HERPETOCULTURE NOTES

ANURA — FROGS

***RANA SYLVATICA* (Wood Frog). CLUTCH SIZE MEASUREMENT.** Obtaining total counts of eggs or larvae within ranid egg masses can be challenging due to the three-dimensional structure of egg masses and high numbers of eggs and larvae. Nevertheless, herpetologists are occasionally faced with this daunting task when, for example, determining annual female reproductive output or the proportion of fertilized eggs per mating. Several methods are currently used to count exact numbers of eggs and larvae within ranid egg masses, each suitable for different applications (Browne and Zippel 2007. ILAR J 48:214–234). The Displacement method provides estimates (but not exact counts) of total egg numbers and cannot be used to count larvae. The Image Analysis and Direct Counting methods can provide exact counts of both eggs and larvae but require chemical egg gel dissociation and can be very tedious (e.g., hand-counting larvae dropped from the tip of a pipette). Finally, "flattening" methods have also been used for ambystomatid and ranid egg masses, which involve photographing or counting egg masses that are pressed between gridded glass plates (Harris 1980. Copeia 1980:719–722) or food storage containers (Karraker 2007. Herpetol. Rev. 38:46–48); however, the grid lines themselves can obscure some eggs or embryos. In addition, "flattening" methods cannot be used to count larvae.

Here, I describe an alternative method of counting *Rana sylvatica* eggs and larvae that does not involve egg gel dissociation or a gridded press. *Rana sylvatica* egg masses may contain up to 2000 eggs, and therefore direct quantification can be challenging. I used white miniature ice cube trays (Cubette Ice Trays 180, China) as a tool to count these large egg masses and hatched larvae. To count eggs and embryos within an egg mass, I cut small sections and gently rolled them over the ice cube tray. By pressing a finger lightly over an egg mass section, eggs can be separated into the ice cube tray's cells and viewed as a single layer. Each cell of the tray held up to seven eggs. Eggs were easily visible (Fig. 1), which facilitated rapid counting using a mechanical tally counter. Alternatively, eggs in the ice cube tray can be photographed and digitally counted using ImageJ. The ice cube tray method eliminates the problems associated with grid lines and does not require chemical egg gel dissociation to spread the eggs in a single layer. The contrast between the dark embryos and white tray also permits the approximate staging of embryos, with or without a hand lens.

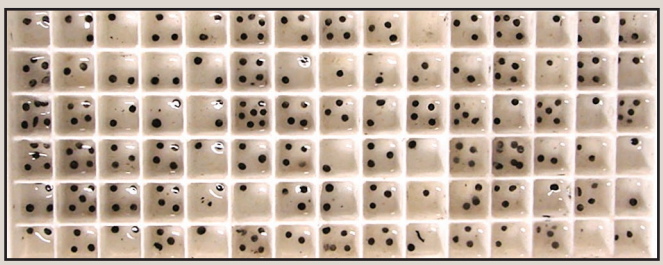

FIG. 1. *Rana sylvatica* eggs prepared for counting using the ice cube tray method.

The ice cube tray method can also be used to rapidly count larvae. *Rana sylvatica* larvae were distributed among the cells of the tray using a plastic disposable pipette with the tip widened or larvae were gently poured from a small specimen cup. Up to six newly hatched larvae can be easily counted per cell. When counting by hand, this method is superior to dropping larvae from a pipette because of the increased accuracy due to the ease of counting, as counts can be performed by counting larvae from left to right in each cell of every row. The ice cube tray method may also be preferred when performing digital larval counts because clumping of larvae can be prevented by adjusting the larval density in individual cells using a pipette, thus providing a more useful photograph for digital analysis. I reared larvae that were counted using this method to metamorphosis in the laboratory; mortality rates for these larvae were low and did not differ from those that were not counted using this method.

All procedures adhered to national and international standards on animal welfare and were compliant with the legal requirements of the United States and the Institutional Guidelines of Penn State University (IACUC permit numbers 42015 and 33346). Animal collection was permitted by the Pennsylvania Game Commission (NC-028-2012) and the Pennsylvania Fish and Boat Commission (Scientific Collector's Permit 483 Type 1).

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TESTUDINES — TURTLES

***PYXIS ARACHNOIDES ARACHNOIDES* (Common Spider Tortoise). EGG SHELL REPAIR.** All turtles are oviparous, producing pliable, hard-expansible or brittle egg shells (Ewert 1985. *In* Gans et al. [eds.], Biology of the Reptilia Volume 14: Development A, pp. 75–241. John Wiley and Sons, Inc, New York). The functions of the egg shell are to protect, allow adequate gas and water exchange, and serve as a mineral reservoir for the developing embryo (Palmer and Guillette 1991. *In* Deeming and Ferguson [eds.], Egg Incubation: its Effects on Embryonic Development in Birds and Reptiles, pp. 29–46. Cambridge University Press, Cambridge). In captivity, brittle or hard-shelled reptile eggs can be cracked or broken during oviposition or shortly thereafter. Damaged eggs can be a result of poor nesting sites (Kohler 2005. Incubation of Reptile Eggs. Krieger Publishing Co., Malabar, Florida) or improper handling when retrieving them (Gurley 2003. Keeping and Breeding Freshwater Turtles. Living Art Publishing, Ada, Oklahoma. 300 pp.). Additionally, changes in humidity during incubation can cause an egg to swell and crack (Packard 1994. *In*