

CONSERVATION RESEARCH REPORTS

Museum Specimens Reveal the Coincidence of Neotropical Amphibian Declines and the Emergence of the Chytrid Fungus

Amphibians highlight the global biodiversity crisis because ~40% of all amphibian species are currently in decline. Species have disappeared even in protected habitats (e.g., the enigmatic extinction of the Golden Toad, *Incilius* [formerly *Bufo*] *periglenes*, from Costa Rica). The emergence of a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), has been implicated in a number of declines that have occurred in the last decade, but few studies have been able to test retroactively whether Bd emergence was linked to earlier declines and extinctions. CHENG ET AL. (2011. *Proceedings of the National Academy of Sciences of the United States of America* 108:9502–9507) described a noninvasive polymerase chain reaction (PCR) sampling technique that detects Bd in formalin-preserved museum specimens. The authors detected Bd by PCR in 83–90% (n = 38) of samples that were identified as positive by histology. They examined specimens collected before, during, and after major amphibian decline events at established study sites in southern Mexico, Guatemala, and Costa Rica. A pattern of Bd emergence coincident with decline at these localities was revealed — the absence of Bd over multiple years at all localities followed by the concurrent emergence of Bd in various species at each locality during a period of population decline. The geographical and chronological emergence of Bd at these localities also indicated a southbound spread from southern Mexico in the early 1970s to western Guatemala in the 1980s/1990s and to Monteverde, Costa Rica by 1987. The authors found evidence of a historical “Bd epidemic wave” that began in Mexico and subsequently spread to Central America and described a technique that can be



Many amphibians have disappeared in recent years, even from apparently ideal habitat in protected areas. The enigmatic extinction of the Golden Toad (*Incilius periglenes*) in Costa Rica is a prime example. A fungal pathogen (*Batrachochytrium dendrobatidis*) has been implicated in many recent population declines, but whether the chytrid fungus was linked to earlier declines and extinctions has been unknown.

used to screen museum specimens from other amphibian decline sites around the world.

Demise of a Swedish Population of Adders

MADSEN AND UJVARI (2011. *Herpetological Conservation and Biology* 6:72–74) cited their previously published reports in 1999 and 2004 on how the introduction of 20 males into a severely inbred and isolated population of Adders (*Vipera berus*) halted its decline toward extinction. The introduction significantly enhanced the population’s genetic variability, which resulted in a dramatic increase in offspring viability and a rapid increase in numbers. Unfortunately, a new and unprecedented development is threatening the population’s survival. In 2004, permission was granted by the Swedish Nature Conservation Agency of the County Administrative Board to build a house and an adjacent 1-m tall brick wall across the habitat occupied by the Adders. The construction of the house and brick wall in 2006 prevented the majority of the snakes from undertaking their annual migration within the study area, resulting in the extirpation of > 75% of the snakes. This reduction seriously impedes the survival of this unique population.



These male Adders (*Vipera berus*) are engaged in combat, presumably for reproductive rights. The red marks visible on one male allow for individual recognition. This photograph was taken before the recent population collapse.

Life History and Seasonal Variations in Chytridiomycosis in Crawfish Frogs

To fully comprehend chytridiomycosis, the amphibian disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), scientists must understand how Bd affects amphibians throughout their remarkable range of life histories. Crawfish Frogs (*Lithobates areolatus*) are a typical North American pond-breeding species that forms explosive spring-breeding aggregations in seasonal and semipermanent wetlands. However, unlike most species, when not breeding, Crawfish Frogs usually live singly — in nearly total isolation from conspecifics — and obligately in burrows dug by crayfish.



Crawfish Frogs (*Lithobates areolatus*) are most vulnerable to the chytrid fungus when in crayfish burrows that penetrate the water table or when they breed in seasonal or semipermanent ponds.

Crayfish burrows penetrate the water table, and therefore offer Crawfish Frogs a second, permanent aquatic habitat when not breeding. Over the course of two years, KINNEY ET AL. (2011. *PLoS ONE* 6(3):1–10) sampled for the presence of Bd in adult Crawfish Frogs. Sampling was conducted seasonally, as animals moved from post-winter emergence through breeding migrations, then back into upland burrow habitats. During the study, 53% of Crawfish Frog breeding adults tested positive for Bd in at least one sample; 27% entered breeding wetlands Bd positive; 46% exited wetlands Bd positive. Five emigrating Crawfish Frogs (12%) developed chytridiomycosis and died. In contrast, all 25 adult frogs sampled while occupying upland crayfish burrows during the summer tested Bd negative. One percent of postmetamorphic juveniles sampled were Bd positive. Zoospore equivalents/swab ranged from 0.8 to 24,436; five of eight frogs with zoospore equivalents near or >10,000 are known to have died. In summary, Bd infection rates in Crawfish Frog populations ratchet up from near zero during the summer to over 25% following overwintering; rates then nearly double again during and just after breeding — when mortality occurs — before the infection wanes during the summer. Bd-negative postmetamorphic juveniles may not be exposed again to this pathogen until they take up residence in crayfish burrows, or until their first breeding, some years later.

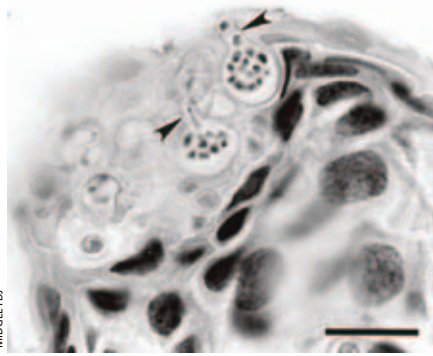
Strategies for Controlling Chytridiomycosis

Rescuing amphibian diversity is an achievable conservation challenge. Disease mitigation is one essential component of population management. WOODHAMS ET AL. (2011. *Frontiers in Zoology* 8(8):1–23) assessed existing disease mitigation strategies, some in early experimental stages, which focus on the globally emerging chytrid fungus *Batrachochytrium dendrobatidis* (Bd). The authors found that the effects of exposure to Bd occurred on a spectrum

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Ventral hindlimb skin of a Harlequin Toad (*Atelopus varius*) from western Panama showing symptoms of chytridiomycosis. Two sporangia (spore-containing bodies of *Batrachochytrium* sp.) containing numerous zoospores are visible within cells of the stratum corneum. Each flask-shaped sporangium has a single characteristic discharge tube (arrow) at the skin surface. Exiting zoospores are visible in the discharge tubes of both sporangia. Hyperkeratosis is minimal in this acute infection. Tissues were fixed in neutral-buffered 10% formalin, embedded in paraffin, sectioned at 6 μ m thick, and stained with hematoxylin and eosin. Bar = 35 μ m.

from transient commensal to lethal pathogen. Management priorities are divided between halting pathogen spread and developing survival assurance colonies, and prophylactic or remedial disease treatment. Epidemiological models of chytridiomycosis suggest that mitigation strategies can control disease without eliminating the pathogen. Ecological ethics guide wildlife disease research, but several ethical questions remain for managing disease in the field.

Because sustainable conservation of amphibians in nature is dependent on long-term population persistence and co-evolution with potentially lethal pathogens, the authors suggested that disease mitigation not focus exclusively on the elimination or containment of the pathogen, or on the captive breeding of amphibian hosts. Rather, successful disease mitigation must be context-specific with epidemiologically informed strategies to manage already infected populations by decreasing pathogenicity and host susceptibility. They proposed population-level treatments based on three steps: (1) Identify mechanisms of disease suppression; (2) establish parameters for epizootiological models of disease and population dynamics that can be tested under semi-natural conditions; and (3) begin a process of adaptive management in field trials with natural populations.

Can Invasive Burmese Pythons Inhabit the Southeastern United States?

Understanding potential for range expansion is critical when evaluating the risk posed by invasive species. Burmese Pythons (*Python bivittatus*) are established in southern Florida and pose a significant threat to native ecosystems. Recent

studies indicate that climate suitable for the species exists throughout much of the southern United States. **DORCAS ET AL.** (2011. *Biological Invasions* 13:793–802) examined survivorship, thermal biology, and behavior of Burmese Pythons from southern Florida in a semi-natural enclosure in South Carolina, where winters are appreciably cooler than in Florida, but within the predicted region of suitable climate. All pythons acclimated to the enclosure, but most died after failing to seek appropriate refugia during sub-freezing weather. The remaining snakes used refugia but died during an unusually cold period in January 2010. Although all snakes died during the study, most survived extended periods at temperatures below those typical of southern Florida, and none exhibited obvious signs of disease. This study represents a first step in evaluating the results of climate matching models and addresses factors that might affect range expansion in this invasive species.

Cold-induced Mortality of Invasive Burmese Pythons in Southern Florida

A recent record cold spell in southern Florida (2–11 January 2010) provided an opportunity to evaluate responses of an established population of Burmese Pythons (*Python bivittatus*) to a prolonged period of unusually cold weather. **MAZZOTTI ET AL.** (2011. *Biological Invasions* 13:143–151) observed behavior, characterized thermal biology, determined fate of radio-telemetered ($n = 10$) and non-telemetered ($n = 104$) Burmese Pythons, and analyzed habitat and environmental conditions experienced by pythons during and after a historic cold spell. Telemetered pythons had been implanted with radio-transmitters and temperature-recording data loggers prior to the cold snap. Only one of 10 telemetered pythons survived the cold snap, whereas 59 of 99 (60%) non-telemetered pythons for which data were available survived. Body temperatures of eight dead telemetered pythons fluctuated regularly prior to 9 January 2010, then declined substantially during the cold period (9–11 January) and exhibited no



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Invasive Burmese Pythons (*Python bivittatus*) are wreaking ecological havoc in southern Florida. Although in this instance, the snake is prey of an American Alligator (*Alligator mississippiensis*), pythons are efficient predators that are likely to imperil native species that include alligators.

further evidence of active thermoregulation, indicating they were likely dead. Unusually cold temperatures in January 2010 were clearly associated with mortality of Burmese Pythons in the Everglades. Some radiotelemetered pythons appeared to exhibit maladaptive behavior during the cold spell, including attempts to bask instead of retreating to sheltered refugia.

Vulnerability of a Peripherally Isolated Population of Wood Turtle in Iowa

The North American Wood Turtle (*Glyptemys insculpta*) is a semi-aquatic species that is considered rare, threatened, or endangered over much of its range. **SPRADLING ET AL.** (2010. *Conservation Genetics* 11:1667–1677) monitored a particularly vulnerable peripherally isolated population in Iowa over a seven-year period. The authors compared population census size, estimated from mark-recapture data, age structure determined from morphology, and genetic variation using microsatellites of this peripheral isolate with data from a population nearer the core of the species range in West Virginia. They also compared gene flow between the Iowa population and a nearby population in Minnesota. Genetic data indicated that the Iowa population is isolated, unique, and diverse. Although the Iowa population has lower allelic richness, lower heterozygosity, and smaller genetic effective population size than does the West Virginia population, the difference is not dramatic despite its lower population size, position at the periphery of the species range, and biogeographic history. The Iowa population is not inbred, and the genetic signature is not indicative of a recent population bottleneck. However, interpretations of recent population dynamics based on genetic data may be unduly encouraging in long-lived species such as *G. insculpta*. Field data suggested a nearly complete lack of recruitment in Iowa. A number of environmental and anthropogenic factors, including recent increases in summer flooding during egg incubation, might have a more negative impact on the Iowa population than on the West Virginia population.



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An isolated population of Wood Turtles (*Glyptemys insculpta*) in Iowa is not inbred and no evidence points to a genetic bottleneck. However, recruitment to the population is near zero, which suggests that a number of environmental and anthropogenic factors might have a particularly negative impact on this population.