Cryopreservation of *Anopheles stephensi* and *A. gambiae* Eggs

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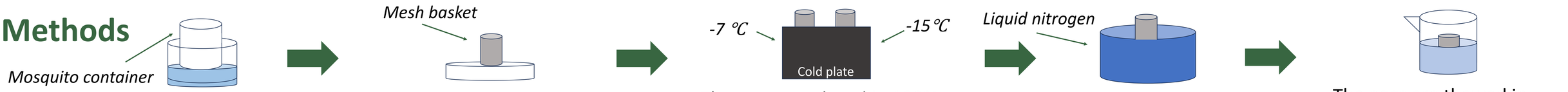
Project Goals

The goal of this project is to **replicate** a previous protocol and **cryopreserve** the eggs of *A. stephensi* and *A. gambiae*. The ability to cryopreserve mosquito eggs would aid in the **research of malaria** and other diseases by allowing researchers to **preserve the genotype** of the mosquitos [1][2].

Vitrification

Cryopreservation is a method of **cold storage** where samples are stored in **liquid nitrogen** and retain viability when thawed. Vitrification is a method of cryopreservation where samples are **rapidly frozen** and skip the ice-formation stage of freezing.

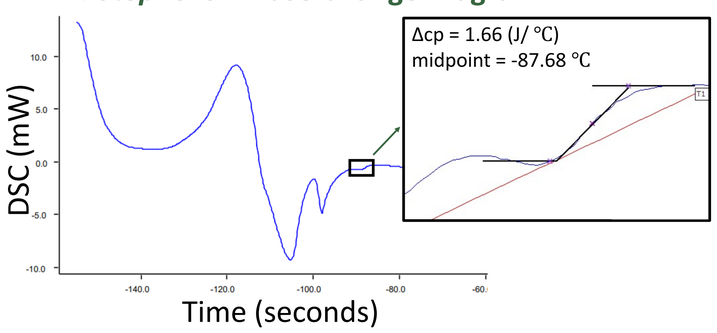
Methods



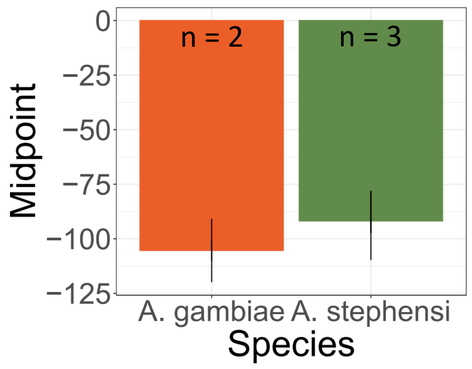
1. Mosquitos are allowed to oviposit in water.
2. The eggs are filtered into a mesh basket and dried on ice.
3. The eggs are placed in 100% methanol at -7℃ for 7 minutes and at -15℃ for 15 minutes.
4. The eggs are frozen in liquid nitrogen.
5. The eggs are thawed in water. Survival is checked after 24 and 48 hours [1].

Vitrification Results

A. Stephensi Phase Change Diagram



Midpoints of Species' Phase Change

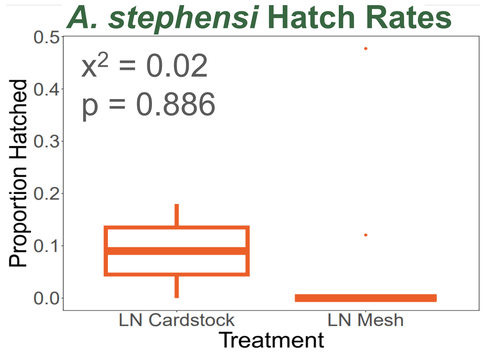


- A cryomicroscope was used to check if the eggs vitrified by observing a phase change as the sample thawed.
- The box indicates the phase change of a trial with *A. stephensi*, showing that the vitrification was successful.
- The vitrification of the *A. stephensi* was closer to the predicted value (-90 ℃) than the *A. gambiae.*

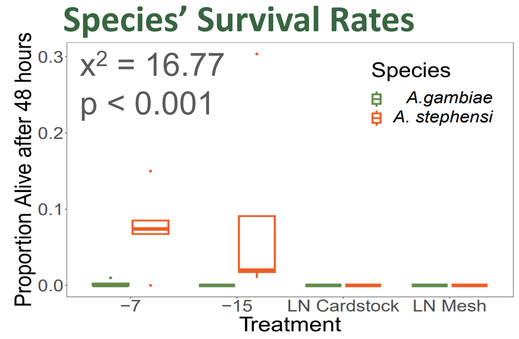
Survival Results

- Out of 9 trials, many *A. stephensi* hatched after liquid nitrogen treatments, while none of the *A. gambiae* did.
- All larvae died before 24 hours.
- Both species survived in the -7℃ and -15℃ trials.
- *A. stephensi* showed higher survival rates than *A. gambiae*.
- No larvae survived 48 hours after liquid nitrogen treatment.

A. stephensi Hatch Rates



Species' Survival Rates



A. stephensi

A. gambiae

Discussion & Future Directions

- *A. stephensi* eggs showed that cryopreservation of mosquito eggs is possible, although no larvae survived up to 24 or 48 hours.
- *A. stephensi* is survived after cryopreservation while *A. gambiae* did not because *A. stephensi* vitrifies more consistently.
- In the future, we plan to figure out why the larvae are dying and try to bring their survival up to 24 hours.
- We plan to continue editing the protocol to make it functional for the *A. gambiae*.

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References

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