitive Host



Intermediate Host



Seasonal Transmission?

**Waved whelks sampled from site on Georges Bank n=30:
100% infection rate**

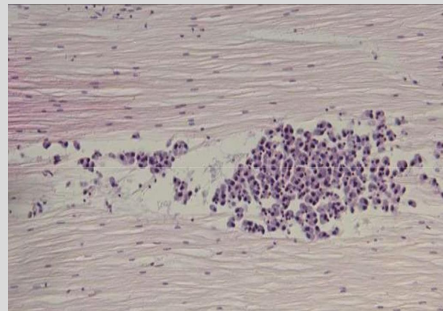
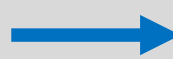


Photo Kevin Stokesbury

Year Round Transmission
Nearest Neighbor  Density Dependent ?

Acknowledgements



- Scallop Industry Partners
- Connamesset Farm Foundation
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