# Online Resource 3

# Stratiomyidae Drying Oven Temperature Experiments

#### Methods

To test whether the temperature was an important factor in revival, we first conducted a microcosm experiment pairing conditions in a drying oven and ambient lab conditions (Sep 2021). There were two temperature treatments: i) summer temperature, in which *Odontomyia* Meigen, 1803 larvae were placed in the drying oven at 40°C, and ii) winter temperature, in which *Odontomyia* larvae were left to naturally dry on a countertop at the ambient air temperature (23°C) in the lab. Seven larvae from the small, medium, and large size groups (as described in Online Resource 2) were used for each treatment (summer temperature treatment n=21; winter temperature treatment n=21). Each individual larva was placed in its own 20mL scintillation vial with a mesh lid. The larvae began the drying cycle submerged in 1mL of deionized water, remained dry for five days, and then were rehydrated using 5mL of deionized water and monitored for revival.

Next, a second oven experiment was conducted to test *Odontomyia*’s ability to revive from drying at high temperatures for longer periods of time. Five 500mL mason jars were filled half-way with a mixture of sand and gravel substrate to provide similar substrate to what they would have for aestivation in their natural habitat. Ten larvae of the following sizes were placed in the jars: five small (4–8 mm length), three medium (9–12 mm), one large (13–16 mm), and one extra-large (17+ mm). For airflow, the jars were lidded with a mesh fabric square underneath the screw-on ring. The jars were placed in the drying oven at 32°C for one day to allow larvae to sense the temperature increasing, leaving the oven door open for the first few hours to allow acclimation. Then, the jars were dried for 17 days at 40°C. The substrate was moist (but not saturated) when larvae first were placed in the jars, and larvae were moving around on top of the substrate when the jars were placed in the drying oven. After 17 days, the jars were removed from the drying oven, and larvae were removed from the jars, rehydrated, and observed for potential revival.

#### Results

No larvae survived the first drying oven and lab experiment with treatments of different seasonal temperatures where no substrate was added to the scintillation vials. Larvae from both the summer and winter temperature vials became very brittle upon drying, often cracking into pieces. After complete desiccation and adding water, 0% of the larvae revived.

At the beginning of second drying oven experiment, where substrate was added to the jars, the larvae remained mobile for at least three hours (the duration that the oven door was left open). After the drying period, 92% of larvae were found on or just below the sediment surface, with many in the interstices of surface gravels, indicating some burrowing activity during drying. However, survival again was low; only one larva was revived out of 50 (2% survival), and this single survivor was the largest individual used in the experiment.

#### Discussion

Both drying oven experiments had very low survival (0–2%). Although one larva survived aestivation in a 40°C drying oven for 17 days, the drying rates used were likely too fast or too intense for most larvae to successfully aestivate. Although Stratiomyinae larvae are known for their ability to survive in extreme environments and utilize heat shock protein gene expression to overcome temperature stress (Garbuz et al. 2008), the rapid change in temperature in the drying oven may have limited these larvae’s ability to adjust. Larvae may have lost tissue water abnormally quickly, resulting in high mortality. Other aquatic insects use temperature to know when to disperse away from a drying environment (Boersma et al. 2019). However, the rapid change in temperature during these experiments could have been overwhelming, preventing the signal to aestivate and resulting in death. Additionally, increases in water conductivity during drying can cue invertebrates to aerially disperse to a new habitat (Bogan et al. 2015). The extremely low levels of water and moisture in the vials and jars may have prohibited *Odontomyia* larvae from perceiving a conductivity cue to start aestivation, if they indeed use this cue.

#### References

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