# Online Resource 2

# Stratiomyidae Drying Frequency Microcosm Experiment

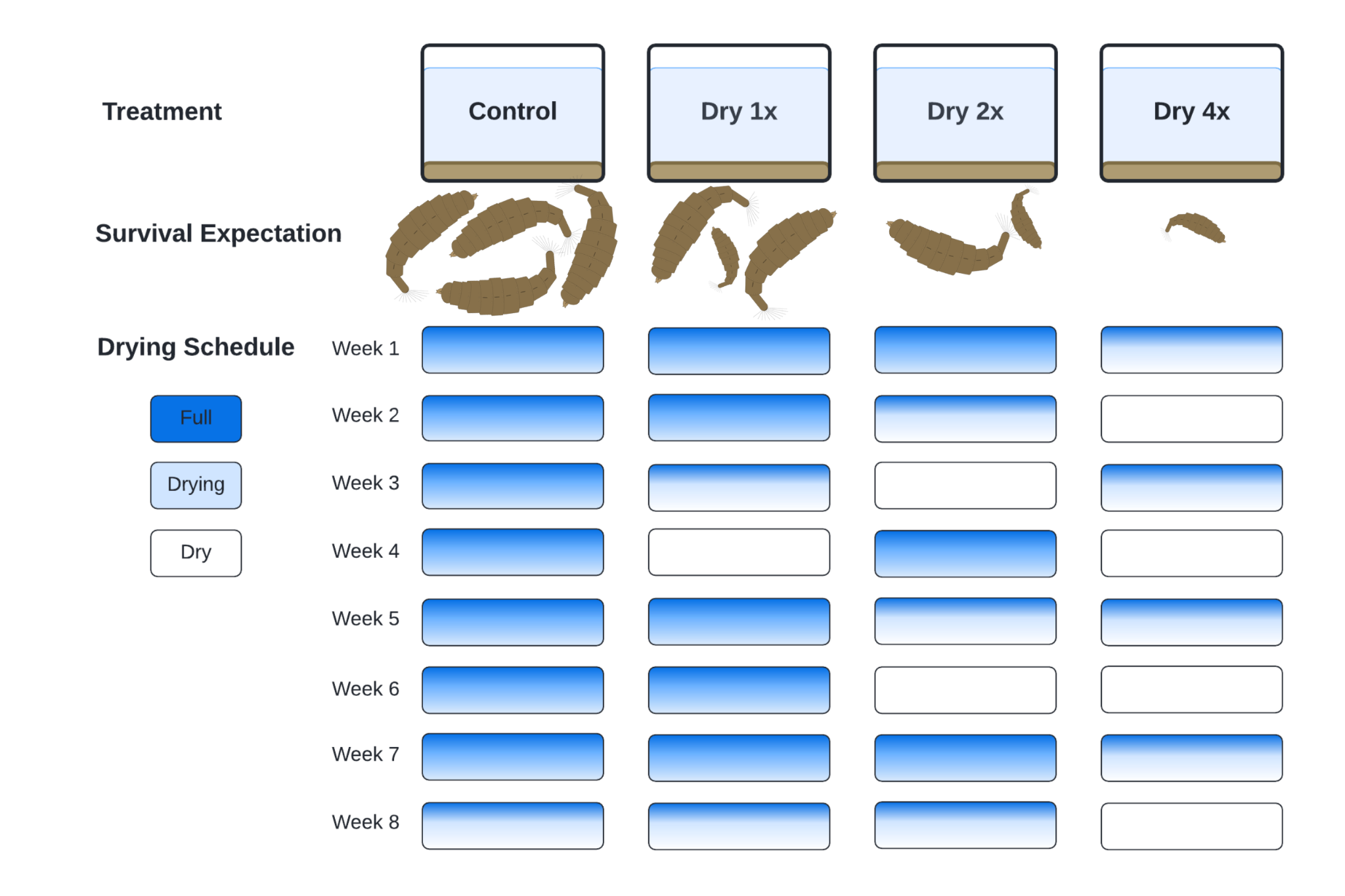
#### Methods

Microcosms were 5.7L plastic boxes (34 cm length x 21 cm width x 12 cm height), hereafter referred to as ‘tanks.’ Each tank was filled with one 0.75L scoop of sand and one 0.75L scoop of gravel. Then, 3L of deionized water and 45mL of algae slurry were added. The tanks sat for 72 hours before larvae were added to allow the algae slurry to inoculate the tanks with algal food resources. The tanks were randomly placed in rows inside a laboratory fume hood. The fume hood was used to increase the evaporation rate for the drying treatments. One HOBO Pendant

waterproof temperature and light data logger (Model UA‐002‐64, Onset Computer Corp) was placed in each tank to monitor temperature and light conditions throughout the fume hood.

Larvae were collected from one rock pool in the South Fork of Alamo Canyon, Organ Pipe Cactus National Monument, Arizona, USA, in September 2021. They were transported back to the lab, sorted by size, and deposited in tanks. Each tank received five small (4–8 mm length), three medium (9–12 mm), one large (13–16 mm), and one extra-large (17+ mm) larvae, totalling ten larvae mimicking the natural size distribution observed in the source rock pool (total n = 160). Five extra larvae replicates were created to calculate a conversion from wet mass to dry mass (n = 50).

Larvae were placed in tanks encompassing four drying treatments. One treatment was the control, which never dried for the duration of the experiment. The other treatments were 1x, which dried once, 2x, which dried twice, and 4x, which dried four times during the experiment (Figure OR2.1). The experiment ran for eight weeks. There was one week of acclimation at the start, but during the second week, the 4x tanks began drying. Tanks that were not scheduled to dry were topped off with deionized water every four days. Conductivity was measured using a hand-held meter (Milwaukee C65 pocket conductivity tester) weekly. At the end of the eight-week experiment, all tanks were dried and the substrate was sieved for remaining larvae. Remaining larvae were weighed and measured when possible. We tested for differences in tank environmental factors (conductivity, temperature, light) using ANOVA in RStudio.

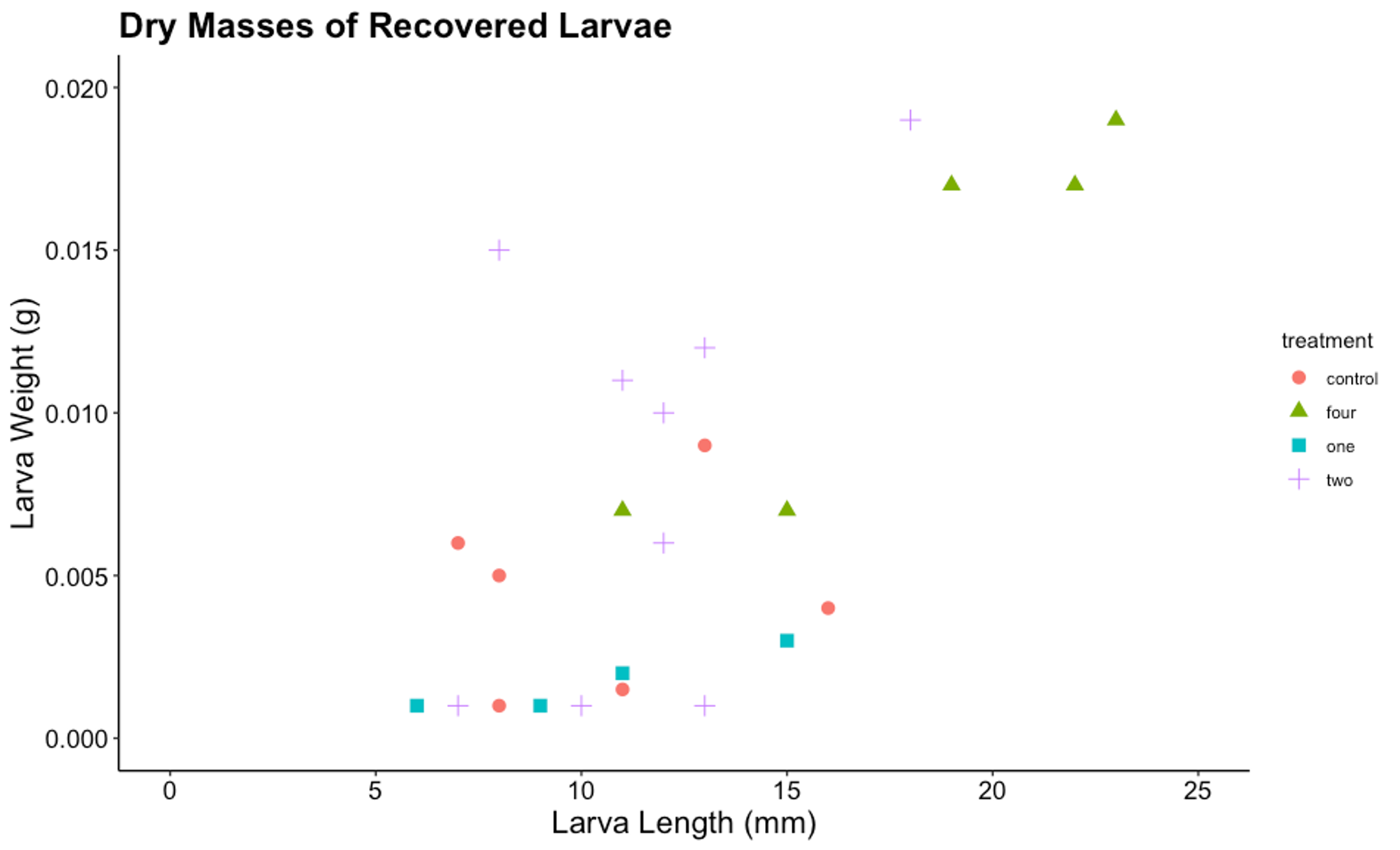


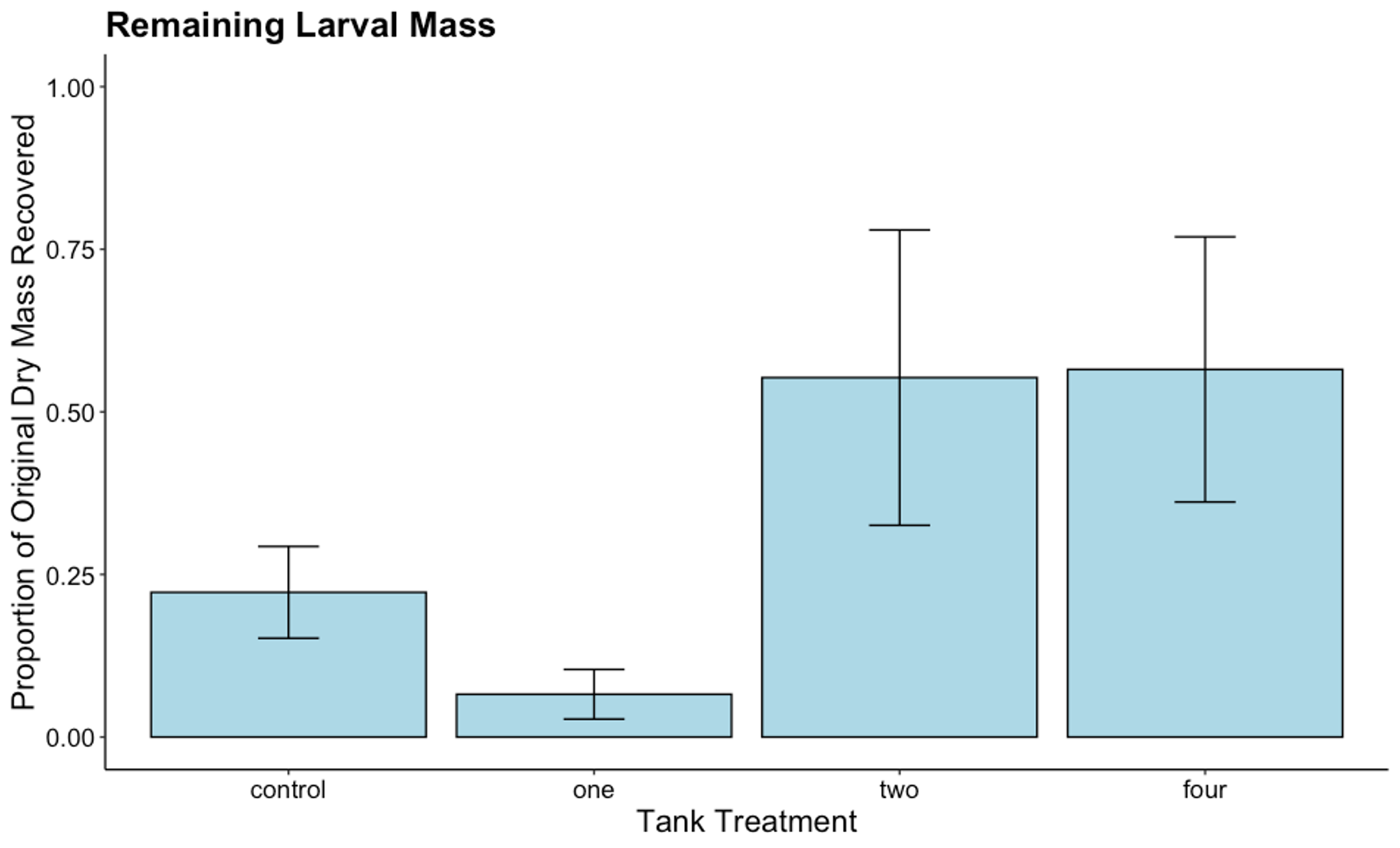
**Figure OR2.1** Experimental setup: Larvae were situated in tanks comprising four treatments, each with a different drying frequency. As drying frequency increased, we expected tanks would have lower survival and the surviving individuals would be smaller.

#### Results

Survival in the microcosm tanks was very low. None of the larvae pupated, so there was no emergence. Only three larvae (< 2%) were alive at the end of the experiment, two from a control treatment and one from a 1x treatment. The surviving larvae were between 11–16 mm in length and weighed between 0.008g and 0.022g. The larvae recovered via sieving were quite brittle and often broken, with some pieces so small they could not be weighed or measured with the tools in the laboratory. In two tanks, no larvae were recovered.

There was no clear pattern of which treatment had the best body condition or most mass recovered due to the low quantity of biomass recovered (Figure OR2.2a-b). Further, because we do not know what body decomposition rates were per treatment, we cannot determine what caused the patterns of remaining biomass (Figure OR2.2b). Treatments did not differ in conductivity (ANOVA, F3,253 = 0.468, p = 0.71) though not all treatments could be tested simultaneously due to some treatments being dry. Similarly, temperature was dependent on whether a tank was dry (air temperature) or wet (water temperature). Temperature differed by treatment (ANOVA, F3,21903 = 815.2, p < 0001), increasing predictably with drying frequency as dry tanks tracked the laboratory temperature more often than wet tanks. Light also differed by treatment (ANOVA, F3,21903 = 32.79, p < 0.001), though we believe this is due to inconsistencies in the light loggers’ positions (facing up or down) after refilling turbulently.





**Figure OR2.2 a)** Due to the difficulty of recovering larval bodies, body condition (weight as a function of length) patterns across treatments were difficult to assess. **b)** We do not know the decomposition rates within the tanks or how long larvae were deceased in the tanks before we weighed them, therefore we cannot confidently test for differences in the proportion of the original dry mass that remained in the tanks at the end of the experiment.

#### Discussion

The drying frequency experiment had very low survival. The drying rates used were likely too fast or too intense for most larvae to successfully aestivate. The drying rate used was modelled on small pools in the field which were the most similar to the microcosm tanks, though they were the most extreme examples of rock pool drying that we observed at Organ Pipe Cactus National Monument. Larvae may need more time to sense drying is occurring, or perhaps they only live in such small, rapidly drying pools after they have been pushed out of larger pools during monsoon-caused floods. Previous research that dried Stratiomyinae larvae in the lab (using desiccation chambers) resulted in roughly 3% survival after four months of aestivation with disturbances (Miller 1968). Under the fume hood, larvae may have lost tissue water abnormally quickly due to the high wind and rapid air movement, resulting in high mortality.

#### References

Miller, P. L. (1968), 'On the occurrence and some characteristics of Cyrtopus fastuosus Bigot (Dipt. Stratiomyidae) and Polypedilum sp. (Dipt. Chironomidae) from temporary habitats in western Nigeria'. *Ecologist’s Monthly Magazine*, 106, 233–238.

Meigen, J.W. (1803), 'Versuch einer neuen Gattungs Eintheilung der europäischen

zweiflügligen Insekten'. *Magazin für Insektenkunde*, 2, 259–281.