Thermal knockdown protocol

1. Use adults eclosed 72 hours prior
2. Place in individual, labeled 5-mL plastic vials
3. Immerse plastic vials in water bath set initially to 28°C
4. Allow to acclimate for 15 minutes
5. After 15 minute prior, start timer and increase water bath temperature 1.0°C / 2 mins (to match the 0.5°C per minute used by Jorgenson and Overgaard, 2019) until 38°C is reached, then stop increasing.
6. Note time (in minutes/seconds) it takes for an individual to knockdown:
   1. Mosquitoes will naturally fall on their back. When this happens, gently rotate/tap the vial for five seconds. If they do not right themselves during this time, consider this as knocked down. If they flip over with a gentle rotation/ tapping of the tube, let them continue
7. Immediately after knockdown, record time (minutes/seconds since end of acclimation period) and place sample in individual, labeled PCR tubes. Store in -80°C.