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Successful Treatment of Chytridiomycosis

By Donald K. Nichols and Elaine W. Lamirande

Batrachochytrium dendrobatidis is a recently discovered species of chytridiomycete (chytrid) fungus (Longcore et al., 1999) that has been isolated from many different amphibian species with fatal skin infections (Longcore, 2000). In experimental studies with dendrobatid frogs (*Dendrobates tinctorius* and *D. auratus*), we showed that *B. dendrobatidis* can be a primary pathogen (Nichols et al., 2001). The earliest clinical sign of chytridiomycosis in dendrobatid frogs is excessive shedding of skin; ante mortem diagnosis of chytrid infection can often be made through microscopic examination of shed skin and/or skin imprints (Nichols et al, 2001).

In another study, we experimentally infected juvenile *D. tinctorius* and then, once excessive skin shedding had begun, we treated them topically with one of three antimicrobial drugs: trimethoprim-sulfadiazine (TMS), miconazole, or itraconazole (Nichols et al., 2000). A powdered form of itraconazole was suspended in 1% methyl cellulose to make a 1% suspension of itraconazole; this was then diluted with 0.6% saline to a final concentration of 0.01% itraconazole in suspension. A 1% solution of miconazole was diluted with 0.6% saline to a final concentration of 0.01% miconazole. A 48% solution of TMS was diluted with saline to a final concentration of 0.1%. Infected frogs were treated by immersion in one of the three solutions or suspensions for five minutes per day for either eight (miconazole) or 11 (itraconazole and TMS) consecutive days. At the end of the study, all frogs were euthanized and examined histologically.

Although treatment with TMS prolonged the frogs' lives compared to untreated controls, it did not eliminate the chytrids from the frogs. All frogs treated with either miconazole or itraconazole were cured of chytrid infection. However, the miconazole treatments were poorly tolerated by the frogs which caused us to end that part of the experiment earlier than planned; in retrospect, this was probably due to ethyl alcohol in the original 1% stock solution.

We have subsequently used topical baths in 0.01% suspensions of itraconazole to successfully treat other experimentally infected *D. tinctorius* (Nichols and Lamirande, 2000) and naturally infected *Litoria caeulea* (unpublished data). This appears to be a highly effective treatment for chytridiomycosis in captive juvenile and adult anuran amphibians. Further studies are needed to determine if this treatment is efficacious and safe to use in tadpoles and caudates.

Itraconazole is a potent drug with fungicidal activity against a wide spectrum of fungal organisms. Therefore, it should not be indiscriminately used to treat wild populations of amphibians or to prophylactically treat captive animals.

Itraconazole is commercially available as a 1% solution (Sporanox, Janssen Pharmaceutica, Inc.), rather than the suspension that we formulated. This solution contains hydrochloric acid and propylene glycol as solvents and has a pH of 2; we do not know if this solution is safe to use on amphibians. Our experience with the frogs' reactions to the diluted miconazole solution clearly illustrates that caution must be exercised when attempting to use various drug formulations to treat amphibians.

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The Frog Princess and Other Projects

By Mark Pestov and Vladimir Anufriev

The Nizhny Novgorod Society for the Protection of Amphibians and Reptiles (NNSPAR) was formed in 1998 as a department of the large nonprofit organization "Dront" Ecological Center. NNSPAR now has more than

50 members. It is one of the largest organizations of its type in Russia.

Nizhny Novgorod province is situated 400 km east of Moscow. Its total area is 42,000 km^2. The vegetation varies from the southern taiga subzone to the semi-steppe zone. There are 12 species of amphibians and 7 species of reptiles recorded on the province's checklist.

During the last 10 years, 5 species of amphibians have been listed as threatened in the red list of Nizhny Novgorod province (*Salamandrella keyserlingii, Triturus vulgaris, T. cristatus, Bombina bombina* & *Pelobates fuscus*). Declines have taken place in districts with high levels of habitat destruction, especially around the towns. Other problems include pollution in freshwater streams and ponds. Some species like *Triturus vulgaris* and especially *T. cristatus* are caught by children in spring and many of them die. Many people have a negative image of more common species like frogs and toads.

We started our activities with the "Frog Princess" project (the Frog Princess is a hero of popular Russian folklore). The project includes research, publications in the mass media, children's pictures, photos, stories about amphibians and reptiles & practical activities for the protection of amphibians and reptiles.

Nizhny Novgorod Provincial Government (through the Department of Nature Protection and Management) financially supported the project in its initial stages (1999). In 2000 we got a grant from the ROLL project for the continuing of our activity (ROLL is a joint project of the US Agency for International Development and the Institute for Sustainable Communities). During its development, more than 1000 people from 40 districts (especially pupils) were involved in the project. In 2001, we were successful in the new round of the ROLL project. We got a grant (about $30,000) for the dissemination of our experience in the Frog Princess project for another 5 Russian provinces.

Since 1999, members of our Society have begun a special research program to determine the status of amphibian and reptile populations in the province. The Department of Nature Protection and Management and the ROLL Project have also financed this program. The results will be published as a book in spring 2001 (in Russian).

Another recent project was the "Children's Garden for the Siberian Newt". There are only 30 isolated populations of this species in European Russia. Two of them are recorded in our province and are separated from each other by 100 km. The abundance of this species in the province is now extremely low. In 1999-2000, only 200 breeding pairs were observed in both populations. Last summer, our Society constructed 19 artificial ponds (like anti-fire ponds for forestry) along the depressions of forest roads, which are used for the reproduction of the Siberian newt in both populations (8 ponds for one population and 11 for the other). All ponds are 10-200 m^2 in area and 1 m deep. We hope to communicate the results of this action through *Froglog* in the future. We would like to conserve the *Salamandrella keiserlingii* population through the making of new artificial populations of this species in one of the nature reserves in the north part of the region too.

Another planned project is for *Hyla arborea arborea*. It would be a great success to reintroduce this species into the southern part of our region. Unfortunately, there is no information about this species in the fauna of our province. It was known to live to the west and east of our region (Perm and Moscow provinces) 100-200 years ago. A very interesting reserve landscape exists for it. It is karst with a lot of small caves and untypical (more southern) vegetation and a specific microclimate. We also plan to make some films about the most interesting species of amphibians and reptiles and about the problems of species conservation in our region too.

We are especially interested in contacting colleagues who have practical experiences with the problems of amphibian conservation and protection (including reintroduction and management), and persons who are interested in natural history film-making in Russia.

For more information contact: "Dront" Ecological Center, Kostina str., 2, Office 164, 603134, Nizhny Novgorod, Russia.
Web site: **www.dront.ru** (in Russian).

Parasitic Copepods Responsible for Limb Abnormalities?

By Leong Tzi Ming

Of the various hypotheses proposed to be the cause of abnormalities in amphibians, four (parasites, toxins, predation and UV-B) have been supported with reasonable evidence (Reaser & Johnson, 1997). Recent research into deformity–inducing parasites has focused primarily on trematodes (eg. Sessions, 1998; Johnson et al, 1999). Apart from trematodes, other forms of parasites have been known to use amphibians as hosts for the completion of their life-cycle. These include parasitic copepods of the genera *Argulus* (Goin & Ogren, 1956) and *Lernaea* (Martins & Souza, 1996). However, there have been no accounts of abnormalities arising from parasitic infestations by such copepods.

In January, 2001, a batch of tadpoles belonging to the copper-cheeked frog *Rana chalconota* (Schlegel) was collected from a disused fish pond in Sukabumi, West Java, Indonesia. This is a common species, which occurs from lowland to above 1200 m and has a preference for stagnant waters such as fish ponds (Iskandar, 1998). From this batch, a total of 214 tadpoles were counted (Gosner Stages 26-42, total lengths 15.1-49.2mm). Among them, 144 tadpoles (67.3%) were found to have at least one to as many as 15 individual copepods (*Lernaea cyprinacea*) attached to their bodies and/or tails. Of the tadpoles with the externally visible copepods, eight tadpoles were observed to exhibit various types of limb abnormalities. Brachymely was recorded for three tadpoles (Stages 36-39), which had relatively shorter right hind limbs. Polymely was recorded for four tadpoles (Stages 36-41), two of which further exhibited polydactyly, and another further exhibited brachymely. The eighth tadpole (Stage 42) exhibited ectromely, whereby the entire right hind limb was missing, with no signs of scar tissue.

Although the incidence of abnormal tadpoles was low (0.06% of infected tadpoles), this is possibly the first time in which amphibian abnormalities are associated with parasitic copepods. While *Lernaea* has been found to parasitise fish, such as *Poecilia reticulata*, its affiliation with tadpoles has not been documented in Java (Iskandar pers. comm.). Observations of the most common points of attachment on the tadpole host by the copepod parasite agree closely with studies by Martins & Souza (1996). These two main 'hot-spots' are around the mouth and vent. It is the parasites around the vent, which are most likely to have caused malformations in limb development, as the site of anchorage (whereby the embedded head of the copepod is fastened sub-dermally) may be the

region of limb-bud development. Further study into this probable cause needs to be carried out to better ascertain the role of the copepod parasite in limb malformations.

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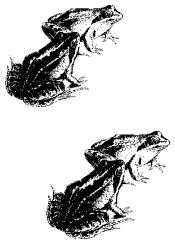

Reduced Genetic Diversity in Swiss Populations of the Italian Agile Frog, *Rana latastei*

By Trent Garner and Peter Pearman

Rana latastei (Boulenger) is endemic to only four countries in central Europe but is found predominantly in northern Italy, where the distribution is limited primarily to the drainage basin of the Po River. A few populations exist within Croatia and Slovenia, and the species range extends slightly into Switzerland (Kanton Ticino). The IUCN classifies this species as lower risk and near threatened, but within Switzerland *Rana latastei* is considered to be a red-list species by the Bundesamt für Umwelt, Wald und Landschaft. In the spring of 2000, we initiated a study of the genetic diversity present in Swiss populations, with the goal of identifying potential conservation units within Ticino. We also compared the genetic diversity of Swiss populations to that detected in a limited sample of larvae from the northeastern part of the Italian range. We used seven dinucleotide microsatellite loci (Garner & Tomio, in press) to generate allele frequency data for 13 Swiss populations and four Italian populations. Most Swiss populations were sampled for over 15 individuals (Switzerland total N = 199), while the largest sample for any Italian population was five individuals (Italy total N = 12). Even with Italian populations so under-represented, we found Swiss populations to be genetically depauperate when compared to Italian populations. Both the percentage of polymorphic loci and the unbiased estimate of mean heterozygosity per locus were statistically lower in Swiss populations, while Swiss populations were fixed at two of the seven loci used for analysis. Genetic distance measures showed that Italian populations differed substantially both from Swiss populations and from each other, while genetic distances between Swiss populations were, by comparison, small (Garner & Pearman, submitted).

Such genetic depletion in amphibian populations has been noted before (Seppa & Laurila 1999), and correlates with lowered larval fitness (Hitchings & Beebee 1997, Rowe *et al*. 1999). Switzerland exists at the periphery of the species range and peripheral populations often exhibit depleted genetic diversity. At present, we are sampling across the entire species range to determine if Switzerland is a unique situation, or if breeding ponds located around the entire periphery of the range always exhibit a paucity of genetic diversity. If so, populations located at the periphery deserve special conservation consideration, especially if genetic depletion correlates with lowered fitness. In the end, we hope to derive the pattern of nonadaptive genetic diversity across the entire species range. We can then address specific issues of adaptive variation between breeding locales, with the goal that species management can be implemented with both historical genetic and adaptive variation in mind.

Acknowledgements
Many thanks to Kurt Grossenbacher for showing PP breeding locales in Ticino, and to Nicola Bressi for sending us larvae from Italy.

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Habitat Fragmentation and Amphibian Species Richness in Riparian Areas of the Parana River, Argentina

By Paola M. Peltzer & Rafael. C. Lajmanovich

Riparian areas and wetlands adjacent to the waterways of the Parana river offer favourable conditions for the existence and distribution of many species of amphibians. Moreover, human impact (agriculture, turning of marshes into steppes, setting up of settlements, deforestation) has gradually altered the conditions of the floodplain woods. Remarkably, the study area is a remnant of the Paranaense Province of the Amazonian Phytogeographic Dominion, mainly represented by mixed fluvial forests. Several authors have suggested that amphibians may be particularly sensitive to habitat degradation and fragmentation in agricultural and industrialized regions because of their complex life history patterns, metapopulation dynamics, and because of the fact that during their ontogenesis they are exposed to various environmental factors. Fragmentation could also affect the spatially and temporally dynamic

nature of amphibian populations and result in lower species richness.

We studied the evidence for changes in anuran species composition caused by habitat fragmentation in riparian areas of the Middle Parana River (31º30´S; 60º20´W - Entre Ríos province, Argentina). Our hypothesis was that anuran species richness would be negatively affected by fragmentation and would be strongly influenced by fragment characteristics.

The most consistent result of this study was that all anuran guilds had a negative association with the presence of human dominated ecosystems. Sites with remnants of fluvial mixed forest showed the greatest species richness. The 20 recorded amphibian species appear to vary with respect to climatic factors (temperature and relative humidity), habitat size, time of isolation, vegetal heterogeneity, level of disturbance and noise presence. Amphibian species on the surveyed sites appear to vary with respect to area-sensitivity because species inhabiting different habitat patches dropped out of assemblages in a similar order with decreasing island size.

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Froglog Shorts

Conference announcement: *'The times they are a changing'* Climate change, phenological responses and their consequences for biodiversity, agriculture, forestry, and human health, December 5 to 7, 2001, Wageningen, The Netherlands. Visit the website for more details: **http://www.dow.wau.nl/msa/epn/conference**

For details of the 2001 Natural History Workshops at the University of Wisconsin-Milwaukee Field Station, check out the website at: **www.uwm.edu/Dept/fieldstation**

For details of grant opportunities from Cleveland Metroparks Zoo, visit: **www.clemetzoo.com**

Papers are invited for the ASTM symposium on *Multiple Stressor Effects in Relation to Declining Amphibian Populations* which will be sponsored by ASTM Committee E47 on Biological Effects and Environmental Fate and convened April 16-17, 2002 in Pittsburgh, Pennsylvania. More information is available from Greg Linder, USGS/BRD/CERC, HeronWorks Field Office, Brooks, OR 97305 USA. Tel: (503) 390-3916, fax: (503) 390-3916; e-mail **linder2@open.org** or **linder2@usgs.gov**

Conservation News

- A mass mortality among frogs in Nova Scotia, Canada, is thought to a result of a long, very cold winter.
- Conservationists are using drift fences and pitfall traps to rescue tiger salamanders that are being killed in large numbers as they cross roads in Bow Valley Provincial Park, near Calgary, Canada.
- Cane toads (*Bufo marinus*) apparently now threaten endangered Iriomote wildcats (*Felis iriomotensis*) on Iriomotejima Island in Okinawa, Japan. Only about 100 are thought to remain. The first cane toad was captured on the island in December 2000. They probably arrived there from a nearby island, where they were first introduced to control sugar cane pests. There is also concern for other island predators and for the island's native amphibians and reptiles, which may well end up on the menu. See **http://jin.jcic.or.jp/atlas/nature/nat21.html** & **http://www.japanupdate.com/previous/01/06/07/story4.shtml**

Publications of Interest

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The report "**Population Declines of Amphibians in Latin America**", describing the three international workshops held in November 1999 to discuss the amphibian decline problem is now available (cf. Lips et al. 2000, Froglog 37:1-2). *To request a copy of this English-Spanish report, contact Bruce Young at* **bruce_young@abi.org**

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