

Banisteria, Number 25, 2005

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MORTALITY OF LARVAL SPOTTED SALAMANDERS (*AMBYSTOMA MACULATUM*) IN A CENTRAL VIRGINIA ROAD RUT PUDDLE—Several species of frogs and salamanders in the mid-Atlantic region use vernal pools for egg deposition and larval development (Mitchell, 2000). Road rut puddles of various sizes that often act like vernal pools are used extensively by amphibians such as Spring Peepers (*Pseudacris crucifer*), Wood Frogs (*Rana sylvatica*), Green Frogs (*Rana clamitans*), American Toads (*Bufo americanus*), Fowler's Toads (*B. fowleri*), Red-spotted Newts (*Notophthalmus viridescens*), Marbled Salamanders (*Ambystoma opacum*) and Spotted Salamanders (*A. maculatum*) (pers. obs.). Such ephemeral aquatic environments are susceptible to early drying that could result in complete mortality of the entire cohort of offspring. Drying pools are also attractive to predators such as crows, herons, and raccoons (pers. obs.). Mortality from disease organisms in such environments has not been previously reported. The biology and effects of various diseases such as ranaviruses (Family: Iridoviridae), ichthyophoniasis and chytridiomycosis have been studied in places other than the mid-Atlantic (Docherty et al., 2003). In Virginia, diseases have caused mortality in Southern Leopard Frogs (*Rana sphenoccephala utricularia*) in Virginia Beach and larval Wood Frogs (*R. sylvatica*) in Augusta County (D. E. Green, pers. comm.). Multiple die-offs of Spotted Salamanders in the southern Appalachians have been attributed to ranaviral epizootics (Converse & Green, 2005). In this paper, I describe a mortality event for *A. maculatum* larvae in a central Virginia road rut puddle and note possible disease agents that may have caused this die-off.

On 3 June 1998, I observed large numbers of dead *A. maculatum* larvae in two shallow road rut puddles on a plant nursery road 1.6 km W Midlothian, Chesterfield County, Virginia. One pool measured 5 m long x 1 m wide x 4 cm deep, and the other measured 7.5 m long x 1.3 m wide x 5 cm deep. Each pool had a clay substrate with little organic matter or algae. There was no emergent vegetation and the water was clear. Most of the margins of each pool were bordered by grass. Mixed hardwoods and pine on both sides of the road afforded shading in the morning and late afternoon. The afternoon temperature was about 32° C and there had been no rain for at least two days. I was unable to take water temperature but it was warm to the touch. The number of dead *A. maculatum* larvae was not counted, although there were about 2-3 scores of them. Not all of

the larvae were dead, as there were numerous live individuals among the dead ones. Those that had died were floating at the surface and lying on the substrate, nearly all with the ventral side up. Several were bloated around the head and neck. The live larvae were colored and behaved normally; swimming speed and response to touch was normal. Most of these had reduced gills and were nearing metamorphosis. One *N. viridescens* adult was in each of the two pools. Frogs in these pools were a juvenile Northern Green Frog (*Rana. clamitans*) and several adult Northern Cricket Frogs (*Acris crepitans*). I found no dead individuals of any of these species. No tadpoles of any anuran were present.

I had originally hypothesized that the mortality event was due to excessive heat. However, bloating of the gular skin and ventrum in ambystomid larvae are common signs of ranaviral infection (Converse & Green, 2005). I also found *A. maculatum* larvae in other warm aquatic sites during the same week that exhibited no mortality. The area within the nursery where the pools were located was apparently not subject to fertilizer, herbicide, or pesticide applications (W. H. Mitchell, pers. comm.). Such mortality events should be recorded because numerous reports of amphibian population die-offs are due to disease outbreaks (Daszak et al., 1999; Carey, 2000; Chinchar, 2002). Detailed descriptions of the event should be taken, and freshly dead amphibians should be promptly frozen or fixed for diagnostic examinations. The frozen samples I salvaged were accidentally discarded later. My observation of a mortality event in larval spotted salamanders with ventral and gular subcutaneous edema, although in a road rut puddle, suggests that infectious disease may have contributed to the deaths. Furthermore, the paucity of larval anurans in the road rut puddles suggests they had died several days earlier and the dead larval spotted salamanders were detected near the end of a ranaviral epizootic (D. E. Green, pers. comm.). Thus, other observations of such dead amphibians should be treated as a die-off and the proper procedures and specimens taken for definitive analysis.

ACKNOWLEDGMENTS

I thank David E. Green for his comments on this note and for the citations he provided.

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- Joseph C. Mitchell
Department of Biology
University of Richmond
Richmond, Virginia 23173