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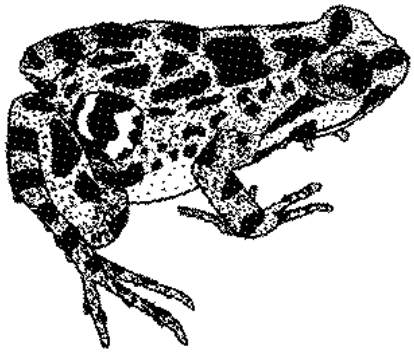
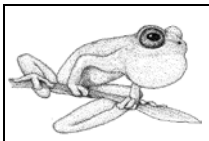


FROGLOG

Newsletter of the Declining Amphibian Populations Task Force

December 1998, Number 30.



SSC Amphibian Network to be Restructured

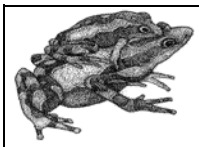
As described in the latest issue of Species (#30), the IUCN Species Survival Commission (SSC) has been pursuing the development of a Global Amphibian Campaign, in partnership with Flora and Fauna International and Conservation International. The goals of the Campaign are to focus attention on amphibian conservation, undertake research on the status of amphibian species (where the planned Campaign centrepiece is a global IUCN Red List assessment), and raise funds. SSC should benefit by gaining access to the resources needed to better develop and support the activities of its amphibian volunteer network, as well as more effectively coordinate its amphibian conservation activities with two key partners.

At the most recent meetings of the SSC Steering and Executive Committees, the Campaign project concept was endorsed, with the caveat that before moving ahead, SSC must address the structural and organizational questions of how the amphibian network should evolve. Three main issues were raised:
(1) the advisability of maintaining the current structural link between amphibians and reptiles;
(2) the relationship of the DAPTF to the Specialist Groups and the future of the DAPTF as it nears completion of its mandate;
(3) the advisability of maintaining a regional structure.

In addressing these issues, the Executive Committee recommended that SSC reorganize its amphibian work under the umbrella of a global Amphibian Specialist Group, within which a regional sub-structure could be maintained as and where appropriate. Through such a structure, SSC's amphibian work could be more focused and effective, fundraising efforts could be enhanced through the establishment of a global profile and targetted regional activity could still be maintained.

Consultation on this recommendation with the SSC amphibian network is vital. As a first step, the Board of the DAPTF was consulted at their recent meeting in Guelph, Canada. The Board endorsed the Committee's proposal, adding the possibility of the Task Force continuing as the research arm of a global Specialist Group. In addition, SSC continues to seek the advice and input of individual members. Your opinions on what sort of internal structure a global Amphibian Specialist Group should have (e.g. taxonomic vs. regional), as well as the advantages and disadvantages that you see in this type of structure, are important.

Please send your comments to: The SSC Chair's Office, c/o Canadian Wildlife Service, Ottawa, Ontario K1A 0H3, CANADA.
Fax: 1-819-9537177
E-mail: **ssc_iucn@ec.gc.ca**

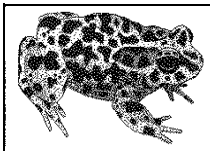


US Government Takes Big Leap

From Jamie Reaser

Mr. Bruce Babbitt, Secretary of the United States' Department of the Interior, recently announced the formation of the federal Taskforce on Amphibian Declines and Deformities (TADD), the US federal agency response to the phenomena of amphibian declines and deformities. TADD's mission is to promote and coordinate federal agency activities for elucidating the causes of these problems and addressing the causes when known. Four working groups (International, Science, Conservation and Education), open to all representatives of TADD member agencies, have been formed. By focusing on federal government programs and activities, TADD has the potential to compliment and support DAPTF's grassroots research and management initiatives. In the coming months, DAPTF experts will have the opportunity to brief TADD members on the state of scientific knowledge and management activities, as well as to help identify particular opportunities for federal agency response to the problems. DAPTF members who are US federal agency personnel are encouraged to take an active role in TADD, providing the necessary bridge for communication and cooperation between the two groups.

For further information, contact: Jamie Reaser at the US Department of State, e-mail: **sprgpeeper@ aol.com** or **jreaser@state.gov**



Status of Amphibians in Bermuda

By Donald W. Linzey

Bermuda, which lies just 1000 km off the coast of North Carolina, consists of a series of interconnected islands of limestone overlying a volcanic seamount. The islands are surrounded by coral reefs and a platform of calcareous sands. Due to the porous nature of the rock, Bermuda has no streams or rivers. There are also no heavy industries that could be local sources of heavy metal pollutants. Due to the year-round mild climate, hibernation is not necessary. There are also no natural predators.

Since March, 1995, a major long-term research project has been underway to determine the status of Bermuda's three species of amphibians. These include the giant toad (*Bufo marinus*) and two kinds of whistling frogs (*Eleutherodactylus johnstonei* and *E. gossei*). Few data exist on these species in Bermuda.

Natural history and ecological studies, in particular, are sparse. No data exist on population sizes and distribution other than brief accounts given by Wingate (1965). This study was initiated in response to the alarm of many Bermudians that one or more of these species were declining in numbers.

Initial efforts focused primarily on gathering baseline data on seasonal parasite loads and foods. Parasite analyses have revealed high parasite loads. One nematode and two trematodes never before found in Bermuda have been recorded (Linzey *et al* 1998a; 1998b). A highly significant increase over the 1992 infection rate of an intestinal trematode, *Mesocoelium monas*, in *Bufo marinus* was recorded beginning in 1995. This high level of infection has been maintained through August 1998. The results of extensive food habit analyses of *Bufo marinus* and *Eleutherodactylus johnstonei* have recently been published (Linzey *et a* 1998c). Considerable data have also been gathered on lengths, weights, sex ratios, and reproductive condition (size and development of reproductive organs, numbers of eggs etc.) of both species. Egg masses of *Eleutherodactylus* (which are laid on land and undergo direct development) have been monitored, and fungi and predators affecting survival of the eggs have been identified. A technique has been developed by which fluid can be withdrawn from within an individual egg and its pH can be determined. These data now form the most comprehensive database ever assembled for Bermudan amphibians.

Results of this study so far have revealed some potentially serious problems and have required the opening of several new lines of investigation. One species of whistling frog (*E. gossei*) has not been seen since June 1994. During each of our 11 research trips, we have searched for this species in areas where it was known to be abundant prior to 1994. We have neither observed nor heard this species. Since it inhabited some of the most acidic sites on Bermuda, it has been speculated that acid rain may have made these sites too acidic for this species to survive. Whether it still exists in small areas on Bermuda or whether it has already become extinct is not known at this time. Efforts will continue to locate individuals either visually or by listening for their distinctive call.

During every research trip to Bermuda, soil, water and tissue samples are taken from each of 15 study sites. The Bermuda Department of Agriculture, Fisheries and Parks has provided a list of all pesticides, herbicides, and fungicides registered for use in Bermuda. Preliminary analyses have revealed such chemicals as pp-DDD, pp-DDE, op-DDT, PCBs, lindane, dieldrin and polyaromatic hydrocarbons such as napthalene, fluorene, phenanthrene, pyrene and benzo-a-pyrene at several of the study sites. Preliminary heavy metal analyses are currently being performed for 8 heavy metals (cadmium, chromium, copper, iron, lead, manganese, nickel and zinc).

Preliminary studies of two toads from the same study site in Bermuda have revealed significant pathological changes in tissues when compared to tissues from *Bufo marinus* taken from within its natural range in Mexico. Granulomas were identified in one Bermuda specimen. Conditions indicative of a significant decrease in leucocytes, the body's main line of defense against disease-causing organisms and a possible indication of immune system suppression, were found in the two toads and also in two of three whistling frogs examined. Specimens from two additional study sites were secured in August, 1998, and their tissues are currently undergoing examination. Preliminary data indicate a significantly greater mitogen response in toads from the least polluted study sites. An attempt is being made to secure selected tissues from *Bufo marinus* and *Eleutherodactylus johnstonei* from within their natural ranges in Central America and the Caribbean. Pathological and immunological studies will be conducted in much greater detail during the next several years if adequate funding can be obtained.

Deformities of the appendages were rare in Bermuda prior to August 1998. Of the hundreds of specimens examined during this study, only one adult *Bufo marinus* was found to have a missing left hind limb that appeared never to have been formed. No *Eleutherodactylus* have exhibited any deformities. However, in August 1998, 25% of the toads examined from two study sites exhibited deformities of the hind limbs (missing toe, stubbed toes, extra spur on toe, etc.). The cause of these deformities is unknown at present. One of these toads was taken in a large banana patch where we had previously recorded a whistling frog kill in progress in 1996. After discovering dead and dying frogs in 1996, we ran several transects and verified a kill rate of approximately 20%. A variety of fungicides, herbicides, and pesticides (including snail and slug bait and rat poison) are used on a regular basis at this site (as they are by many other banana growers in Bermuda). It has been speculated that the deformities and frog kills may be the result of chronic exposure to one or more of these substances. As our pesticide and heavy metal analyses proceed, we hope to be able to identify the major causative factor(s) in the deformities and frog kills and to link it to its source (for example, copper or zinc in certain pesticides).

This study is now being expanded to cover a portion of northeastern Tennessee and the entire state of Virginia. Regional study sites are being established from which seasonal soil, water, and tissue samples will be obtained during the spring (April) and fall (October) for two years. Two species of amphibians will be monitored: Fowler's toad (*Bufo woodhousei*) and the spring peeper (*Pseudacris [Hyla] crucifer*). Regional cooperators are being requested to select preferably one study site where both species occur. All samples will be analyzed for pesticides and for heavy metals. All data will be correlated with studies that will be continuing in Bermuda.

This study may also play a critical role in assessing the significance of the air-borne particulate matter that affects Bermuda. Major weather patterns in the North Atlantic move in an easterly direction, thus transporting air masses from the United States towards Bermuda (Jickells *et al* 1982). During the summer and autumn, the semi-permanent sub-tropical Azores /Bermuda high dominates the Bermuda area. This high pressure prevents many of the frontal systems which originate over North America from reaching Bermuda. During winter, with a weak or absent sub-tropical high, frontal systems pass regularly. This situation becomes firmly established over the islands during December and persists for about three months. Late autumn and spring are the transitional seasons. During June to December, pH values are generally high; however, from December to May, the reverse is true.

The author would like to thank the Bermuda Zoological Society, the Bermuda Biological Station for Research, the Bermuda Department of Agriculture, Fisheries and Parks, and many individuals who have assisted this investigation in numerous ways.

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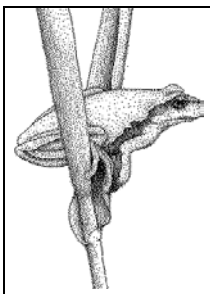

Disease and Pathology Working Group Report: A breakthrough in the hunt for a cause of amphibian declines

By Andrew A. Cunningham, Working Group Chair

At the time of its formation back in 1991, the DAPTF was given two major remits: to determine whether global amphibian population declines were a real phenomenon and to attempt to elucidate the causes of these global declines. Sadly, the first question has been answered affirmatively, allowing the DAPTF to move on to the task of monitoring these declines and investigating their cause or causes. The latter is proving to be a very tough nut to crack, but, as the Chair of the Disease and Pathology Working Group (PWG), I am pleased to announce at least one major breakthrough in this respect: the finding of a novel fungal epidemic of amphibians causing mass mortalities associated with population declines in a range of species in Australia and Central America.

Many of you will already have heard this news. The findings published in the Proceedings of the National Academy of Sciences of the United States of America in July 1998 (Berger *et al* 1998), were reported in many scientific publications, including Nature (Halliday 1998) and Science (Kaiser 1998), and were given extensive media coverage in both Australia and the U.S.A. The story is, however, worth repeating here as the findings and, more to the point, the interpretation of the data, are of major significance to the declining amphibian populations conundrum. Furthermore, this work is partly a consequence of the DAPTF nurturing what has been termed "an exemplary example of international scientific collaboration" (Halliday 1998).

Perhaps the most precipitous of the amphibian population declines have been in the rain forests of Queensland, Australia and Central America. As this has become increasingly apparent over the past decade, so the intensity of research on amphibians in these areas has increased. These studies have demonstrated that amphibians were disappearing from areas not because of migration, aestivation or some other relatively innocuous factor, but because they were dying. And dying in large numbers. The first mass mortality incidents were seen in Australia in 1993, prompting herpetologist Keith MacDonald, virologist Alex Hyatt and veterinarian Rick Speare to seek funding from the Australian authorities to fund a veterinarian, Lee Berger, dedicated to investigating the cause(s) of these deaths. Detailed post mortem examinations of the inevitably limited supply of suitable carcases (dead frogs donít stay fresh or visible for long in tropical rain forests) gradually allowed a picture of infection to be built up that was common to almost all mortality incidents investigated. An unknown parasite was detected in the skin of the animals, but, despite rigorous and extensive testing, little else was found. Gross lesions were rarely seen, in fact macroscopic abnormalities were almost non-existent!

Microscopically, the parasite resembled either a fungus or a protozoan and in order to identify it, samples were sent (via the PWG network) to a protozoan taxonomist, Peter Daszak, in the U.K. At the same time, transmission experiments carried out by Dr. Berger and colleagues in Australia indicated that the skin parasite was highly pathogenic and did indeed kill adult frogs. It was now early 1997 and, in one of those fortuitous accidents of history, the PWG was approached by Karen Lips, a biologist monitoring amphibians in Central America. Dr. Lips had found large numbers of dead frogs in her study site in Panama and required assistance in investigating the cause of the mortality incident. Fortunately, e-mail connections enabled a swift response and put Dr. Lips in touch with Earl Green, an experienced veterinary pathologist and former Chair of the PWG, in the U.S.A.

It wasn't long before Dr. Green
was reporting similar findings in the Panamanian frogs as those described in the frogs from Queensland and shortly afterwards Dr. Daszak indicated that the organism from Australia was a chytrid fungus and not a protozoan. What was equally important was that, in both cases, the frogs were dying in epidemic proportions and in areas where rapid, documented declines were occurring, often to localised extinctions. It was obvious that both research groups had to get together to pool data and to make direct comparisons of the parasites concerned, in order to establish their identification and whether or not the same pathogen was infecting frogs on both continents. Through the DAPTF, Dr. Val Beasley and Prof. Rex Hess of the University of Illinois offered financial and logistical assistance for such a gathering and American Airlines and Conservation International paid for the air flights required. George Rabb, a founder of the DAPTF and Director of Brookfield Zoo played a major part in facilitating the week-long meeting of the scientists concerned.

An intensive week of comparative work, mostly at the electron microscopical level ensued and resulted in the conclusions that (1) organisms infecting frogs in Australia and Central America are indistinguishable on the basis of pathogenesis, light microscopy and ultrastructure, and (2) the organism concerned is a member of a previously-undescribed genus of the chytridiomycete order, *Chytridiales*. DNA sequencing work conducted by Louise Goggins (Australia) and Mark Ragan (Canada) confirmed the taxonomic position of the Australian parasite, although sequencing of the Central American fungus has yet to be performed.

So what does all this mean? Firstly, the paper resulting from the work detailed above contains the first

convincing reports of amphibian population declines being linked to amphibian mortality, and of this mortality being caused by infectious disease. Secondly, and perhaps most significantly, the same pathogen appears to be the sole infectious agent causing this mortality on two disparate continents and in areas described as pristine rain forest habitats. The epidemiological evidence from both continents (Laurance *et al* 1996, Lips 1998) suggests that the causative organism is not naturally endemic to these areas, but that the disease has been recently introduced. This, along with the finding of a sole primary pathogen, strongly suggests the absence or relative insignificance of any co-factors (e.g. contaminants, environmental change) that might also be present. If continued scrutiny verifies the current indications that this "new" chytrid was introduced into previously naïve habitats, this introduction was most likely carried out by humans (either directly or inadvertently with organic materials or other organisms such as plants, fish or amphibians). Such anthropogenic introductions of "alien" pathogens is an increasingly recognised problem which can be devastating (Cunningham 1996) and might yet prove to be one of the most challenging threats to herpetological conservation in particular and to wildlife conservation in general (Halliday 1998, Daszak & Cunningham 1998).

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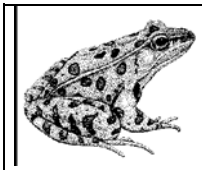

Disease & Immune Function Workshop

From Cindy Carey

A workshop on Amphibian Diseases and Immune Function, sponsored by the National Science Foundation, was held at the San Diego Zoo on July 25-27, 1998. Current observations on the role of disease in amphibian declines and understanding of the immune defenses of amphibians were presented. A review of this subject will be published in the next year. *Reprints of this review will be available from:* Dr. Cindy Carey, Dept. of EPO Biology, Univ. of Colorado, Boulder, CO 80309, USA or by e-mail: **careyc@spot.colorado.edu**

The following consensus statement was produced by workshop participants:

Statement of the Problem

There is compelling evidence for mass deaths among amphibian populations resulting from disease outbreaks, most recently due to viral and fungal pathogens, in diverse geographic locations. The patterns of losses appear to exceed normal population fluctuations and suggest one or more globally emerging disease epidemics that may place existing amphibian populations at risk of extinction. Although amphibians, like mammals, have diverse immune defense mechanisms, the apparent failure of these defenses to prevent infections suggests that new disease agents have been recently introduced and/or some environmental change is either varying the virulence of these pathogens or altering the ability of the immune system to provide adequate defense.

Plan of Action

To avoid population declines that may lead to species extinctions, fundamental research into the nature of specific etiological agents and the mechanisms by which these agents are introduced and spread, the nature of protective immune responses they elicit, the genetic diversity of the immune systems components in various species, and the nature of environmental stressors that may contribute to disease susceptibility is needed. To demonstrate that a selected pathogen is the causative agent of disease, Koch's postulates must be satisfied. Research into the immune responses against a specific pathogen must include studies of both innate and adaptive immunity at different life stages (e.g. larval, metamorphosing and adult) where changes in structure and function of the immune system have been reported. To identify exogenous stressors (in the broadest sense) that may contribute to suppression of immunity and increased susceptibility to disease, model amphibians should be selected for initial laboratory studies to determine sensitive immunological parameters. These studies should be coupled with field collections and field monitoring for rapid detection and identification of pathogens, study of pathogen reservoirs and spread, and putative stressors. The effects of such stressors on host resistance to each selected pathogen following controlled laboratory exposures or field exposures is necessary to demonstrate the impact of each suspected stressor on survival of selected populations.

Signed by:

Lee Berger, David Green, Pauline Brousseau, Alexander Hyatt, Cynthia Carey, James Jancovich, Gregory Chinchar, Joyce Longcore, Nicholas Cohen, Mark Mitchell, James Collins, Allan Pessier, Kathy Converse, Louise Rollins-Smith, Andrew Cunningham, Trenton Schoeb, Peter Daszak, Michael Smolen, Elizabeth Davidson, Andrew Storfer, Louis du Pasquier, Sharon Taylor, David Gardiner.

Swiss study highlights a major problem in the study of amphibian declines

It is widely recognised that, to detect many amphibian population declines, long-term data are required. A recent study of common frog (*Rana temporaria*) breeding populations in Switzerland (Meyer *et al.* 1998) provides such data and illustrates just why they are so important. Three populations were monitored over a 28-year period, from 1970 to 1997; each year, the number of spawn clumps was counted. Two of the populations show no evidence of long-term decline; the third has declined, probably as the result of introduced fish. Most importantly, all three populations show dramatic fluctuations in size. At one site, for example, the number of spawn clumps rose from just over 1000 to over 8000 in 1988 and then declined to about 2500 in 1993, but since then it has returned to its mean value of 3200. In other words, a steady decline over a five-year period can be highly deceptive.

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Tim Halliday, International Director.

DONATIONS

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