

FROGLOG

Newsletter of the Declining Amphibian Populations Task Force
of the World Conservation Union's Species Survival Commission.

June 1997, Number 22.



SEED GRANT DEADLINE

We are pleased to have received a good number of applications for funds from both the general Seed Grant Fund and the Directed Funds provided by our Anonymous Donor. **Please note** that the deadline for receipt of applications for both types of grant has now been set as **15th July 1997**. Applications received after this date cannot be considered.

John W. Wilkinson, International Coordinator.



Disease, Stress and Amphibian Declines

From Cynthia Carey - DAPTF Climate and Atmospheric Change Working Group

Boreal toads (*Bufo boreas boreas*) experienced a massive die-off in the Rocky Mountains of Colorado in the late 1970's and early 1980's. No toads have since been seen in the die-off localities. "Red-leg syndrome", caused by a variety of bacteria or fungi, has been identified as the proximal cause of death. We have been testing how environmental perturbations might cause immunosuppression and increased vulnerability to disease.

UV-B exposure should be high for boreal toad eggs, tadpoles and toadlets and, although there is no evidence to suggest that this was a factor in the mass die-offs, it is important to know if UV-B is now a potentially hazardous factor. In collaboration with researchers from the National Biological Service in Columbia, the levels of UV-B irradiance at breeding sites between 9,800 and 11,400 ft are being compared with tolerance levels of 5 hr

daily exposure to graded levels of UV-B in the laboratory. Preliminary findings indicate that boreal toad toadlets are more tolerant than are tadpoles of this species, and that both the toadlets and tadpoles of the boreal toad are more tolerant than are the same stages of Woodhouse's toad (*Bufo woodhousii*), which is found in Colorado at lower elevations. Boreal toad tadpoles raised indoors with no UV-B exposure were markedly less tolerant than those raised in natural sunlight. No evidence has yet emerged that UV-B levels are responsible directly for toad mortality. Investigations are underway, however, into the sublethal effects of UV-B on immune function and vulnerability to disease.

Tests for 16 different organochlorines in tissues and sediments indicated that these compounds are an unlikely cause of boreal toad deaths. None of these compounds were present above current detection levels.

Some surviving boreal toad populations exist in areas heavily polluted with tailings from silver, gold or molybdenum mines. Metal scans indicated that cadmium, copper, lead and mercury in whole-body concentrations of tadpoles in these populations are among the highest reported in the amphibian literature. Research is continuing to determine how whole-body concentrations correlate with tissue concentrations and how these affect growth, time to metamorphosis and post-metamorphic survival.

Remaining boreal toad populations are too small to justify the removal of animals for study. Environmental effects on immune function are being tested on leopard frogs (*Rana pipiens*), which have experienced extinctions in Colorado and cane toads (*Bufo marinus*), which are widespread and apparently not declining. Since the pH of the precipitation fell significantly during the mass die-off years, we tested whether low pH alone could cause

immunosuppression. Exposure to pH 3.8 for two weeks resulted in significant immunostimulation of lymphocytic mitogenic responses in cane toads and serum complement levels in leopard frogs. Immunostimulation has been observed in frogs subjected to environmental stresses, but the mechanism involved and its relationship to increased vulnerability to disease is not fully understood. No animals of either research species became sick when injected with 10^6 *Aeromonas hydrophilia*, a common pathogen. Low pH alone is therefore an unlikely cause of increased vulnerability to infection.

Cold body temperatures, a continual consequence of life for amphibians at high altitudes, were tested for their effects on immunosuppression. Exposure to 5°C for 5 months significantly depressed proliferative responses of lymphocytes to mitogens and lowered serum complement levels in leopard frogs, but exposure to 10°C for 6 weeks had no effect on these parameters in cane toads. Injection of 10^6 *Aeromonas hydrophilia* again resulted in no deaths. Exposure to cold alone is therefore unlikely to increase vulnerability to infection in these two species.

Laboratory experiments conducted thus far have utilized one experimental variable. Animals in the field, however, are simultaneously exposed to several variables which could act synergistically or antagonistically. Future experiments are designed to address the combination of factors on immune function and vulnerability to disease.

Report on research conducted under a DAPTF Seed Grant award. Contact: Cynthia Carey, Department of EPO Biology, University of Colorado, N122 Ramaley, Campus Box 334, Boulder, CO 80309-0334, USA.

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See also "Publications of Interest", this issue.

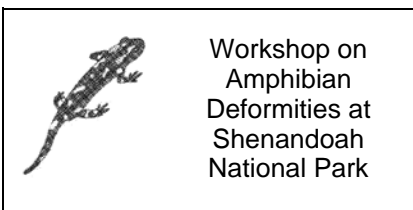


The DAPTF in Prague

There are three DAPTF activities scheduled during the Prague meeting of the World Congress of Herpetology. The previously announced symposium, organized and chaired by Tim Halliday, will be held on Monday, 4 August 1997. On Tuesday, 5 August, we will have an open business meeting. There are two purposes of this meeting: (1) to apprise DAPTF members of activities and plans of the central DAPTF office and leadership as represented by the Board, and (2) to give DAPTF members the opportunity to voice their concerns or suggestions to the DAPTF leadership. We encourage as many of you as possible to attend this meeting. The annual DAPTF Board Meeting (this meeting will be limited to Board members) has been scheduled for Thursday, 7 August, so that the Board can act upon any items that arise from the open business meeting.

For those individuals who will be unable to attend the Prague meeting, I invite you to send any concerns, suggestions, or recommendations that you wish to be brought to the attention of the Board directly to me or to any other Board member.

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Workshop on Amphibian Deformities at Shenandoah National Park

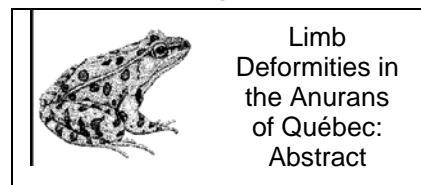
By Martin Ouellet

On April 15-16, 1997, I attended a workshop entitled "Evaluating the increase of amphibian malformations" at Shenandoah National Park, Virginia. Sponsored by the US Environmental Protection Agency, and organized by Joe Tietge, it was convened to address the "apparent" increase in deformed frogs in some areas of North America. It emphasized data collection objectives, field methods, and information networks. Presentations were given by Joe Tietge, Ron Heyer, David Green, Sam Droege, Martin Ouellet, Rick Levey, Dave Hoppe, Ken Muneoka, Stan Sessions, Kathy Converse, Andrew Blaustein, David Gardiner, Dave Mount, Steve Paulsen, and Doug Johnson. Some attendees were mainly interested in deformed frogs in

relation to human health, while others were concerned by the direct relevance to amphibian population decline. My impression is that there are insufficient data to ascertain if deformities constitute a serious threat to amphibian populations. Limb deformities and other abnormalities have been encountered mostly in metamorphosing anurans, with cases having been reported in the international literature since 1740. Attempted predation, trematode parasite infestation, and xenobiotic chemicals (perhaps affecting the retinoic acid pathway) emerged among the leading hypotheses to explain the occurrence of anomalies in the wild. Keeping in mind that habitat destruction is the greatest challenge to preserving amphibian populations, participants agreed that further studies are required to assess the extent of deformities in amphibians, along with their potential causes.

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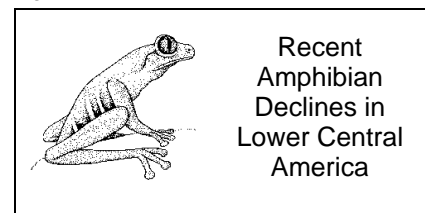
Limb Deformities in the Anurans of Québec: Abstract

Ouellet, M.; Bonin, J.; Rodrigue, J.; DesGranges, J.-L. and Lair, S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *Journal of Wildlife Diseases* 33: 95-104.

High prevalences of hindlimb deformities were recorded in wild-caught green frogs (*Rana clamitans*), northern leopard frogs (*Rana pipiens*), American toads (*Bufo americanus*), and bullfrogs (*Rana catesbeiana*) from agricultural sites exposed to pesticide runoff in the St. Lawrence River Valley of Québec, Canada, between July and September 1992 and 1993. Of 853 metamorphosing anurans examined in 14 farmland habitats, 106 (12%; range 0 - 69%) had severe degrees of ectromelia and ectrodactyly, compared to only two (0.7%; range 0 - 7.7%) of 271 in 12 control sites. However, the variation in the proportion of deformities among sites was too large to conclude that there was a significant difference between control and pesticide-exposed habitats. Clinical signs varied and were characterized by segmental hypoplasia or agenesis of affected limbs. Conspicuous abnormalities interfered with swimming and hopping,

and likely constituted a survival handicap. Because of circumstances and the frequency of these malformations in nine distinct habitats, and in three different species from one of our study sites, we propose a teratogenic action of exogenous factors. Despite the fact that many biotic and abiotic agents are potentially harmful to limb development, agricultural contaminants were suspected as primary aggressors. Thus, clinical examination and frequency of deformities in anurans might be an economical screening tool to assess ecosystem health and the presence of environmental contaminants.

The paper also presents a review of different agents potentially harmful to leg development.



Recent Amphibian Declines in Lower Central America

By Karen R. Lips

The Zona Protectorada Las Tablas, Puntarena Province, Costa Rica is a high-elevation (1900m) site in the Cordillera de Talamanca. It comprises part of the Amistad Biosphere Reserve. I have been studying stream-breeding frogs at this site since 1990. The first sign that something was wrong was when I found six dead and dying individuals of five species between 1992 and 1993. By 1994, previously abundant species (eg. *Atelopus chiriquiensis*) were not as common, but a delayed rainy season was thought to be responsible. When I next returned in 1996, something was clearly wrong: five species were reduced to <10% of past abundance (*Hyla calypsa*, *A. chiriquiensis*, *Eleutherodactylus sp.*), and some tadpoles had abnormalities of the mouthparts. Similarities in the pattern of mortality and the taxa affected between this site and Monteverde suggested that the same agent was responsible (Lips *in press*), but actual cases were not obvious.

One way to determine causation of these mysterious declines would be to collect similar data from multiple sites and to time the surveys so that (at a minimum) records would exist from before, during and after the die-offs. It seemed logical that if the frogs died in Monteverde in 1988 and at Las Tablas in 1994, then sites further to the south might be affected next. In December 1996 - January 1997 I visited the Reserva Forestal Fortuna, Chiriquí Province, Panama. I had previously surveyed the amphibians at

this site in 1993 and again in 1994-95. During those visits, I encountered over 55 species of amphibians. In 1996-97, I conducted 44 surveys of seven streams over a three-week period and encountered only 24 amphibian species. Only two streams had levels of amphibian abundance equal to past years, while the other five were essentially frogless. It seemed that I had arrived at the height of the decline. In addition to reduced numbers overall, I found 54 dead or dying frogs belonging to 10 species. Many of the casualties still had a very life-like appearance; most were found during morning surveys, still sitting in a perched position. Dying individuals were lethargic, had no righting response and exhibited convulsions and trembling of the limbs and head. Several dying adults were somewhat thin, but none had any obvious lesions or wounds, although two *Rana warzewitschii* tadpoles were found swimming in an uncoordinated fashion and one had a bloody lesion on the body. Several of these symptoms are similar to those described for dying frogs from an Australian site that experienced a decline, and from which viruses have been isolated from preserved specimens (Speare *et al.* 1994). I collected and preserved the Fortuna casualties, and sent them to the Animal Health Diagnostic center in Maryland, where D. Earl Green of the DAPTF Disease and Pathology Working Group is coordinating the tissue analysis.

I believe that declines at Las Tablas and Fortuna were caused by the same agent that reduced the montane amphibian fauna of Monteverde, Costa Rica. In all cases the declines occurred suddenly, and rapidly decimated populations of previously common species, especially diurnal and stream-dwelling taxa such as *Bufo*, *Rana* and *Atelopus*. The most parsimonious explanation is that these three sites (which are similar in geology, climate and biota) have suffered similar sudden, massive declines of their amphibian faunas because of the same causative agent. If this is true, then the directional movement suggests a "front" or wave of mortality which is not consistent with mortality due to the introduction of predators, El Niño Southern Oscillation (ENSO) events or habitat destruction, but a pattern more typical of disease epidemics.

I propose to test this "extinction wave" hypothesis by continuing surveys of amphibian populations on both sides of this front. If declines in Monteverde, Las Tablas and Fortuna were found to be caused by a pathogenic virus spreading southward

through Central America, then intervening upland sites should have already suffered considerable loss of their amphibian species, and upland sites east of Fortuna will be affected next.

I believe that it is especially important for all researchers interested in amphibian declines to collect similar data (numbers of adults per metre and/or per minute of survey, presence of larvae and eggs, and condition of larval mouthparts), preserve dead and dying animals in a consistent manner (freezing, 10% formalin, EM fixative and/or 95% ethanol), and to collaborate with pathologists, climatologists and local residents to understand the full scope of possible agents of mortality and to identify their actions.

Lips, K.R. (*in press*) Decline of a tropical montane amphibian fauna. *Conservation Biology*.

Speare, R.; Field, K.; Koehler, J. and McDonald, K. (1994) Review of the presentation: Disappearing Australian rainforest frogs: Have we found the answer? *Froglog* 9.

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Extract from
Alytes

The following is extracted from an editorial by Dr. Alain Dubois marking the 15th year of publication of the journal *Alytes*.

The recent international interest in the problems of conservation of amphibian populations and species has also clearly emphasized the need for a particular effort of the international community, not only to understand the causes of the observed declines and to try and stop or reduce them, but also to increase and speed up our efforts for the mere work of inventory and description of amphibian species on our planet: it is now clear that, if we delay considering this task as a major priority for amphibian specialists of our time, many amphibian taxa will become extinct in the forthcoming years without their having even been collected and recognized by man. This is well exemplified in this issue of *Alytes* (Volume 14, Number 4), which contains the descriptions of several new species, all of which are clearly threatened with extinction, and some of which may even be already extinct as these descriptions are published. It is rather unusual to publish a paper

dealing with the contemporaneous fauna that describes four new species but at the same time states that they were probably already extinct when these descriptions were written. Unfortunately, it is very likely that in the coming future we will have to confront such situations more and more often, as a consequence of ongoing massive destructions or extensive modifications of amphibians' habitats throughout the world. It results from this that basic alpha taxonomic work of inventory and description of amphibian species is an urgent matter that should deserve all our attention, including the creation of academic positions and financial support for field work, collection management and descriptive activity. All well considered, this might be much more urgent than concentrating all efforts on more 'theoretical' issues of biology, however interesting and important the latter may be.



DAPTF
Reports

The DAPTF office is producing a series of occasional reports documenting the amphibian decline phenomenon and the efforts of those persons who have undertaken to study it. The first in the series, a report on the activities of the Philippines Working Group by Angel Alcala, is now available on request from John Wilkinson (address below). A second report, on the status of the amphibians of Georgia (former Soviet Union) by David Tarkhishvili, will be available shortly.

Two copies (or an e-mail version) of all potential contribution, should also be sent to John Wilkinson. Submissions should be concise and directly relevant to the amphibian decline phenomenon. Tables and figures should be kept to a minimum.



Froglog Shorts

Craig Hassapakis, Editor of the new journal *Amphibian and Reptile Conservation*, is always looking for papers on amphibian declines for possible publication. For more information, e-mail him on ARC@byu.edu or visit their website at:

<http://www.byu.edu/~arcon/>

Britta Grillitsch is the Working Group Chair for our new group covering Austria. Contact: Dr. Britta Grillitsch, Lab. Ökotox./Inst. Versuchstr. Veterinärmed., Univ. Wien, Linke

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We are also pleased to have a new working group for Croatia. *Contact the Group Chair:* Sveltana Lupret-Obradovic, Ljudevita Gaja 42, 10290 Zapresic, CROATIA.

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UN Report on the World's Freshwater Resources: The Rio Summit of 1992 stressed the importance of holistic management of the World's freshwater resources, not only for the human population, but also for the preservation of biodiversity. A recent report provides an analysis of the Earth's water supplies and concludes that, in many developing and developed countries, current use of fresh water is not sustainable. There are many instances of local and regional deterioration in water quality and quantity, largely as the result of widespread pollution and over-exploitation. The report emphasises the potentially damaging impact of this trend on biodiversity.

UN (1997) *Comprehensive assessment of the freshwater resources of the World. Report of the Secretary General.* UN Commission on Sustainable Development. Report No. E/CN.17/1997/9.

A new journal, *Advances in Amphibian Research* in the Former Soviet Union is soon to be produced by the DAPTF-CIS Working Group and the Russian Academy of Sciences. *Please contact the Editor:* Dr. Sergius L. Kuzmin, Institute of Ecology and Evolution, Russian Academy of Sciences, Leninsky Prospect, 33, Moscow 117071, Russia.

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DONATIONS We gratefully acknowledge receipt of the following donations from 15 January through to the end of March 1997. An asterisk indicates a response to the challenge grant for global change research Seed Grants announced in Froglog 21.

Organizations: Association of Reptilian & Amphibian Veterinarians; Colorado Herpetological Society; Florida West Coast Herpetological and Conservation Society; Lincoln Park Zoo; Minnesota Herpetological Society; Reptile & Amphibian Magazine; *Institute of Herpetological Research at Santa Barbara Zoo; *The Zoological Society of Philadelphia.

Individual donors: Anonymous; Mary E. Brewster; Don Burton; Thane Caro; Steve Connors; Donald & Eugenia Farrar; John Ferner; Katherine V. Fite; Kathryn Freemark;

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The DAPTF web pages have recently been updated, and contain new links to other amphibian conservation resources as well as some new DAPTF pages. They remain located at the URL:

http://www.open.ac.uk/OU/Academic/Biology/J_Baker/JBtxt.htm

Froglog on the web can be found at:

<http://acs-info.open.ac.uk/info/newsletters/FROGLOG.html>

The DAPTF Media Briefings can be found at: http://www.open.ac.uk/OU/Academic/Biology/J_Baker/DAPTF.Press.html

We welcome any suggestions, and would particularly like to hear from any of our Working Groups with home pages which are not linked to our site. Please contact John Wilkinson (address below).



DAPTF stickers are now available. Each sticker is 4" (10cm) in diameter and pictures the *Neobatrachus* motif (above), plus contact details for the DAPTF office, in black on green. The design is available in both **window-sticker** and **adhesive-backed** form, and each costs £1.00. Please send cheques or international money orders (in British pounds only please) made payable to "DAPTF", to John Wilkinson at the DAPTF office, address below. Remember to state which type(s) of sticker you would like!



Carey, C.; Maniero, G.D.; Harper, C.W. and Snyder, G.K. (1996) Measurements of several aspects of immune function in toads (*Bufo marinus*) after exposure to low pH. In: Stolen, J.S.; Fletcher, T.C.; Bayne, C.J.; Secombes, C.J.; Zelikoff, J.T.; Twerdok, L.E. and Anderson, D.P. (eds.) *Modulators of immune responses: the evolutionary trail.* SOS Publications, Fair Haven, NJ.

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