

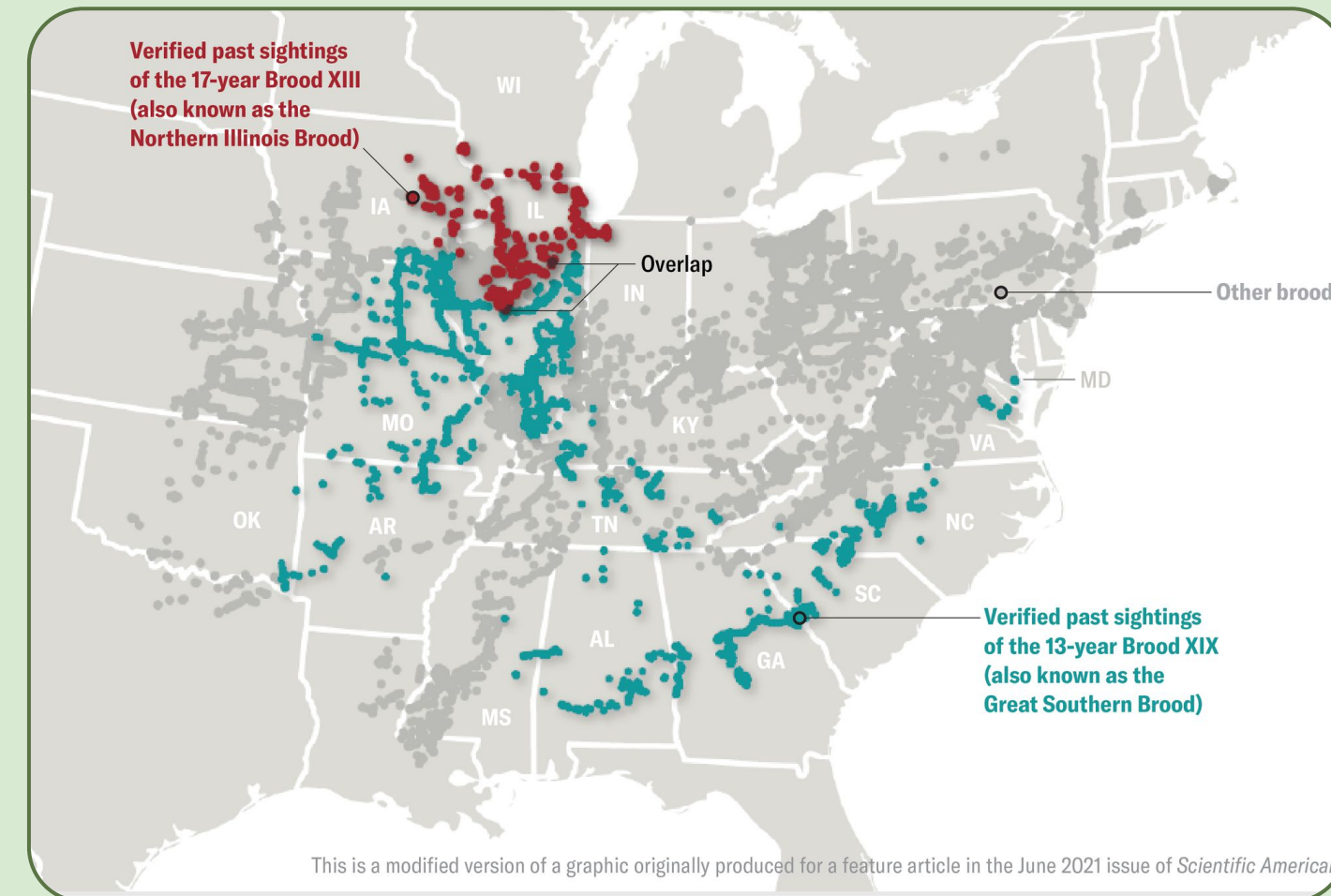
The effect of preservation methods on the viability of microbial symbionts within a periodical cicada (*Magicicada* sp.)

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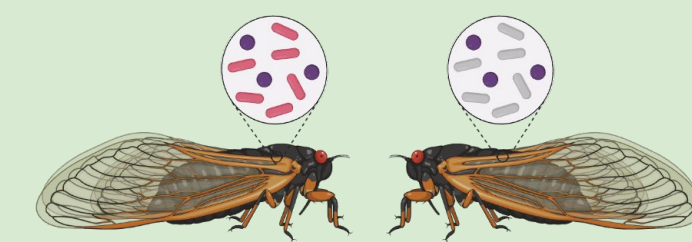
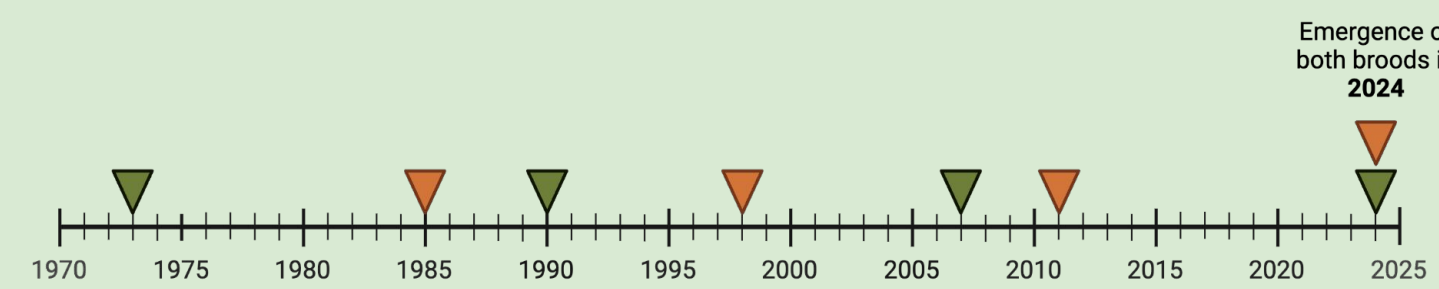
Introduction



The North American periodical cicadas are in the *Magicicada* genus, these broods emerge every 13 and 17 years. They are the **hosts to a variety of microbial symbionts**. (Brumfield et al., 2022)

Due to long periods before emergence, **there is not much data about their microbiome** and at what capacity each microorganism is present.

We are finding baseline results of the cicada microbiome and the effects of different preservation methods on microbial viability so that preserved cicadas may serve as a reference point for continuing studies.



Central question

- The XII and XIX broods that have emerged this year will take another 17 and 13 to emerge again, so what is the most effective cicada preservation method so that microbiome studies may continue after the emergence is over?

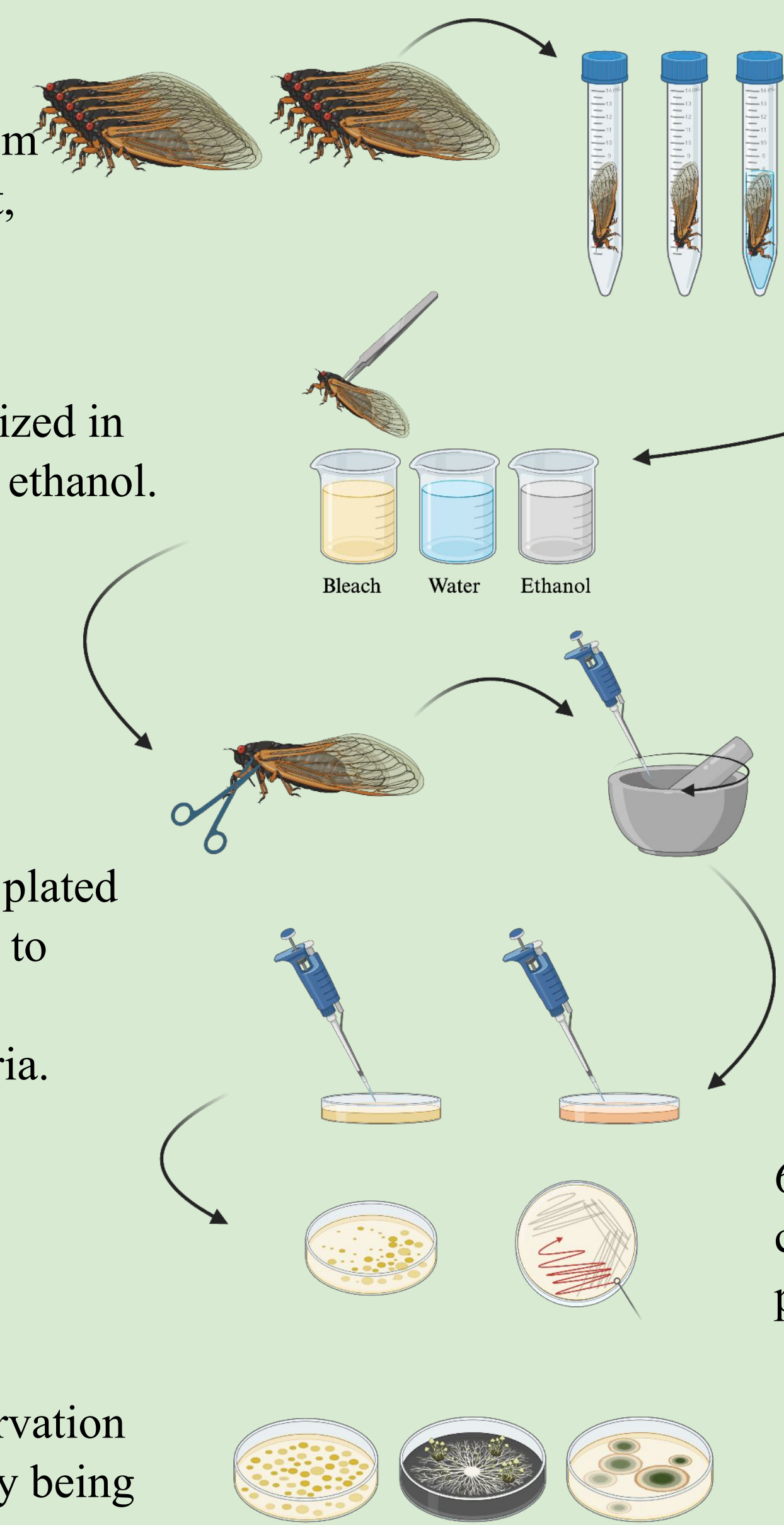
Methodology

1) Thirty *Magicicada* -cassini individuals were collected from Lake of the Woods (Mohamet, IL): 15 males and 15 females.

3) Cicadas were surface sterilized in bleach, sterile water and 70% ethanol.

5) The cicada solutions were plated on MEA plates (+antibiotics) to culture fungi and R2A plates (+fungicide) to culture bacteria.

7) Morphotype data was then analyzed between the 3 preservation methods. Isolates are currently being sequenced for taxonomic assignment.



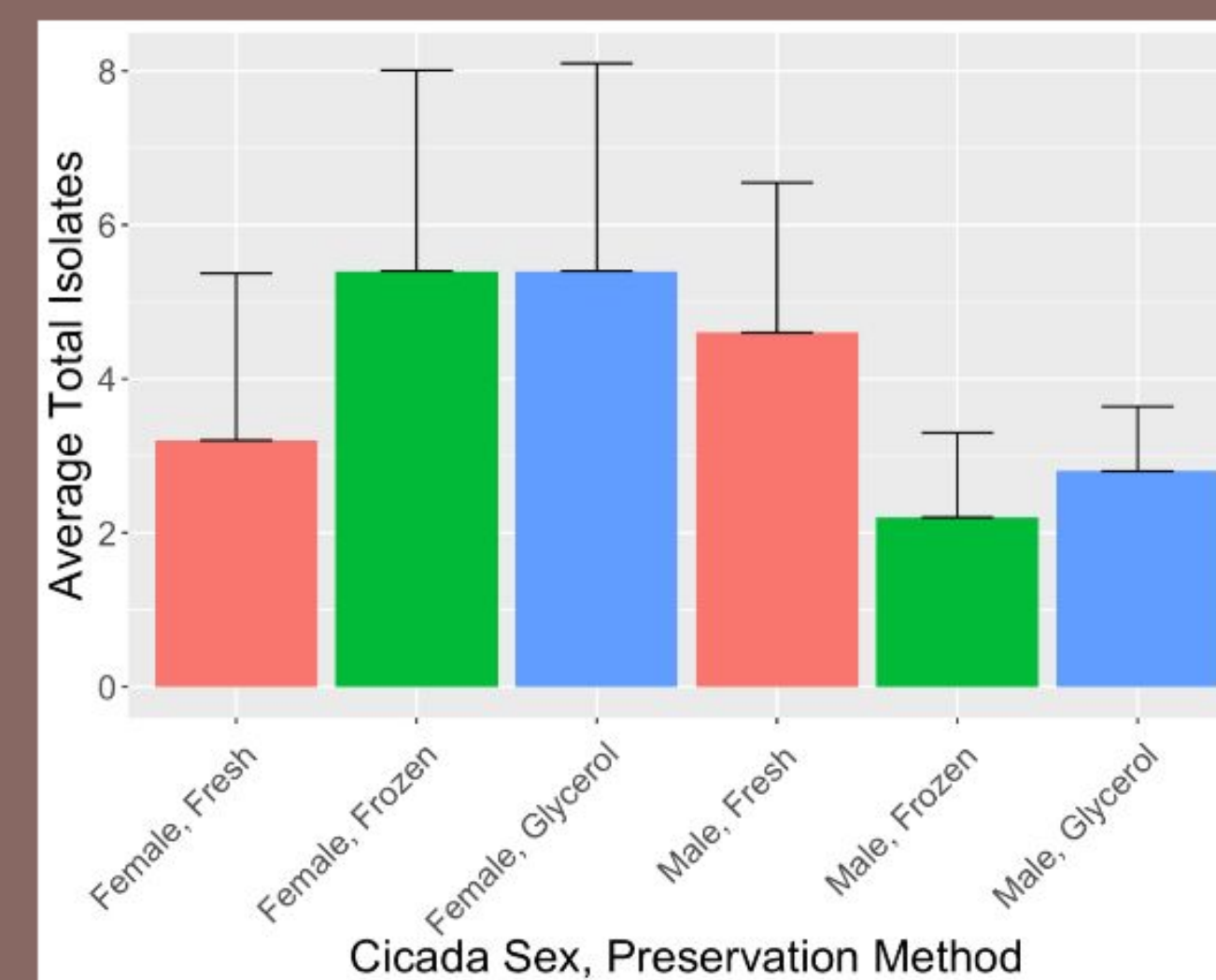
2) Five cicadas of each sex were chosen for 3 preservation methods: fresh cicada (not frozen or preserved), frozen at -20C, and frozen at -20C in a 30% glycerol solution.

4) The wings were removed and the whole bodies were ground in a mortar and pestle.

6) Emerging fungal and bacterial colonies were then subcultured into pure isolates.

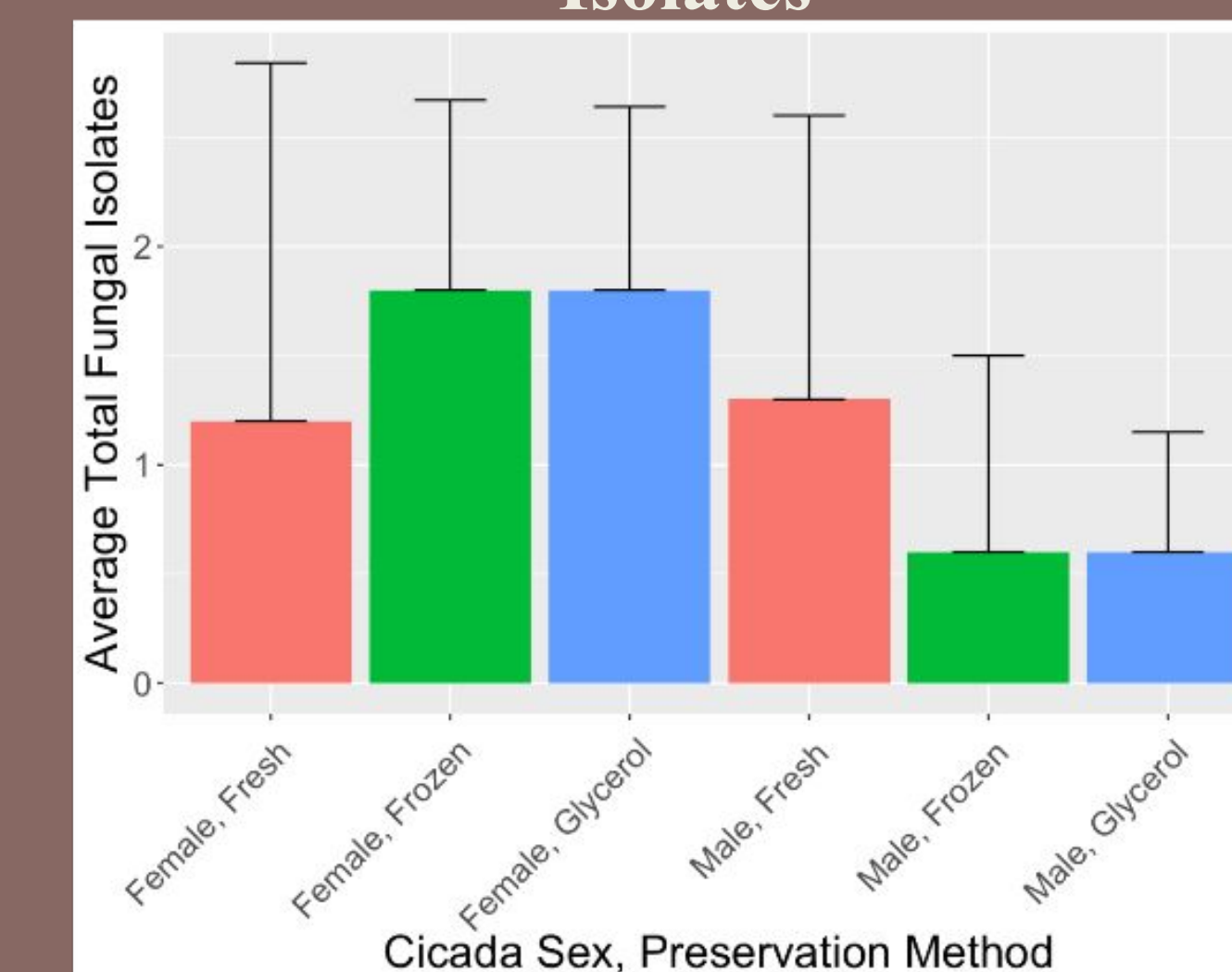
Results

Average Total Isolates



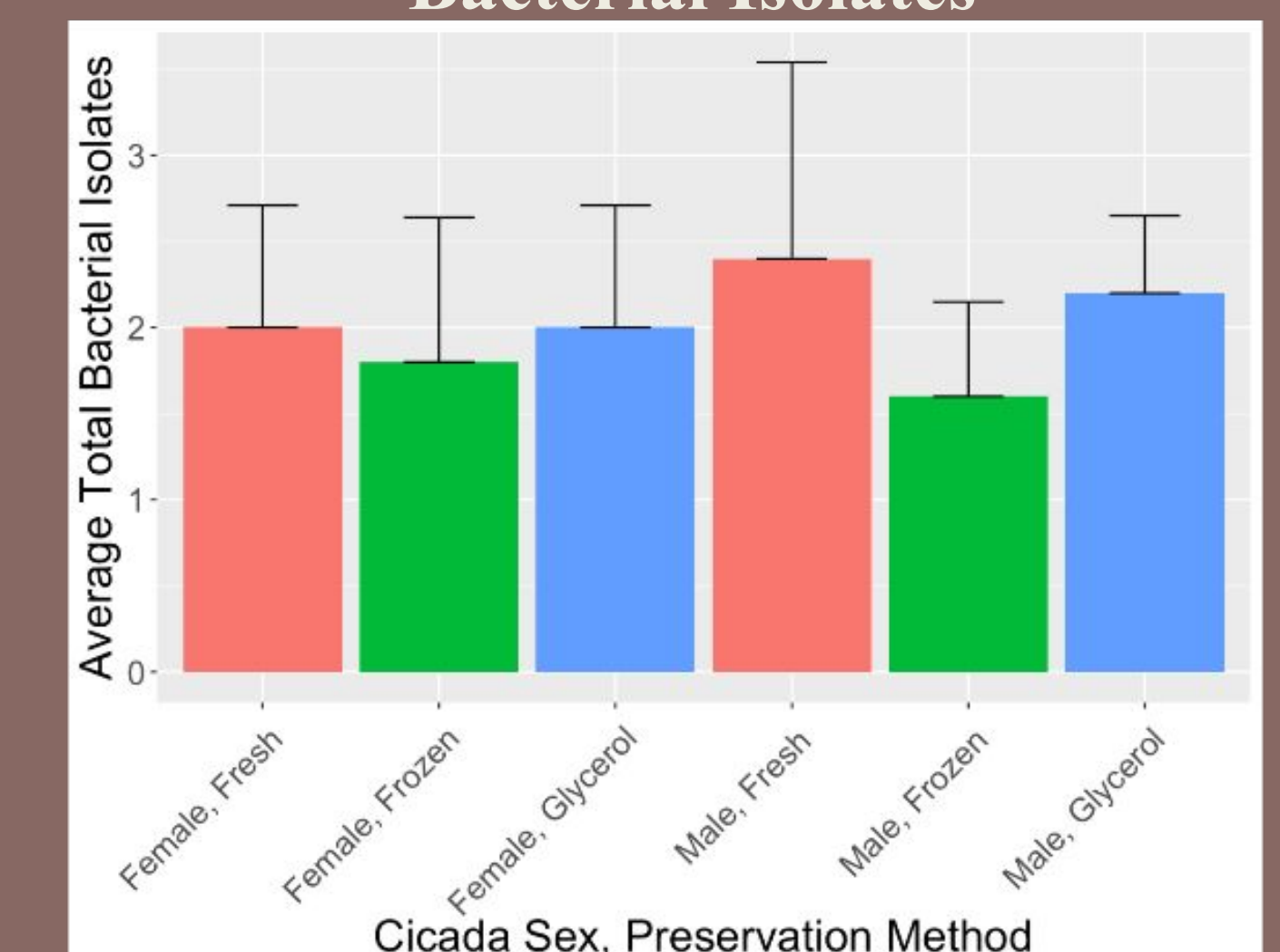
- ANOVA found significant effect of the interaction between sex and preservation method on total isolates recovered (p-value = 0.04)
- Dunnett's test showed that for males only, the Frozen method significantly reduced the recovery of total isolates (p-value = 0.03)

Average Total Fungal Isolates



- ANOVA found significant effect of the interaction between sex and preservation method on total fungal isolates recovered (p-value = 0.046)
- Dunnett's test showed that for males only, the Frozen and Glycerol methods significantly reduced the recovery of total fungal isolates (p-value = 0.04 for both)

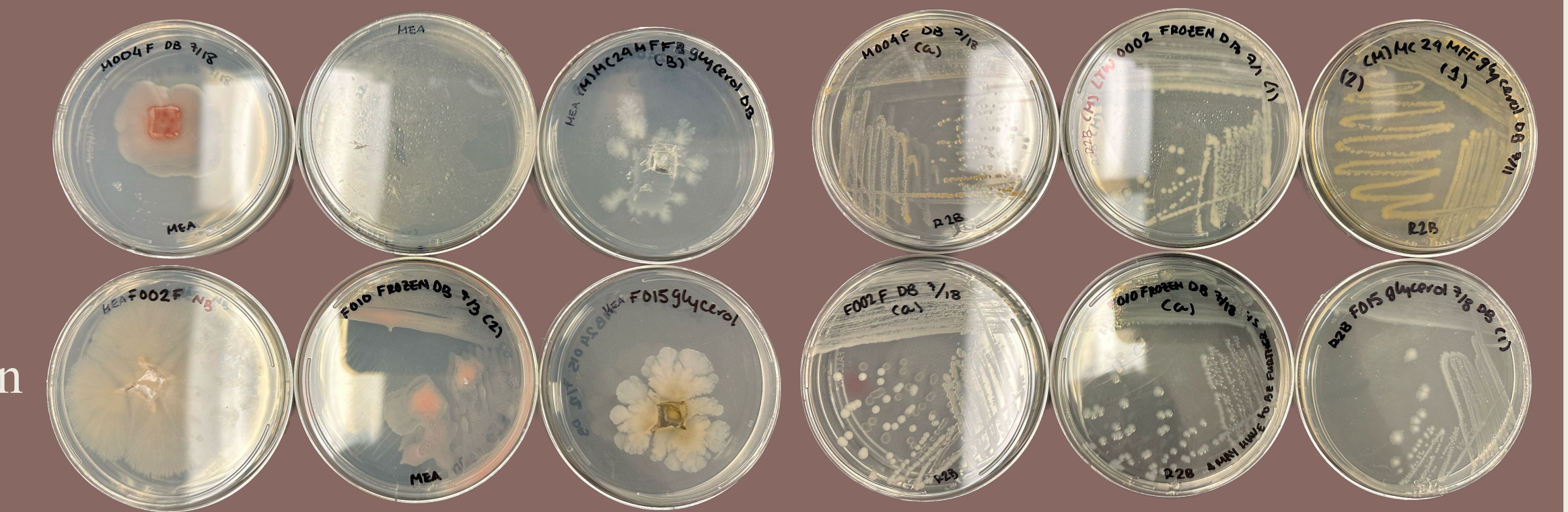
Average Total Bacterial Isolates



- ANOVA found no significant effect of sex, preservation method, nor the interaction between the two on total bacterial isolates recovered.

Conclusion

- We can conclude that, for **male cicadas only**, the freezing methods significantly **reduced the recovery of total isolates** as well as fungal isolates.
- On the other hand, **female cicadas remained unaffected** by any of the preservation methods.
- The difference in the effect that preservation methods have between male and female cicadas may be due the frozen and glycerol males being collected earlier in the season- hence they were likely younger and had a longer freezing time than the frozen females.
- Therefore, the age of the cicada and the length of the freezing time may be important factors that play a role in the recovery of microbes.



Acknowledgements



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References

- Brumfield, K. D., Raupp, M. J., Haji, D., Simon, C., Graf, J., Cooley, J. R., Janton, S. T., Meister, R. C., Huq, A., Colwell, R. R., & Hasan, N. A. (2022). Gut microbiome insights from 16S rRNA analysis of 17-year periodical cicadas (Hemiptera: Magicicada spp.) Broods II, VI, and X. *Scientific reports*, 12(1), 16967. <https://doi.org/10.1038/s41598-022-20527-7>

Image by Daniel P. Huffman and John Cooley (2024).