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.

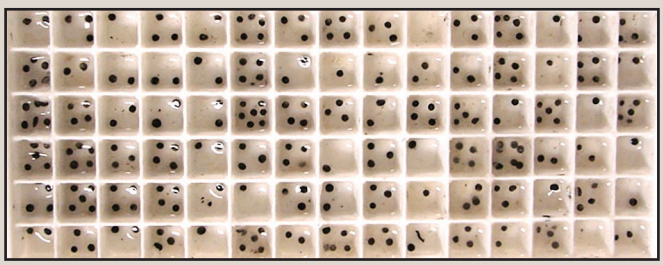

FIG. 1. *Rana sylvatica* eggs prepared for counting using the ice cube tray method.

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*

Murphy et al. [eds.], Captive Management and Conservation of Amphibians and Reptiles. SSAR, Ithaca, New York; Gurley 2003, *op. cit*).

Several methods to repair reptile egg shells have been used in both zoological institutions and private collections, and discussed openly on internet forums. These include the use of super glue, freezer tape, vinyl patches, paraffin wax, skin glue, Neosporin® (Johnson and Johnson Consumer Inc., New Brunswick, New Jersey), petroleum jelly, clear plastic sandwich bags, and tea bag fabric. Published accounts include the repair of *Morelia viridis* eggs with melted paraffin or the use of a conspecific's egg shell (Maxwell 2005. The More Complete Chondro. ECO Herpetological Publishing and Distribution, Rodeo, New Mexico. 317 pp.), a *Varanus acanthurus brachyurus* egg repaired with a piece of a *V. tristis* egg shell (Adragna and Madden 2009. Biawak 3:144–145), a *V. beccarii* egg sealed with hot wax (Fischer 2012. Biawak 6:79–87), and a *V. prasinus* egg repaired with tissue adhesive (Davis 2014. Biawak 8:39–42). Three *Dracaena guianensis* eggs that ruptured during incubation at the San Diego Zoo were repaired with Tegaderm™ (3M Inc., St. Paul, Minnesota), a transparent clear dressing (R. Walton, pers. comm.). Chelonian eggs have been repaired with surgical tape, Neosporin® (Gurley 2003, *op. cit.*), and plastic wrap (McKeown 1997. Vivarium 8[5]:6–7); *Geochelone nigra* eggs have been repaired successfully using tissue glue (C. Adams, pers. comm.).

FIG. 1. Distended membrane of a *Pixys a. arachnoides* egg prior to repair on 20 April 2015.

FIG. 2. *Pixys a. arachnoides* egg repaired with a section of *Rhacodactylus leachianus* egg shell.

A *Pixys arachnoides arachnoides* at the Smithsonian National Zoological Park laid a single egg in August 2014, followed by a second in September and a third in November of the same year. These eggs were incubated at 28°C in a 2:1 substrate mixture of vermiculite and water by weight. After four weeks, each egg was gradually cooled over a few weeks to 15.5°C where it remained for six weeks before being gradually warmed back up to 28°C for the remainder of its incubation. A large lateral crack in the shell of the third egg was first noticed on 16 March 2015, one month after warming the egg back up to 28°C. The crack continued to expand as the embryo developed, and eventually resulted in the rupture of the shell membrane on 20 April. Fluid began to leak from the egg and blood vessels were apparent in the distended membrane (Fig. 1).

At this time, the egg was repaired using the egg shell from a hatched *Rhacodactylus leachianus*. This shell was soaked in warm water to regain its pliability and disinfected with a 10% bleach solution. Once disinfected and rinsed repeatedly, the shell was soaked in AmQuel® (Novalek Inc., Hayward, California), an ammonia detoxifier, and then thoroughly rinsed again with water. Once dried, a piece of the *R. leachianus* shell was cut with scissors and rubbed with paper towels to remove any loose shell membrane remaining. New Skin Liquid Bandage® (Medtech, Jackson, Wyoming) was applied with a brush to seal the edges of the crack where the egg membrane was not protruding and to attach the *R. leachianus* egg shell directly over the distended membrane (Fig. 2).

Approximately three hours after the repair, the egg began sweating and a strong odor from the New Skin Liquid Bandage® was apparent. For these reasons, the egg incubation container was vented daily; an increase from once a week. Although the egg stopped sweating within 24 h, the smell of the adhesive persisted throughout the remainder of incubation. On 9 June 2015, after 215 days of incubation, the hatchling pipped the egg shell above the repaired section. By the following day the tortoise had made no progress and was manually assist-hatched in order to assess its condition. Upon examination, the tortoise had an open umbilicus, and was kept in the incubator on unbleached paper towels and treated daily with Nolvasan (Chlorhexidine: Fort Dodge Laboratories, Fort Dodge, Iowa) for four days, at which point the plastron had fully healed. This hatchling weighed 10 g; for comparison, the egg laid in September incubated for 244 days and the hatchling weighted 12 g.

Although the origin of this crack is unknown, it is suspected that the egg was either damaged during oviposition or became cracked as a result of some aspect of the incubation method (humidity and/or temperature). This eggshell repair method proved successful in this instance, allowing the continued development of the embryo. The strong odor of the New Skin Liquid Bandage® was concerning and may be avoided by using other forms of adhesive.

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SQUAMATA — LIZARDS

***CALOTES VERSICOLOR* (Garden Lizard). AGGRESSION.** Aggressive behavior has been studied in a wide range of animal taxa (Maher and Lott 1995. Anim. Behav. 49:1581–1597; Calsbeek and Sinervo 2002. Oecologia 132:468–477). Aggression in lizards has

been associated with territorial defense (Sheldahl and Martins 2000. Herpetologica 56:469–479), and between males especially during the breeding season (Archer 1988. The Behavioral Biology of Aggression. Cambridge University Press, Cambridge. 272 pp.), and it is evident in different forms such as advertisements using visual displays to physical contact (Martins 1994. *In* Vitt and Pianka [eds.], Lizard Ecology: Historical and Experimental Perspectives, pp. 117–144. Princeton University Press, Princeton, New Jersey). However, in some circumstances, agonistic behavior results in combat between opponents; this behavior can be costly for individuals because of resulting physical injury. The intensity of aggression by territory owners against intruders may vary in accordance with available resources in the habitat for retreat sites from predators, food, and mates (Leuck 1995. Herpetol. Monogr. 9:63–74; Bradbury and Vehrencamp 2011. Principles of Animal Communication, 2nd ed. Sinauer Associates, Sunderland, Massachusetts. 697 pp.). Aggressive behavior in anoles and agamids involves a variety of visual displays, such as headbobs, push-ups, dewlap or gular extensions, and lateral body compression which can advance to physical contact (Jenssen 1977. Amer. Zool. 17:203–215; McMann 2000. Anim. Behav. 59:513–522; Paterson 2002. Herpetologica 58:382–393; Perry et al. 2004. Anim. Behav. 67:37–47; Ammanna et al. 2013. Anim. Biol. 63:47–58). Here, I report for the first time a case of aggressive behavior observed in a female agamid lizard *Calotes versicolor* under captive conditions.

This female was hatched in the laboratory within a group of 15 hatchlings (2 males and 13 females). The hatchlings measured 25.11 ± 0.42 mm SVL ± SE and 44.36 ± 1.12 mm in tail length ± SE) and the body mass ± SE was 470.43 ± 12.53 mg. Sex of the hatchlings was recorded on day 4 by protrusion of the hemipenes as per Harlow (1996. Herpetol. Rev. 27:71–72). The hatchlings were placed in a glass terrarium 60 × 30 × 30 cm (L × W × H) with a 2-mm deep sandy substrate layer and a wooden structure for perching. The hatchlings were provided with food consisting of termites and silk moth larvae. Though the food was supplied *ad libitum*, the hatchlings began dying day by day and some of them were found injured and later died. Five females survived for one month. Among these five, the tails of four were observed becoming shorter in length. A single female in the group was observed chasing and eating the tails of other individuals without killing them.

This dominant female was always found at the top of the perch provided in the cage displaying aggressive behaviors such as push-ups and gular extensions. The dominant female attained maturity (83.48 mm SVL, tail length 141 mm) and was shifted to a new terrarium with an adult male of 115 mm SVL. The female did not attack him nor was any aggression observed. The remaining females with broken tails had the following measurements (SVL/TL, in mm): 70.8/35.66, 82.83/68.25, 74.06/60.64mm.

Tails in lizards are involved in diverse functions such as balance, fat storage, and mating (Ali 1949. Proc. Ind. Acad. Sci. 32:87– 95; Robinson et al. 1970. Mariorana 1977; Vitt et al. 1977. Ecology 58:320–337; Schall and Pianka 1978. Am. Nat. 115:551–566). The directed caudal attacks by the dominant female may have been intended to damage the tails of conspecifics in her group in order to suppress her siblings or as a sort of territory guarding behavior to access the food and basking site in the area provided. Further observations are needed from field studies to determine if such behaviors occur in their natural habitat.

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