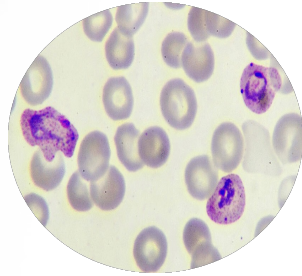


ASSURE 2023:
Gambusia vs. Blackspot
Disease Study
By: Garrett Bohrstedt



Harris, K. (2023). *Gambusia holbrooki* [Photograph]. Unpublished.

Background

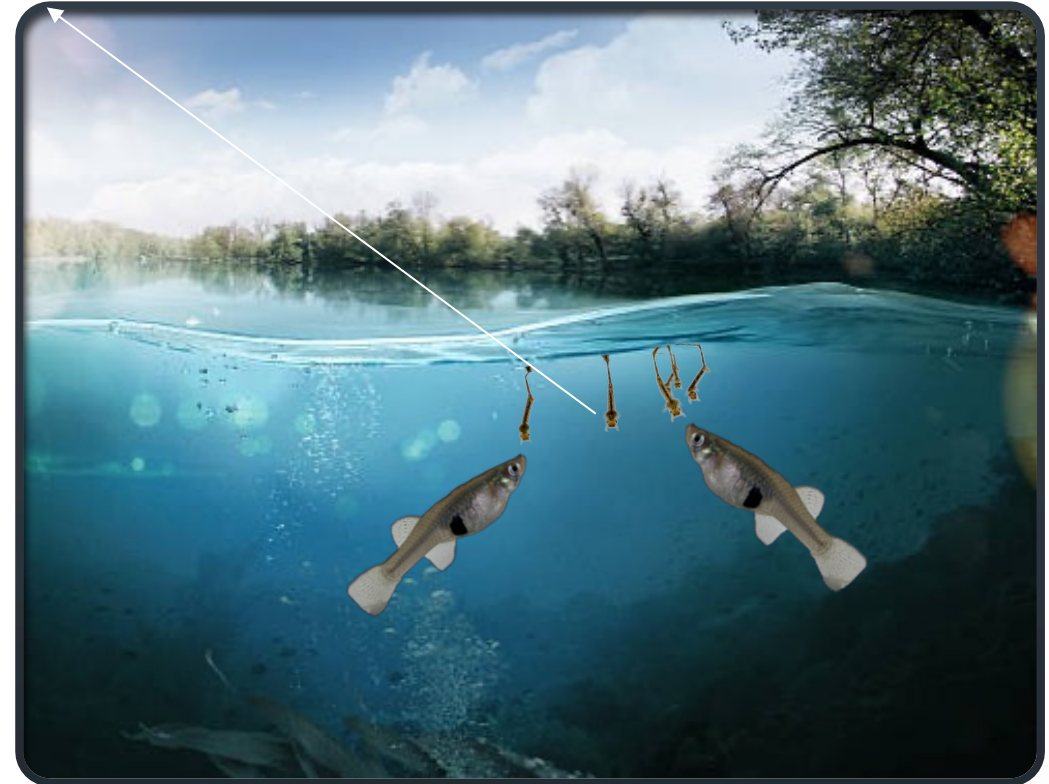


Science News. (2020).
Malaria parasites.
<https://www.sciencenews.org/article/malaria-parasites-may-have-their-own-circadian-rhythms>

- *Gambusia* are also known as mosquito fish
- Important bio control; eat mosquito larvae; helps eliminate vectors for diseases malaria.



Vecteezy. (2017). *Mosquito*.
<https://www.vecteezy.com/png/24077679-bloodsucker-mosquito-isolated-on-transparent-background-png-file>



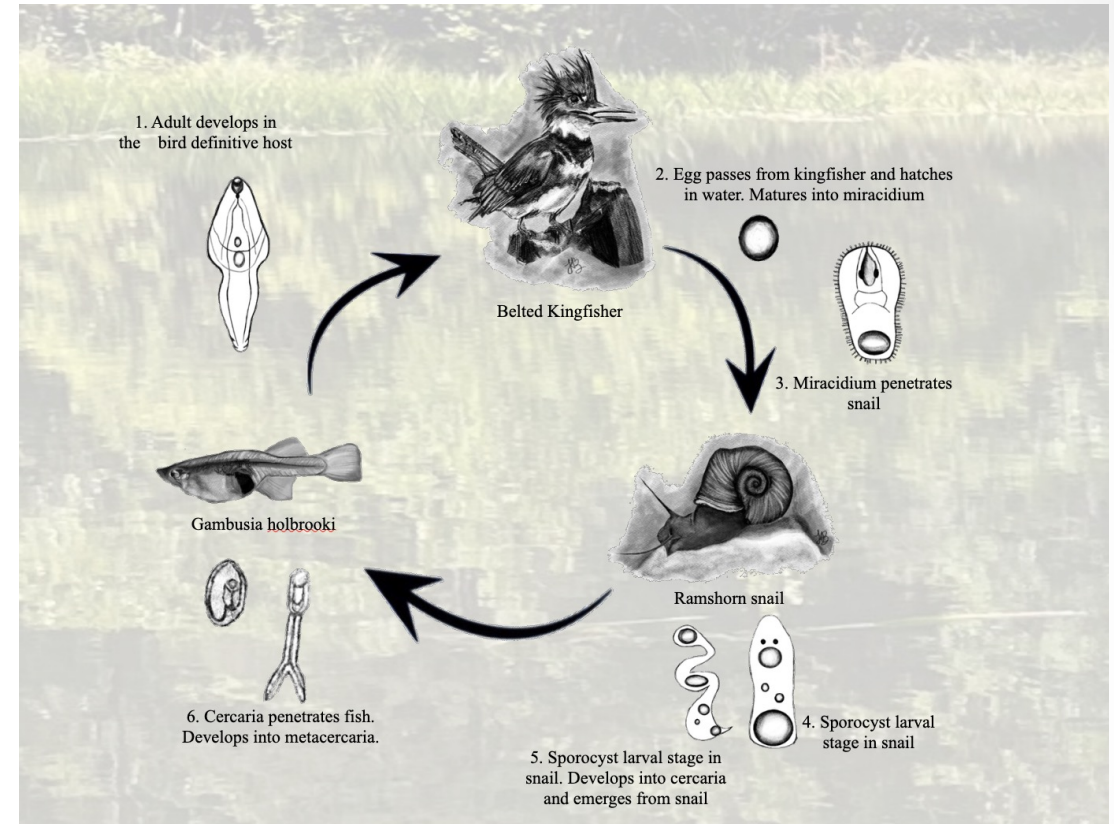
Huff, G. (2023). *Gambusia holbrooki* [Image].
Created using Biorender.

Background

- Blackspot disease is visualized when the *Uvulifer ambloplitis* cercariae (released by ramshorn) bore into the muscle of fish and forming cysts
- Melanin forms around these cysts and gives it this “blackspot” look

Effects:

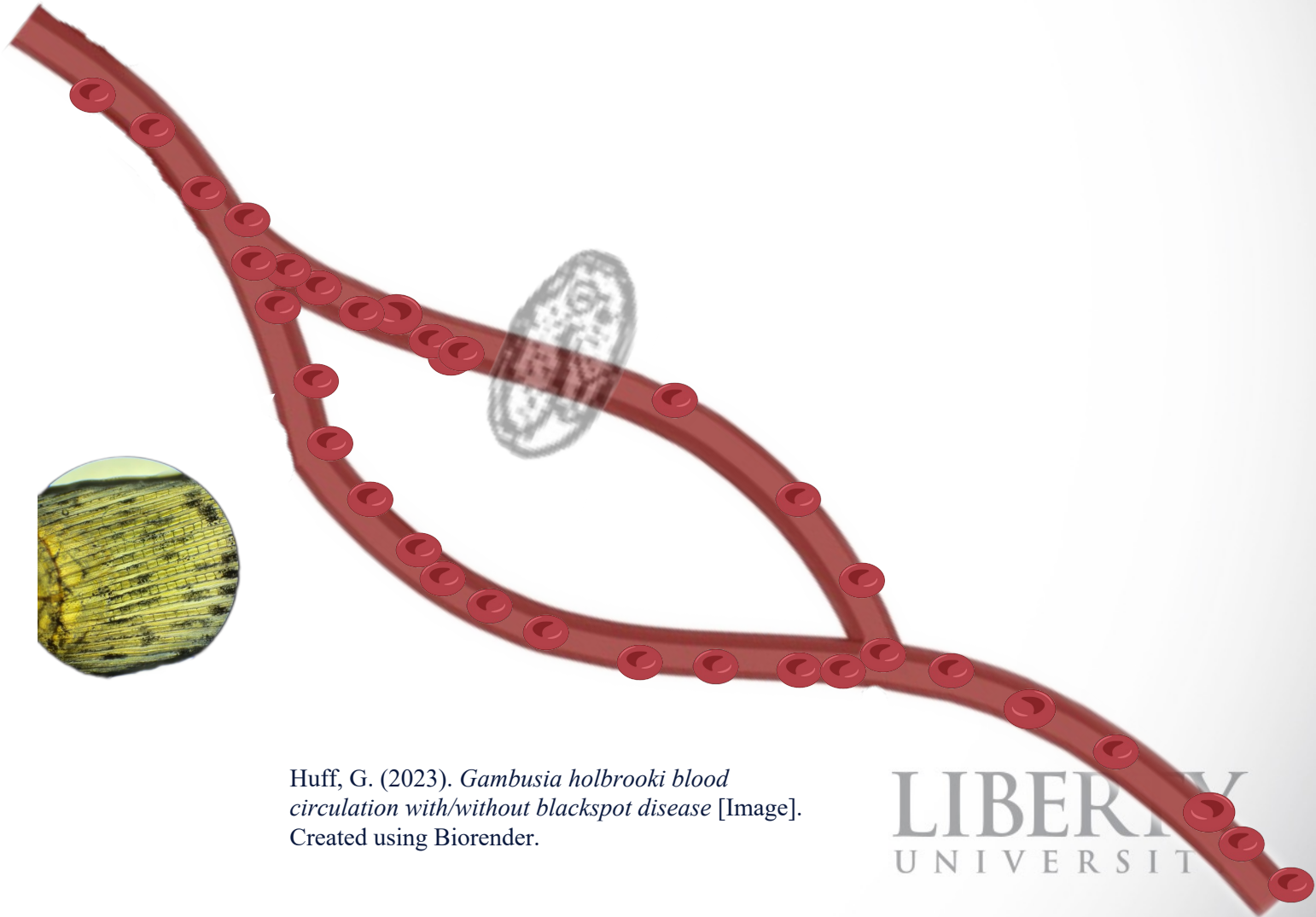
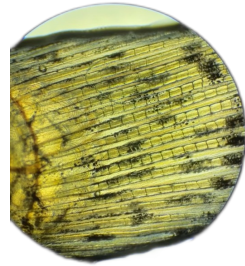
- shoal less (group together)
- Decrease in mating (female prefers male with no blackspot)



Bohrnstedt, H. (2023) *Life cycle of Uvulifer Abloplitus* [image]. Unpublished.

Hypothesis

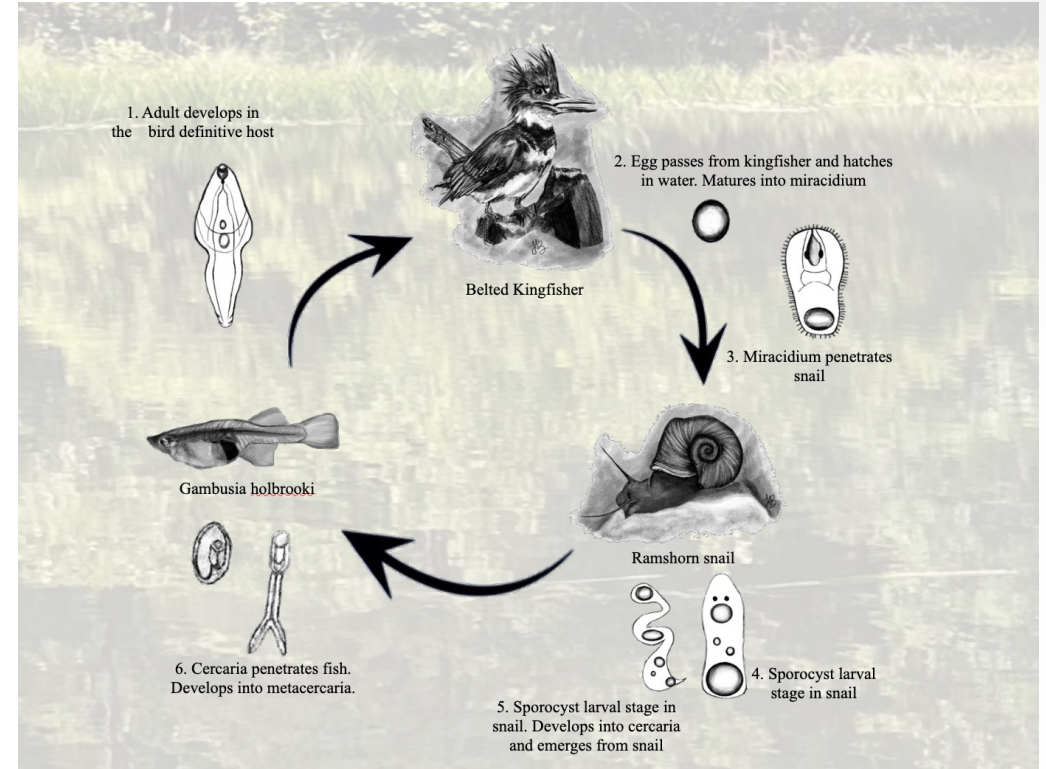
- The presence of Blackspot cysts will result in a substantial decrease in red blood cells navigating through each capillary.



Huff, G. (2023). *Gambusia holbrooki* blood circulation with/without blackspot disease [Image]. Created using Biorender.

If the Hypothesis is True...

- lower blood circulation, worse body condition
- Increase in predation (Bluegill)
Less effective biocontrol



Bohrnstedt, H. (2023) *Life cycle of Uvulifer Ambloplitis* [image]. Unpublished.



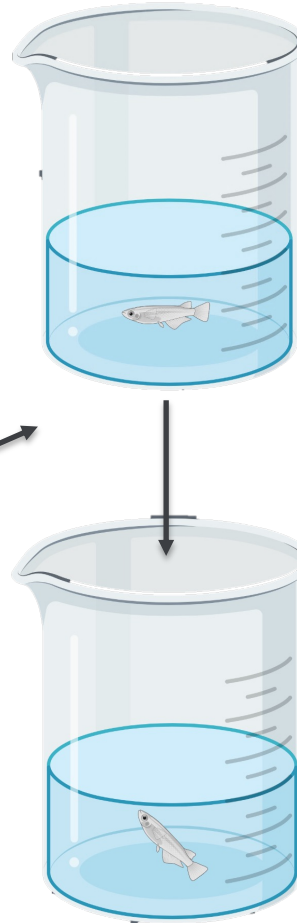
Huff, G. (2023). *Gambusia holbrooki* and bluegill [Image]. Created using Biorender.


Methods

- 46.99 mg/mL for males
- 27.348 mg/mL for females



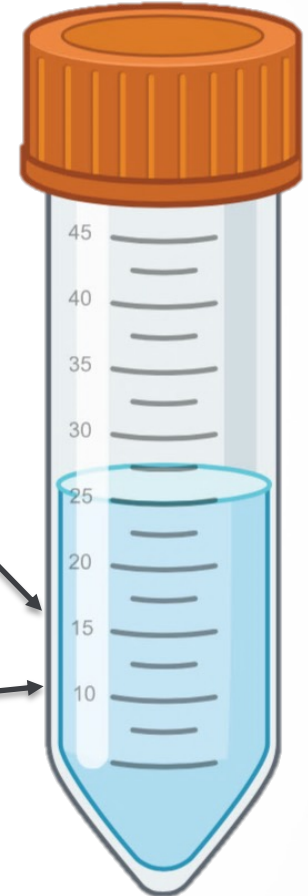
200 mL
Tank water




Tricaine (MS222)

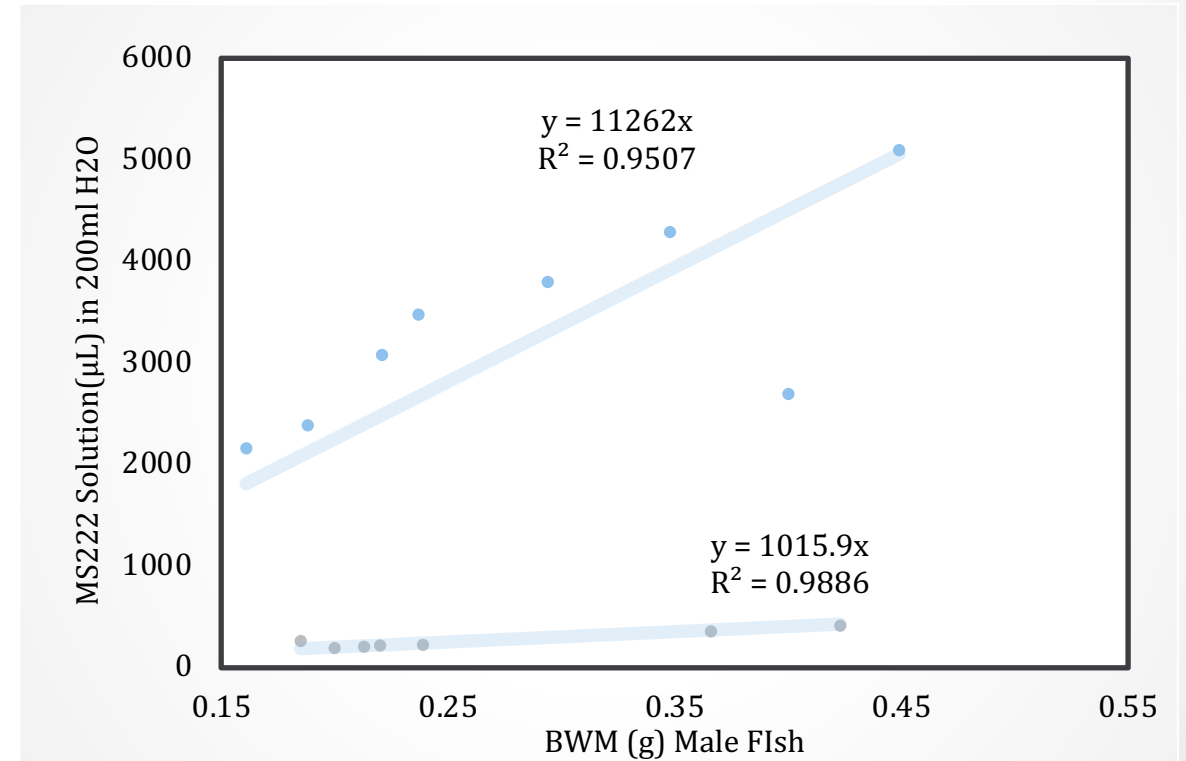
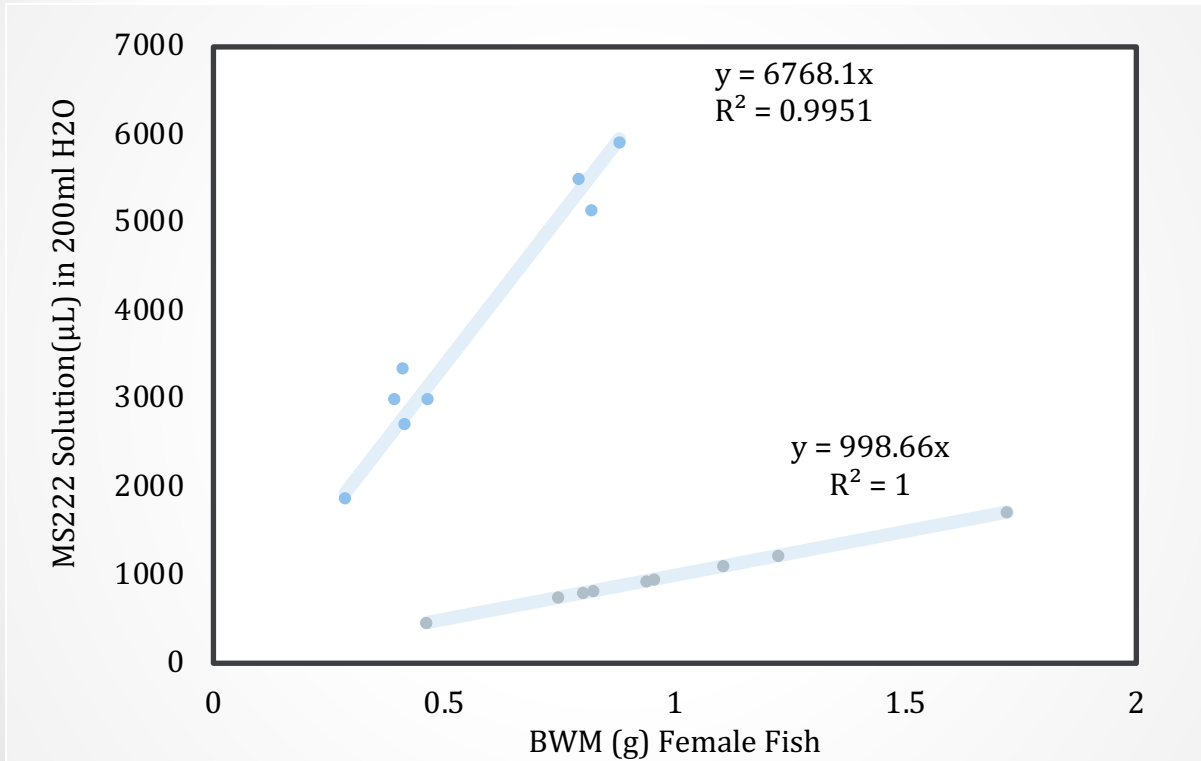


RO water



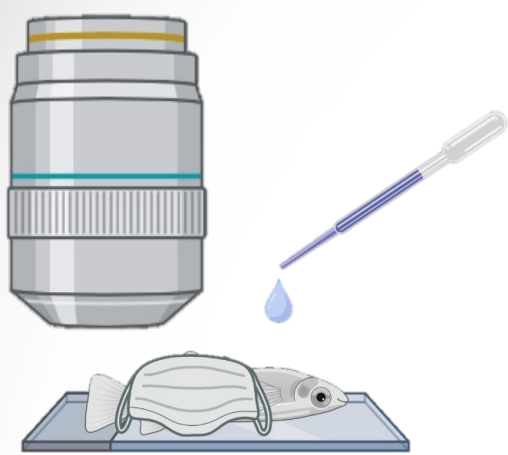
Huff, G. (2023). *Gambusia holbrooki* drug concentrations [Image]. Created using Biorender.

Tricaine (MS222) Solution (μL) in 200 mL H₂O vs. BWM (g) for both Female and Male fish (Gambusia)



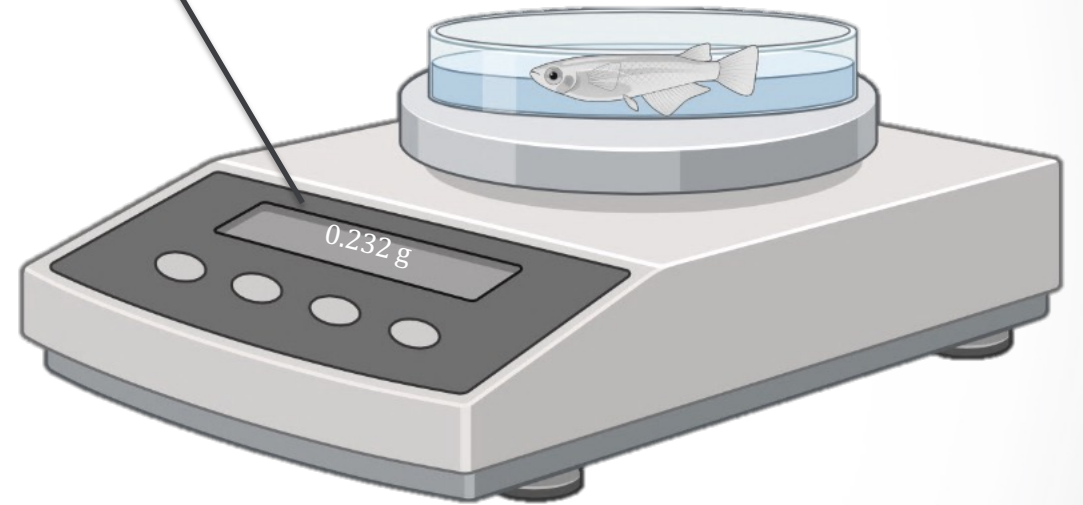
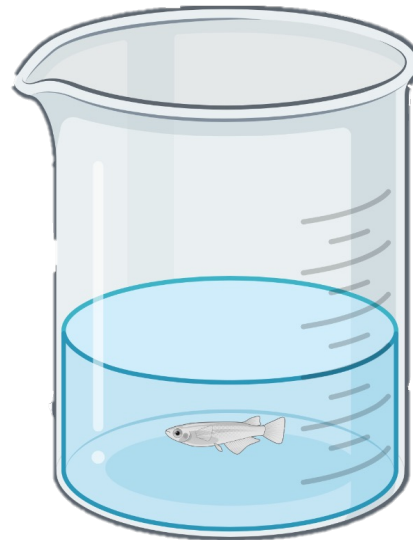
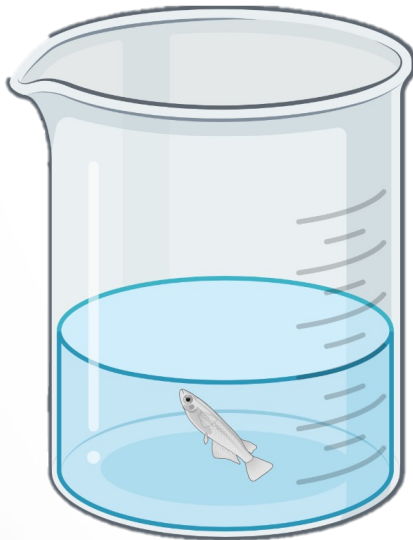
Piddock, A. (2023). *Gambusia holbrooki* drug concentration trial and error graphs [Image]. Created using Excel.

Piddock, A. (2023). *Gambusia holbrooki* drug concentration trial and error graphs [Image]. Created using Excel.



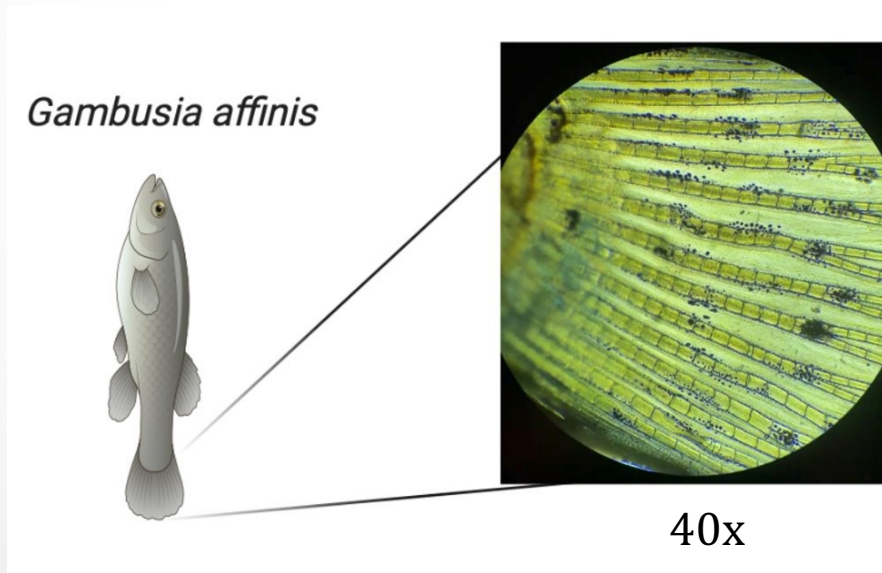
Method:

30s - 60s



Huff, G. (2023). *Gambusia holbrooki* sedation method [Image]. Created using Excel.

1. 40x for 4 seconds (heavy/light pigment)
2. 100x for 15 seconds (filmed)
3. RBCs counted in capillaries (15s -> 60s)



Huff, G. (2023). *Gambusia holbrooki* sedation method [Image]. Created using Excel.

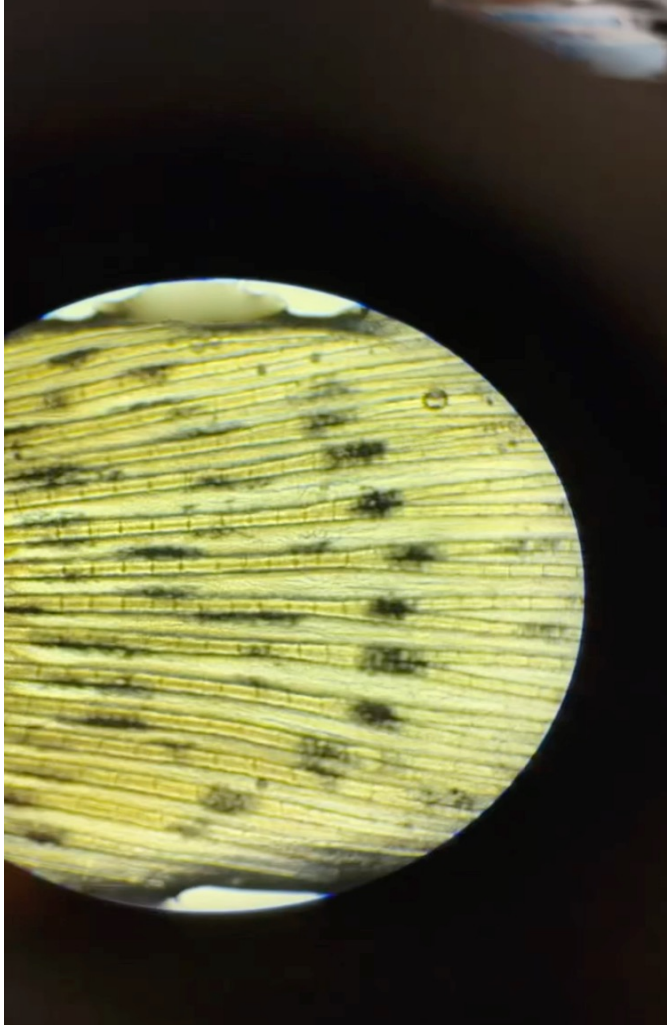


Huff, G. (2023). *Gambusia holbrooki* sedation method [Image]. Created using Excel.

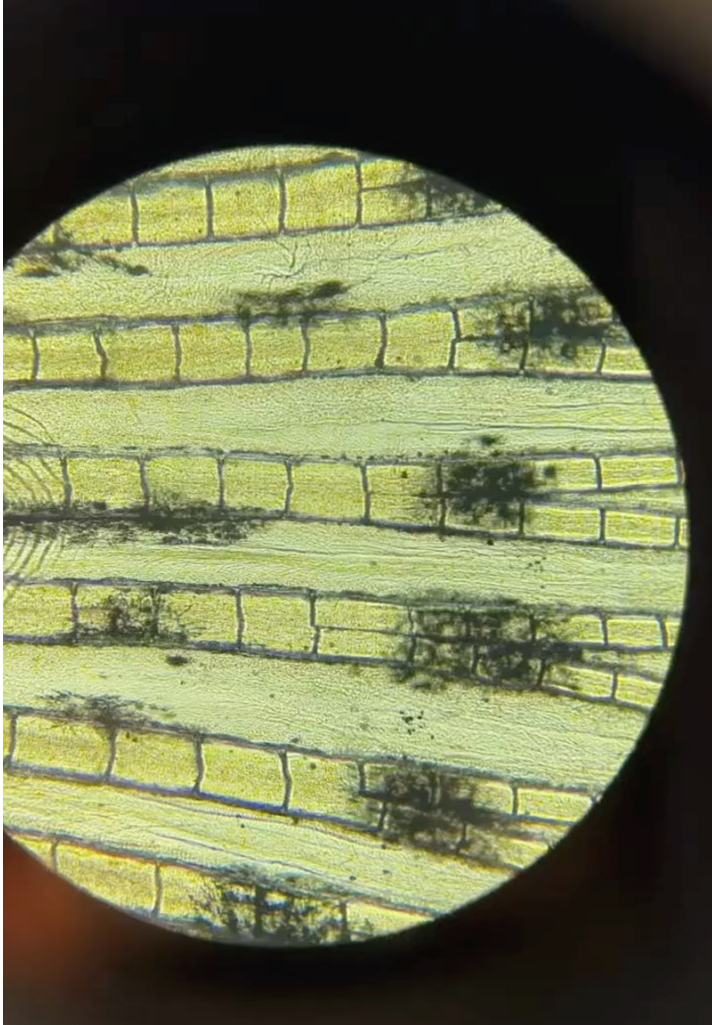


Piddock, A. (2023). *Gambusia holbrooki* sedation method [photo]. Unpublished.

40x and 100x
video examples

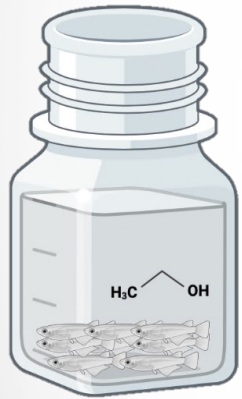
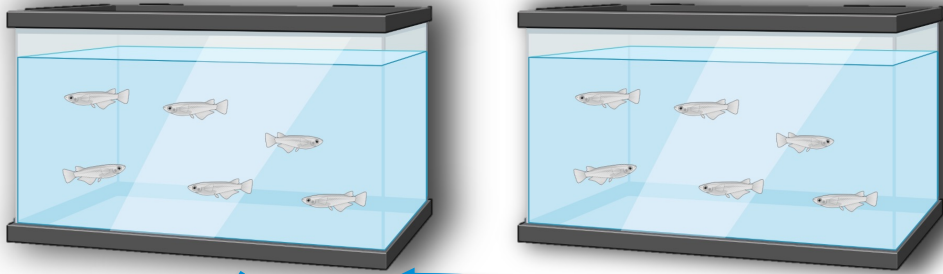


Bohrnstedt, R. (2023). *Gambusia holbrooki* blood circulation [Image]. Unpublished.



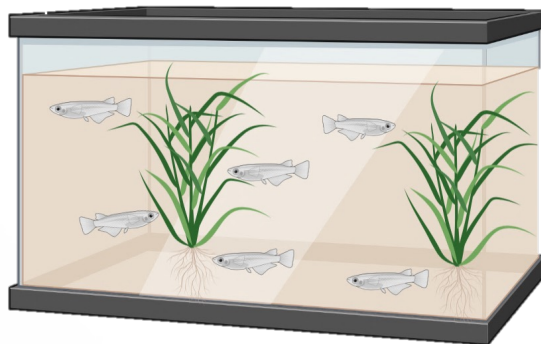
Bohrnstedt, R. (2023). *Gambusia holbrooki* blood circulation [Image]. Unpublished.

Recovery tanks



70% ethanol solution

Kingfisher tank



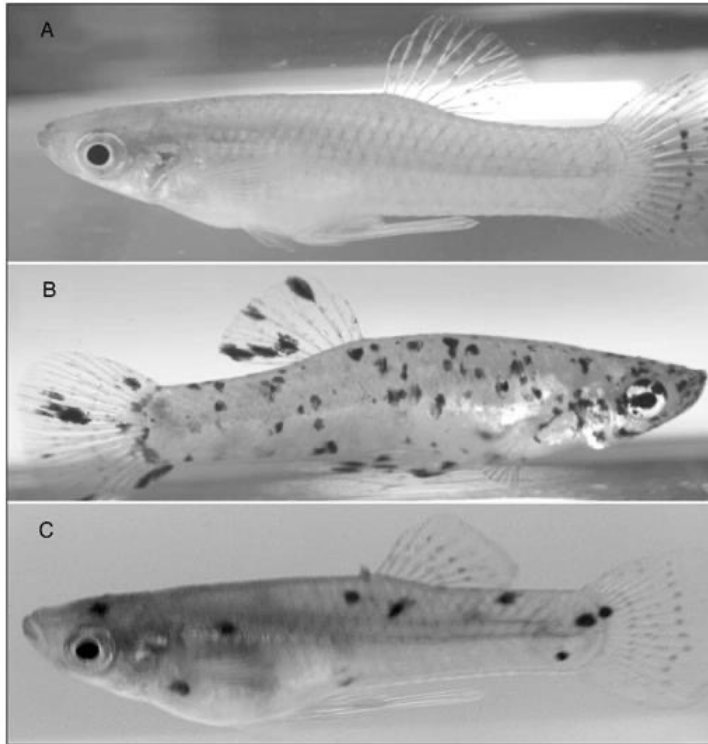
Huff, G. (2023). *Gambusia holbrooki* recovery tanks [Image]. Created using Excel.



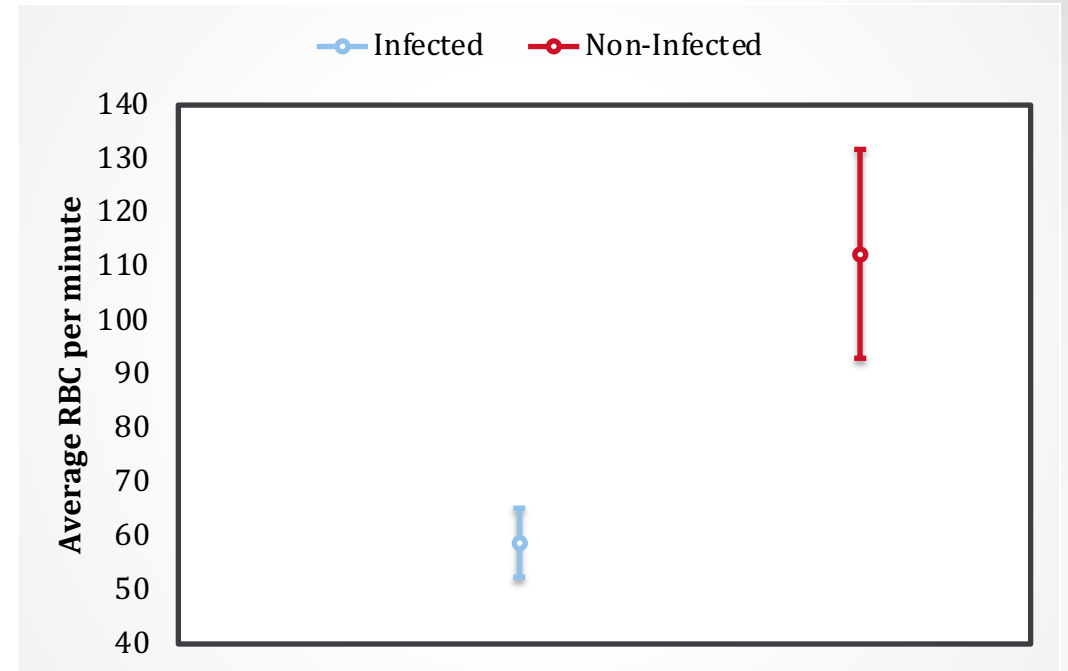
Limitations and Future Research

- Gambusia from kingfisher pond only showed pigment and no 100% confidence of actual blackspot

Blackspot



ResearchGate. (2013). *Blackspot disease*. https://www.researchgate.net/publication/236333490_Heritable_Melanism_and_Parasitic_Infection_Both_Result_in_Black-Spotted_Mosquitofish



Piddock, A. (2023). *Gambusia holbrooki healthy versus infected graph* [Image]. Created using Excel.

Non-blackspot
(pigmented)

Vs

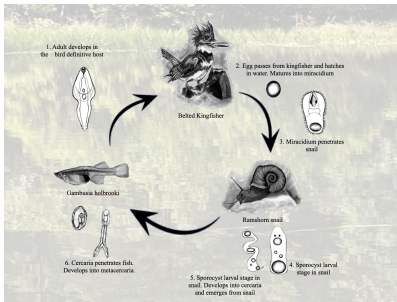


Harris, K. (2023). *Gambusia holbrooki* [Photograph]. Unpublished.

Limitations and Future Research



Harris, K. (2023). *Ramshorn snail* [Photograph]. Unpublished.



Bohnstedt, H. (2023) *Life cycle of Uvulifer Abloplitis* [image]. Unpublished.



Harris, K. (2023). *Ramshorn snail* [Photograph]. Unpublished.

- Future research will be adding a multitude of snails (ramshorn) to the kingfisher tank for the release of cercariae
- 21-day cycle of blackspot will occur, giving more confidence of it and hopefully visuals
- Data set will be compared to this data set of non-blackspot fish or new set later

Resources

Au, R.J., & Berra, T. (1978). Incidence of black spot disease in fishes in Cedar Fork Creek, Ohio. *Ohio Journal of Science*, 78(6), 318-322

Beier, J. C. (1998). Malaria parasite development in mosquitoes. *Annual Review of Entomology*, 43(1), 519–543.

Cabral, J. A., & Marques, J. C. (1999). Life history, population dynamics and production of eastern mosquitofish, *Gambusia holbrooki* (Pisces, Poeciliidae), in rice fields of the lower Mondego River Valley, Western Portugal. *Acta Oecologica*, 20(6), 607–620.

Chappell, L.H., & Secombes, C.J. (1996). Fish immune responses to experimental and natural infection with helminth parasites. *Annual Review of Fish Diseases*, 6, 167-177.

Deaton, R., Martin, R. E., Stoops, S. B., Cureton II, J. C., & Lewis, R. L. (2011). Effects of a trematode infestation on body condition, reproduction and mating behaviors in a livebearing fish. *Behaviour*, 148(8), 967–984.

Hoffman, G.L. (1955). *Neascus nolfi* n. sp. (Trematoda: Strigeida) from the cyprinid minnows with notes on the artificial digest recovery of helminths. *American Midland Naturalist*, 53(1), 198-204.

Horth, L., Gauthier, D., Vogelbein, W. (2013). Heritable melanism and parasitic infection both result in black-spotted mosquitofish. *Southeastern Naturalist*, 12(1), 209-216.

Hunter, G. W., & Hunter, W. S. (1938). Studies on host reactions to larval parasites the effect on weight. *The Journal of Parasitology*, 24(6), 477.

Husen, M.A., Sharma, S. (2014). Efficacy of anesthetics for reducing stress in fish during aquaculture practices- a review. *Kathmandu University: Journal of Science, Engineering and Technology*, 10(1), 104-123.

Kirse, S. C. (2010). *Parasite ecology of fish with black spot disease*. Senior Honors Theses. 184. Liberty University Digital Commons

Lemly, A. D., & Esch, G. W. (1984). Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: Ecological implications. *The Journal of Parasitology*, 70(4), 475.

Tobler, M., & Schlupp, I. (2006). Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei). *Environmental Biology of Fishes*, 81(1), 29–34.

Additional Figure Development by the authors in support of Biorender and drawings of black spot life cycle and ramshorn snails by Hazel Bohrnstedt