Identification of Hepatopancreatic Parasites Afflicting Crayfish and Associated Snails in Local Virginia Streams

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Outline

- Background
- Ecological importance
- What are trematodes?
- Purpose of this study
- Study methods
- Results
- Discussion & Conclusion

Background

- Noticed cysts in crayfish while doing other studies
- Some studies on liver flukes in bobcats and snail infection in northern VA
- Very little studies on parasites in central VA area– bridging the gap
- Focus crayfish as intermediate host
  - Abundant

Ecological Importance

Crayfish have multiple symbiotic relationships

-Cleaning ectosymbionts (Fureder et al., 2009; Creed et al., 2015)

-Parasitic endosymbionts (Procop, 2009)

Clear understanding of freshwater ecosystems

-Crayfish are good determiner of stream health and biodiversity (Reynolds & Souty-Grosset, 2011)

-Better conservation and stewarding efforts

What are Trematodes?

- Complex organisms requiring 2-3 hosts to complete their lifecycle
- Taxonomy and ecology not heavily developed like other organisms (Leung *et al.* 2009, Cribb & Bray, 2011).
- Typically parasites of fauna
  - Liver flukes in birds and cattle
- Can infect humans
  - Cercarial dermatitis (Dodangeh et al., 2019)
  - Raw/undercooked

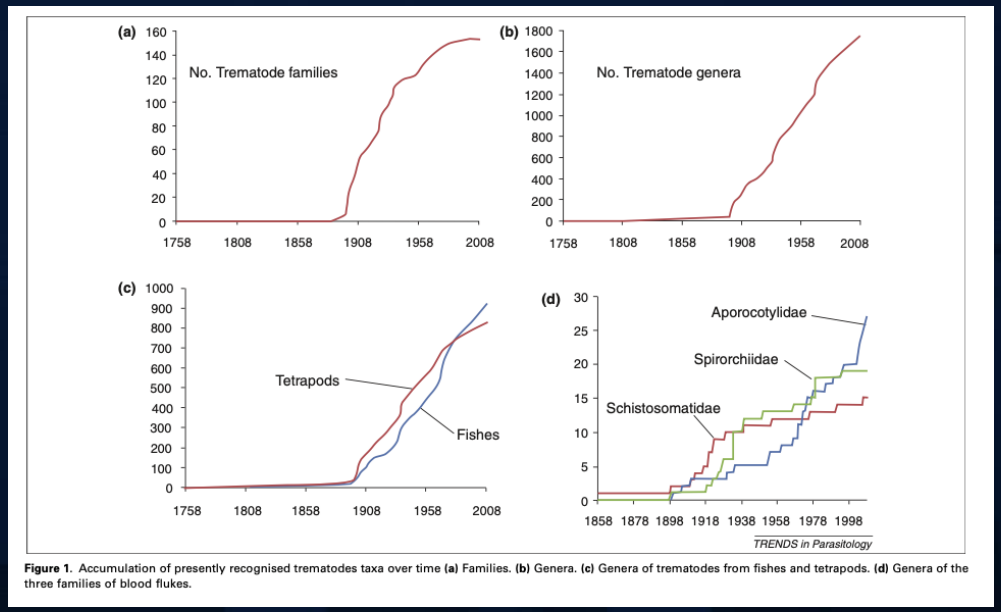


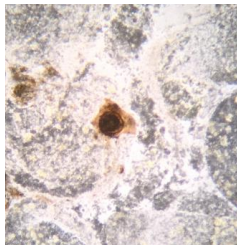
Figure 1. Accumulation of presently recognised trematodes taxa over time **(a)** Families. **(b)** Genera. **(c)** Genera of trematodes from fishes and tetrapods. **(d)** Genera of the three families of blood flukes.

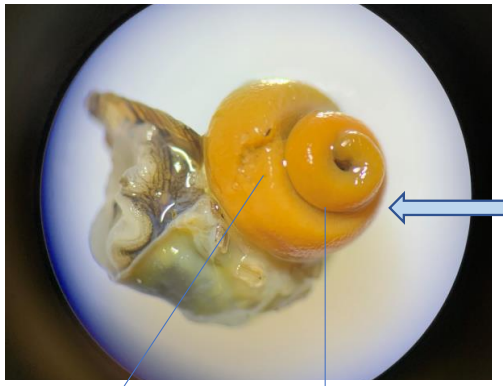
(Cribb & Bray, 2011)

Trematode Life Cycle (snail focused)

Cercariae exit snail through the anus.

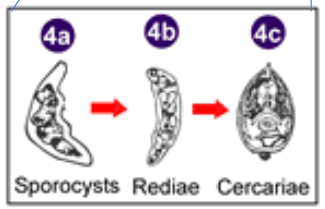
Encystation occurs within crayfish tissue, or any other **secondary intermediate host**.





Digestive-Gonad complex

Miracidia penetrate through snail's tissue, where three lifecycle transformations occur: **sporocyst, rediae, and cercariae.** Maturation and infection occurs in **gonads**.



CDC - Paragonimiasis - Biology. (2019). Cdc.gov. Retrieved 16 October 2019, from https://www.cdc.gov/parasites/paragonimus/biology.html

Eggs hatch into **miracidia** in water

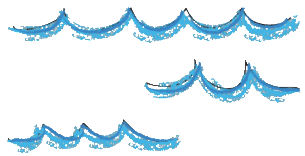


Figure created by Jonathan Tenerovich

Purpose

Gain a clearer understanding of parasitic presence affecting crayfish and snails in local freshwater ecosystems

Q1: What is the prevalence of trematode infection in crayfish?

Q2: What is the prevalence of trematode infection in related snail populations?

Q3: What species of snail, crayfish, and trematode will be identifiable through molecular ID?

Methods

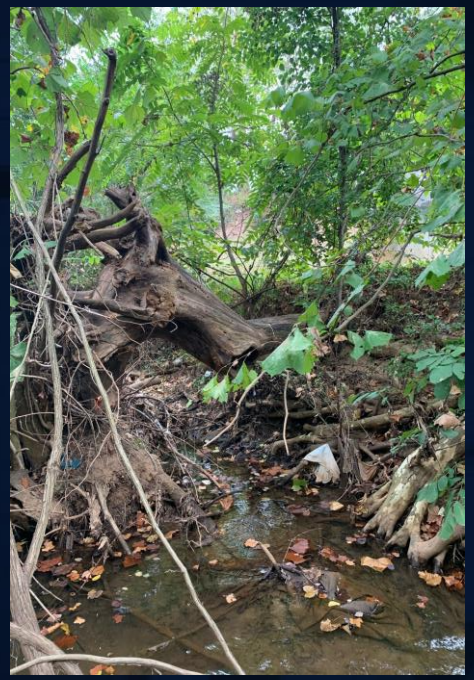
Crayfish hepatopancreas cysts count

Snail cercarial release assay

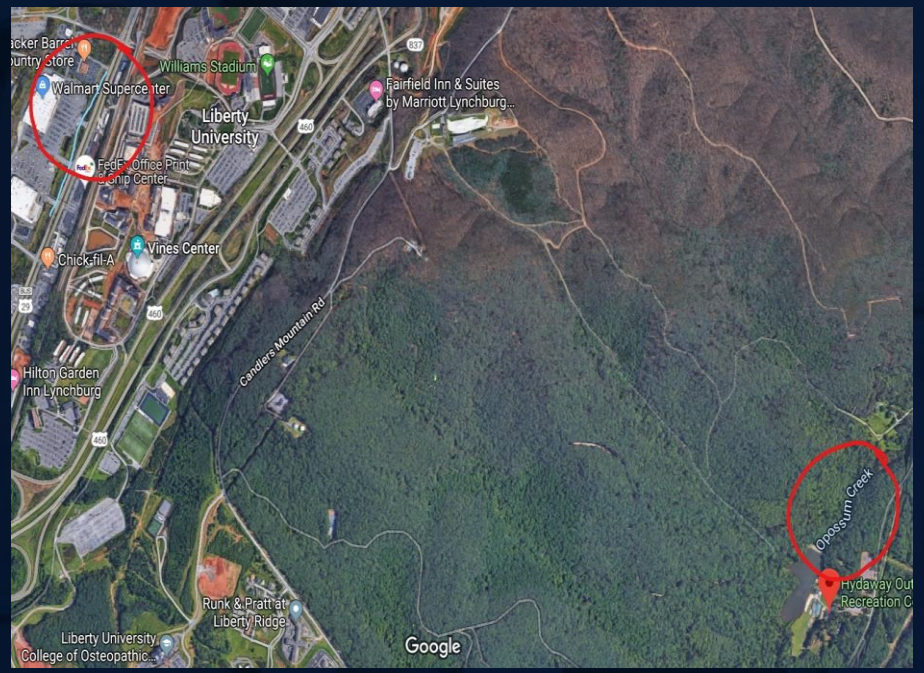
Molecular ID

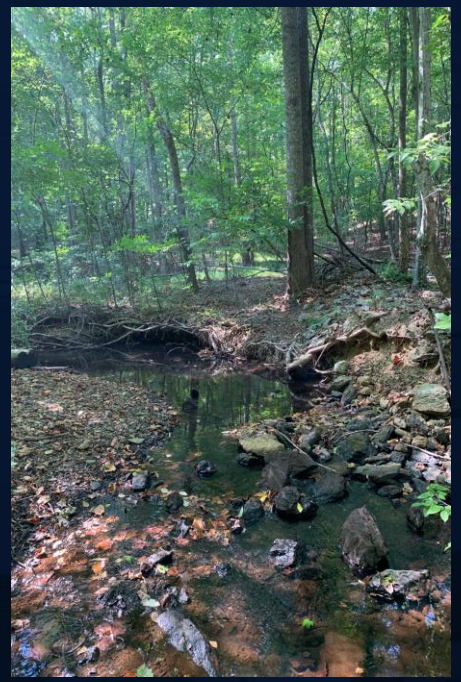
Crayfish Hepatopancreas Cyst Count

- N=30 crayfish between 2019-2020, kept in aquaria
- Sacrificed and dissected for hepatopancreas
- Morphological ID using squash technique



Rock Castle Creek

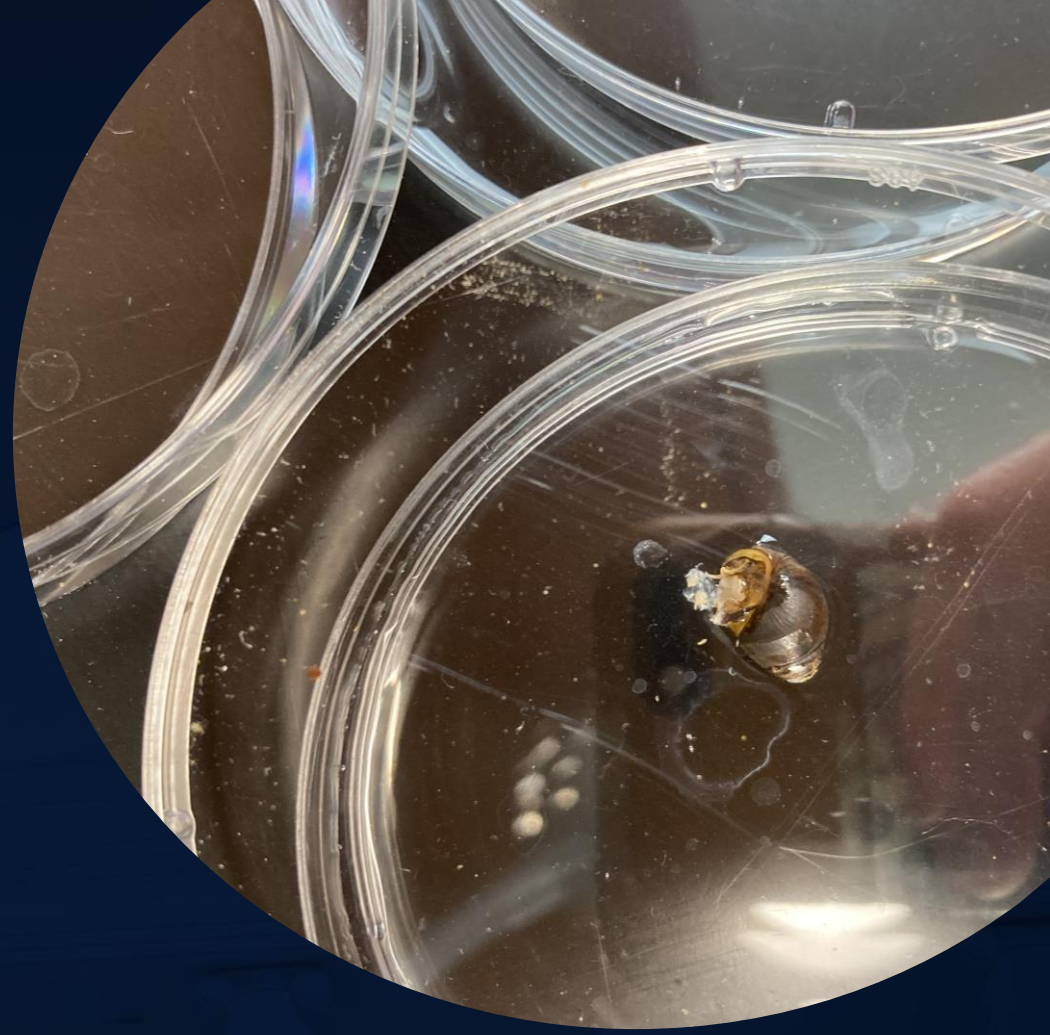




Opossum Creek

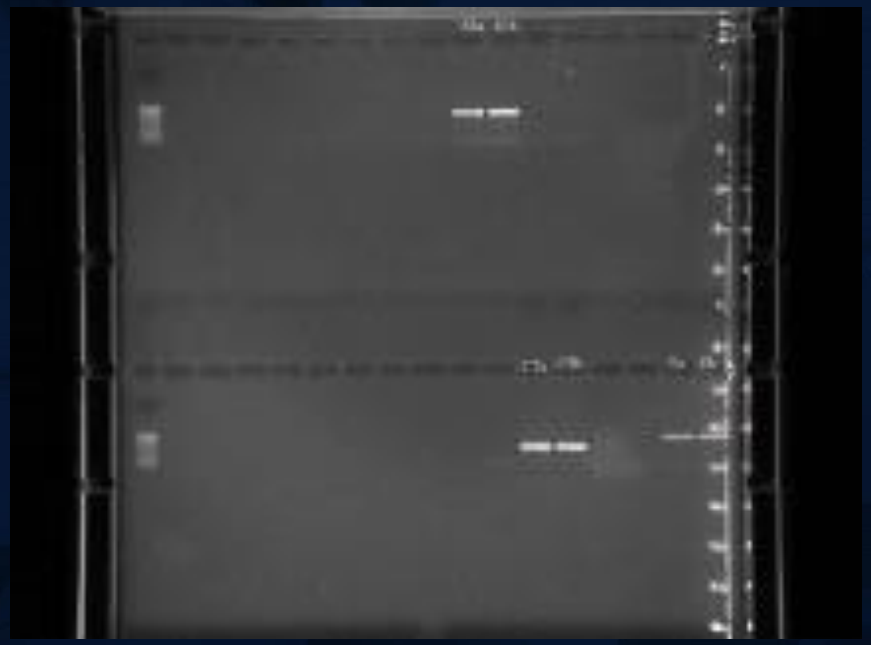
Snail Cercarial Release

- N=304 total across both years
- Initial snails were sacrificed and dissected, examined gonads
- Large number kept alive for cercarial release in Fall 2020
  - Light and temp technique
  - Petri, under heat lamps, monitored temperature
  - Check hourly

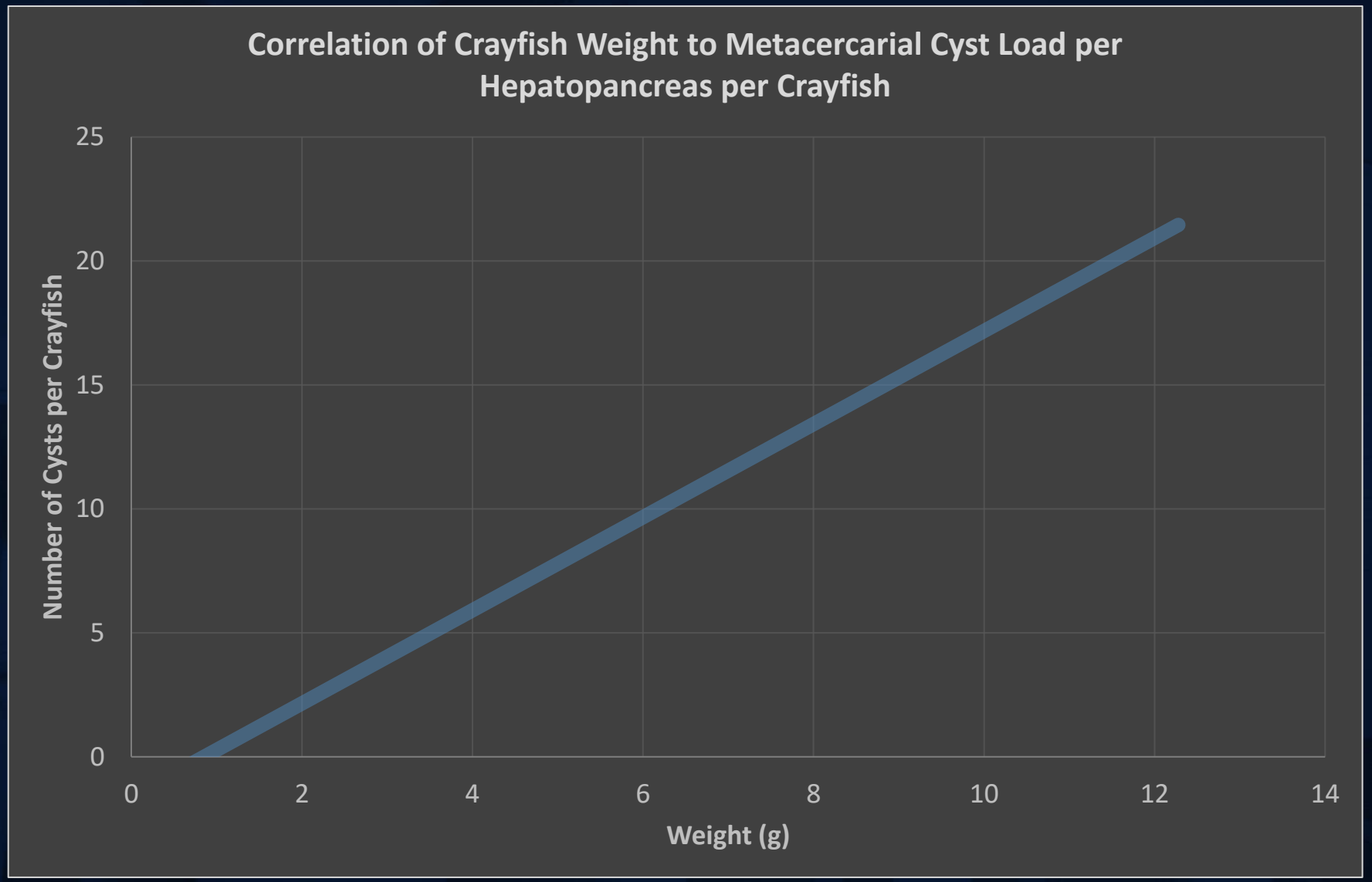


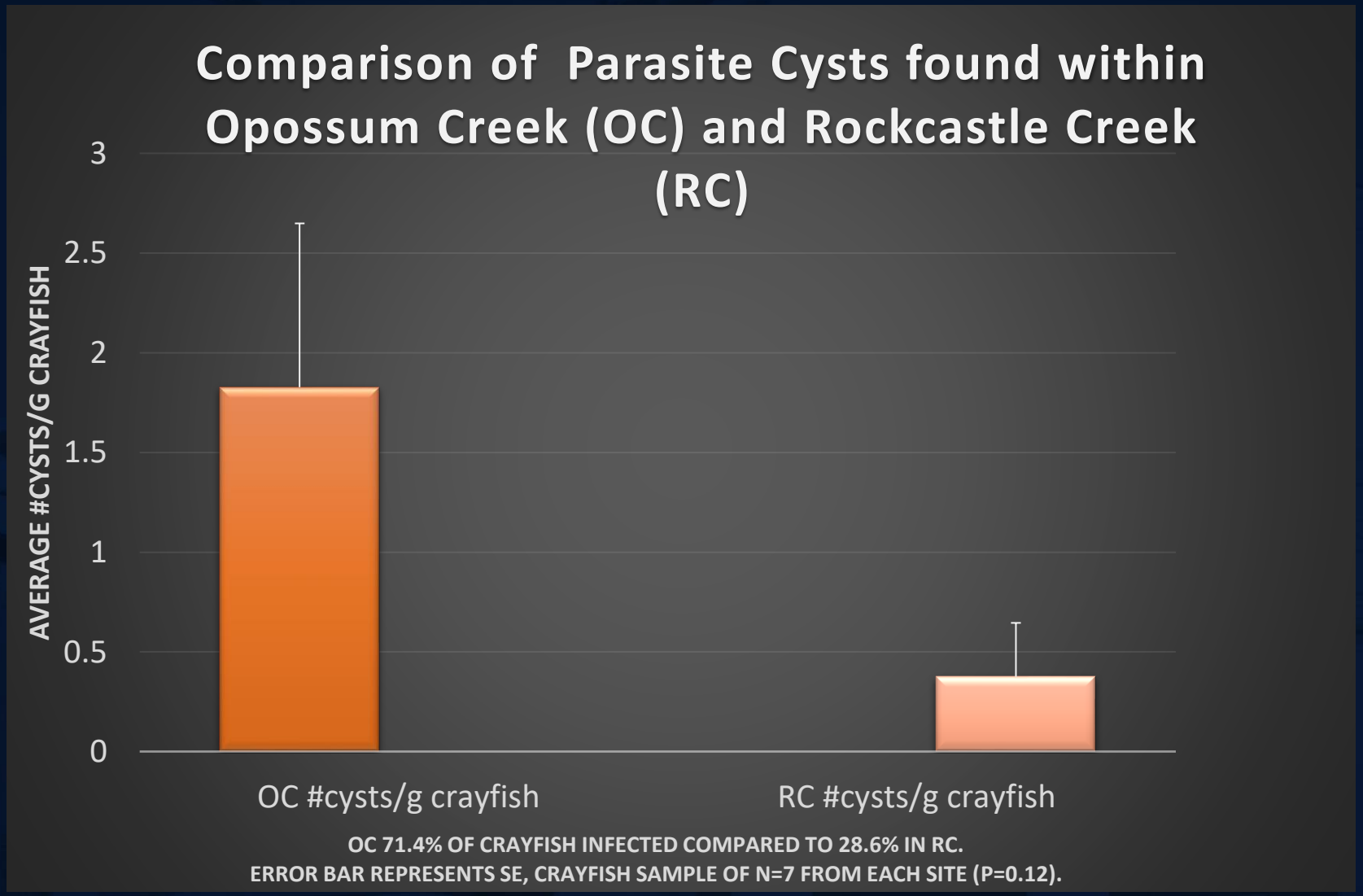
Molecular ID

- DNA extraction & purification (Dneasy Blood and Tissue Kit)
- Amplified via PCR
- Cleaning PCR products, prepped and sent out for sequencing



Results


Correlation of Crayfish Weight to Metacercarial Cyst Load per Hepatopancreas per Crayfish
Number of Cysts per Crayfish
25
20
15
10
5
0
0
2
4
6
8
10
12
14
Weight (g)


Comparison of Parasite Cysts found within Opossum Creek (OC) and Rockcastle Creek (RC)
AVERAGE #CYSTS/G CRAYFISH
3
2.5
2
1.5
1
0.5
0
OC #cysts/g crayfish
RC #cysts/g crayfish
OC 71.4% OF CRAYFISH INFECTED COMPARED TO 28.6% IN RC.
ERROR BAR REPRESENTS SE, CRAYFISH SAMPLE OF N=7 FROM EACH SITE (P=0.12).

Cercarial Release

Found no cercarial release or signs of trematode in 2020

Moved us to molecular work with the cysts

Molecular ID

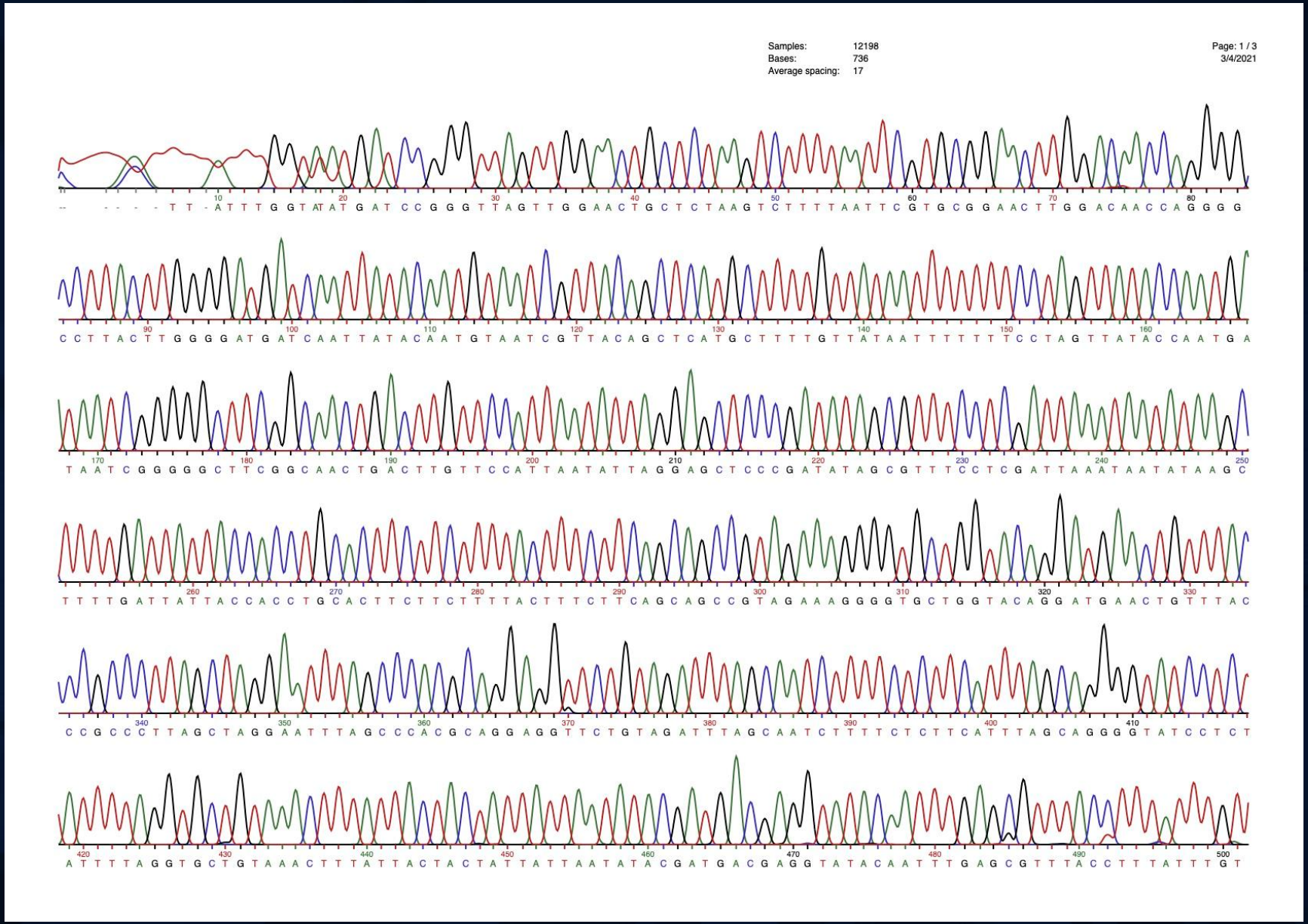
Crayfish: *Cambarus robustus**

Snail: *Leptoxis carinata* (Figured to the right)

Trematode: sequencing came back multiple overlapping peaks resulting in inconclusive ID

Further ID using different primers will/should take place in the future for accuracy and reinforcement of species found

*most probable



From Europhins Genomics sequencing data

Discussion

- Crayfish hepatopancreas cysts
  - Larger the crayfish the longer lived– size & molting (Thorp *et al.,* 2001)
  - Suggests longer exposure to possible trematodes

- Cercarial release not found
  - Density dependent or small infection rate population?
    - Large sample size required in previous studies (Mereta *et al*., 2019; Ciparis *et al.,* 2013)
    - Yields of <5%-10% with samples sizes in the thousands to tens of thousands
  - Potential seasonal or temperature dependency

Discussion

- Molecular ID
  - Pinpoint host specificity & population distributions
  - Trematode: Refinement in methods and extraction quality
  - Value of molecular ID when morphological variability

Conclusion

Q1: What is the prevalence of trematode infection in crayfish?

A: More trematode cysts were found in older crayfish and in crayfish from rural stream.

Q2: What is the prevalence if trematode infection in related snail populations?

A: Inconclusive data found from fall 2020 sample

Q3: What species of snail, crayfish, and trematode will be identifiable through molecular ID?

A: Definite *Cambarus* genus of crayfish, *Leptoxis carinata* snail species, trematode DNA found but specific species not identified in preliminary trial run

References & Acknowledgments

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**All pictures are from the authors unless otherwise noted or referenced

Any Questions?

Additional Talking Points

- Cercarial shedding—little white flecks swimming in water
- Urban found to have more pollution
- Rural more pristine and higher likelihood of more forest species interactions
- 12s, 16s, cytochrome c oxidase, 18s rRNA primers