Morphological and Behavioral Changes of Salamanders Infected with Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*)

Adam L. Crane and Alicia Mathis

Department of Biology, Missouri State University, Springfield, Missouri 65897, USA

Photographs by the senior author.

Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) or Bd has been associated with worldwide amphibian declines and mortality (e.g., Berger et al. 1998, Daszek et al. 2003, Lips et al. 2006). In frogs, the fungus is known to cause gross skin sloughing (Berger et al. 1999), as well as behavioral changes in posture, loss of righting reflex, and lethargy (Carey et al. 2006, Berger et al. 1999). Chytrid also has been found in several salamander species (e.g., *Cryptobranchus alleganiensis*, Briggler et al. 2007; *Eurycea* spp., Gaertner et al. 2009; *Ambystoma tigrinum*, Davidson et al. 2003), including some species that are completely terrestrial (*Plethodon neomexicanus*, *Desmognathus conanti*, Cummer et al. 2005, Timpe et al. 2008; *Batrachoseps attenuatus*, Weinstein 2009), although the prevalence of chytrid in wild populations usually is low (Hossack et al. 2010). Individuals that are infected typically have dark molts that contain Bd zoosporangia (Cummer et al. 2005, Davidson et al. 2003). Other documented symptoms have sometimes included dark spots on the venter (Davidson et al. 2003), frequent and dark molting (Montanucci 2009), flakes of unshed skin around the vent (Cummer et al. 2005), redness on the ventral surface of the digits (Vasquez et al. 2009), skin lesions (Brodman and Briggler 2008), and foot- and limb-loss (Brodman and Briggler 2008). None of these studies, however, reported any behavioral changes associated with chytrid infection. Herein, we describe morphological and behavioral changes that we observed among laboratory-housed salamanders that were infected with the chytrid fungus.

Fig. 1. An adult Ozark Zigzag Salamander (*Plethodon angusticlavius*).

Collection and Maintenance of Salamanders

During October 2008 through February 2009, we collected 86 Ozark Zigzag Salamanders (*Plethodon angusticlavius*; Fig. 1) from a site in southwestern Missouri. In March and April 2009, we collected 46 additional individuals from a site in northern Arkansas. We then transported the salamanders to our laboratory to be used in behavioral experiments. The salamanders were housed in plastic Petri dishes with moist filter paper in an environmental chamber at 17 °C with a 12:12 light-dark cycle (Fig. 2). Salamanders were initially sized and sexed and then fed 4–8 flies (*Drosophila melanogaster*) three times per week. Filter paper was changed biweekly.

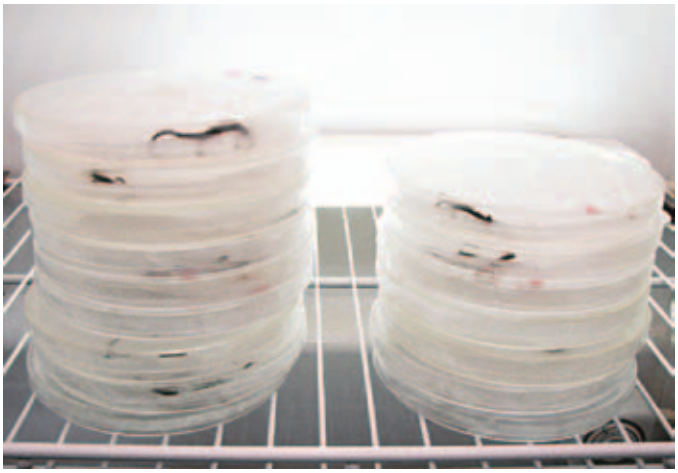

Fig. 2. Ozark Zigzag Salamanders (*Plethodon angusticlavius*) housed in Petri dishes with moist filter paper inside an environmental chamber.

Chytrid-induced Mortality and Treatment

In April 2009, several salamanders died from unknown causes. After the rate of mortality increased sharply in May (Fig. 3), we sent six preserved specimens to Pisces Molecular (Boulder, Colorado) for chytrid testing, and all six tested positive. In July, we began treating the salamanders with an antifungal medication (Itraconazole, aka Sporanox). The salamanders were bathed for 5 min on 11 consecutive days in a 0.01% solution with the addition of 0.6% saline. This treatment has been successful for other chytrid-infected amphibians (Forzan et al. 2008). In our laboratory, these treatments also appeared to be mostly successful, evidenced by the reduction of mortalities. After 11 days, we continued to treat salamanders that displayed chytrid-like symptoms (discussed below) until the symptoms ceased or mortality occurred. In total, slightly over half of the salamanders died by December 2009, at which point the remaining salamanders appeared to be healthy. Many of these mortalities occurred before the Itraconazole treatments began; of the salamanders that were treated, approximately 75% survived, but we do not know how many actually were infected with chytrid.

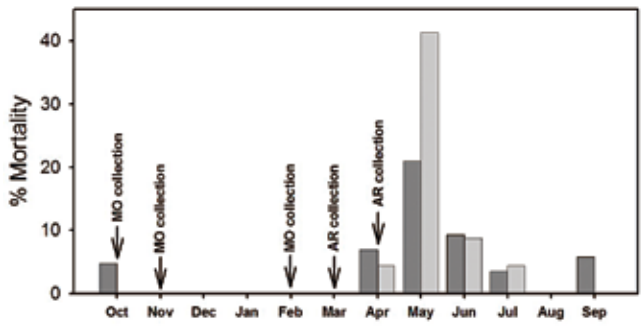

Fig. 3. The percentages of mortality in laboratory-housed Ozark Zigzag Salamanders (*Plethodon angusticlavius*) collected from a site in Missouri (dark gray bars) and a site in Arkansas (light gray bars) during fall 2008 and spring 2009.

Prior to the chytrid-induced mortality in our laboratory in March 2009, we collected several Spotted Salamander (*Ambystoma maculatum*) eggs (Fig. 4) from a site in southwestern Missouri. Eighty larvae (Fig. 5) hatched, and we housed them in aquaria with aerated pond water. The larvae metamorphosed around June 2009 into juvenile salamanders (Fig. 6), and we housed them in Petri dishes lined with moist filter paper that we kept in the environmental chamber with the Ozark Zigzag Salamanders. Subsequently, several Spotted Salamanders died after exhibiting symptoms of chytrid (discussed below). Once individuals began exhibiting symptoms, we initiated anti-fungal treatments — and about 25% of the treated salamanders recovered. This suggests that waiting until salamanders become symptomatic to begin treatments may be too late to achieve a high success rate.

Other studies have reported high mortality of chytrid-infected salamanders in the laboratory. In one study on a terrestrial salamander (*Batrachoseps attenuatus*), laboratory mortality was 100%, whereas wild populations appeared stable according to seasonal variation (Weinstein 2009). An experimental study on another species (*Plethodon metcalfi*) found laboratory mortality rates of 41.7% at 8 °C and 8.3% at 16 °C (Vasquez et al. 2009).

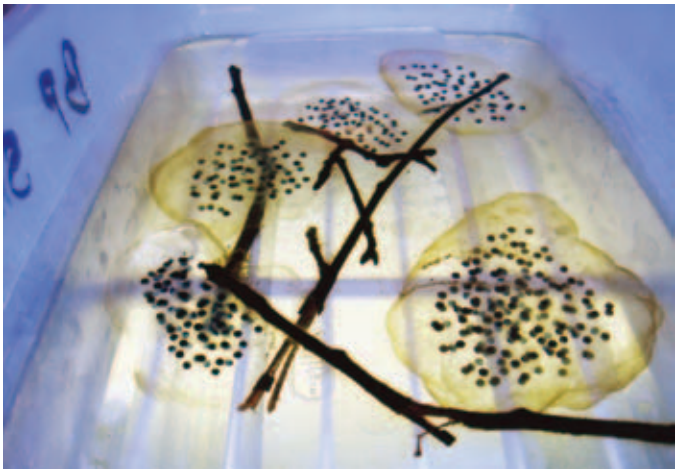

Fig. 4. Spotted Salamander (*Ambystoma maculatum*) egg masses collected from a pond in Missouri and housed in an environmental chamber.

Fig. 5. A larval Spotted Salamander (*Ambystoma maculatum*) housed in an aquarium

Morphological and Behavioral Changes

During the period when the salamanders were ill, obvious morphological changes in Ozark Zigzag Salamanders included sloughing large amounts of skin that were dark in coloration, and occasional loss of part or all of the legs (Fig. 7A). These salamanders also displayed behavioral anomalies char-

Fig. 6. Juvenile Spotted Salamanders (*Ambystoma maculatum*) that had recently metamorphosed in our laboratory.

acterized by raising part of their body as if to avoid contact with the substrate. Specifically, the salamanders would raise their tail (Fig. 7B) or trunk (Fig. 7C) much like the posture that is typically seen during an aggressive display in territorial contests (Jaeger 1984). They also were observed frequently raising their legs (Fig. 7D). In severe cases that typically led to mortality, these behavioral symptoms would progress until the salamanders had difficulty walking (video posted at: www.facebook.com/video/video.php?v=706655963964).

Similar to the Ozark Zigzag Salamanders, the Spotted Salamanders that were infected also shed large amounts of skin (Fig. 7E). In some cases, it appeared that the salamanders eventually died from internal hemorrhaging (Fig. 7F). This occurrence matched the recent discovery that chytrid-induced mortality results from asystolic cardiac arrest (Voyles et al. 2009). In contrast to the symptoms of the Ozark Zigzag Salamanders, we observed no obvious behavioral symptoms in infected Spotted Salamanders, although the speed of their righting responses appeared to be greatly reduced. Thus, we used both skin sloughing and decreased righting response as indicators of infection to determine whether individual salamanders would be treated with Itraconazole.

What was the source of the chytrid?

We do not know how the chytrid entered our laboratory. The Ozark Zigzag Salamanders that we collected were the first to display chytrid-like symptoms in our laboratory, but we cannot be certain they were the original source of the infection. Once the chytrid was in the laboratory, we probably were responsible for spreading it among salamanders. This contamination was likely facilitated by our lack of sterilization of some laboratory equipment and testing chambers.

Conclusions

To our knowledge, the only prior reports of chytrid in the Ozarks are from a stream salamander (the Hellbender, *Cryptobranchus alleganiensis*; Briggler et al. 2008) and from frogs (*Lithobates* spp.) and salamanders (*Eurycea* spp.) in caves (Rimer and Briggler 2010). The source of the chytrid in our laboratory was either terrestrial, via the Ozark Zigzag Salamanders, or from ponds, via either the Spotted Salamanders or Central Newts (*Notophthalmus viridescens louisianensis*), which we also housed in the laboratory. Whatever the source, Ozark Zigzag Salamanders and Spotted Salamanders are susceptible to chytrid, and can die following infection. Chemical treatments of these two species with Itraconazole were somewhat effective; however, some recent research has found that temperature treatments on amphibians can be highly effective (Woodhams et al. 2003, Márquez et al. 2010, Briggler et al. 2009).

Acknowledgements and Ethical Note

These salamanders were originally brought into our laboratory (Missouri Department of Conservation permits 13611 and 13966 and Arkansas Game and Fish Commission permit 101420081) for research that was approved by Missouri State University's IACUC (protocols 2007M and 2008AA). Because these salamanders were exposed to chytrid, we did not return them to the wild; instead, they were euthanized in accordance with officials from MDC, AGFC, and MSU's IACUC. Since the occurrence of these infections, we have worked to prevent future chytrid-related problems in our laboratory. We have improved procedures for quarantining wild-caught individuals and for cleaning and disinfecting equipment, and, since 2009, no further evidence of chytrid has occurred in our laboratory. We are especially grateful to the following people for their assistance and expertise:

Fig. 7. Chytrid-induced morphological (A) and behavioral (B, C, D) changes in Ozark Zigzag Salamanders (*Plethodon angusticlavius*), and morphological changes (E, F) in Spotted Salamanders (*Ambystoma maculatum*).

Mark Wanner (St. Louis Zoo), Jeff Briggler (MDC), Kelly Irwin (AGFC), and Michael Stafford (MSU IACUC). We also thank Jenny Parsons and Rob Hunt for help performing chytrid treatments, and Allison Overmeyer for help with euthanasia.

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MIGUEL A. LANDESTOY

The Hispaniolan Green Treefrog (*Hypsiboas heilprini*) occurs in both the Dominican Republic and Haiti and is Vulnerable according to the IUCN Red List. Recommended actions include *in situ* conservation and conservation education.