

# HALTING THE GLOBAL DECLINE IN AMPHIBIANS: RESEARCH AND PRACTICE

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RESEARCH POSTERS PRESENTED

## **Spatialepidemiology.net/bd and EpiCollect – A global data collection and mapping framework for *Batrachochytrium dendrobatidis***

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To address the global spread of *Batrachochytrium dendrobatidis* it is crucial that we have a dedicated system to collect, map and model the prevalence of infection, in order to aid control measures directed against the pathogen. We have developed a web-based data collection and mapping tool which can be accessed by a range of audiences (scientists, policy makers and the public) which is designed to address a number of questions (spread, epidemiology, evolution, control).

The aim of spatialepidemiology.net/bd is to provide a truly global, community-focussed resource. All data are presented in a geographical context, allowing for identification of areas of potential spread, and temporal analysis allows previous spread to be modelled and used to identify areas of concern. Multiple private databases are available and users can enter and analyse their data independently or in conjunction with publicly available data. These analyses can be made available to policy makers, resource managers, the scientific community and the public.

Data can be entered into the system via the web or by using a novel mobile phone-based collection tool, EpiCollect, which allows field researchers to geo-tag and record epidemiological data on site and for all data to be streamed over mobile networks to the central database, allowing timely recording of *Bd* incidence.

All data on the public site will be downloadable, allowing further analysis and synthesis to be carried out by interested parties.

## **Habitat management and emerging infectious diseases: a study on Canadian soil**

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Although amphibian populations in Canada are under pressure from a wide array of threats, the severest factor affecting them is habitat destruction, alteration, and fragmentation. Wetlands in the prairies are down to 30% of their historical range while in southern Ontario only 10% of wetlands remain. Thus, populations of amphibians relying on these wetlands for survival and reproduction are segregated, condensed, or extirpated. Range-restricted populations are generally more vulnerable to decline because of their increased sensitivity to habitat loss and their low genetic variability. Therefore, emerging infectious diseases (EID) can have devastating effects on these populations. This concern is reflected in research projects at Laurentian University. Past projects include the evaluation of a non-lethal sampling method, which can assess infection by ranaviruses in toe clips, the relationship between human-influenced habitat and the prevalence of *Batrachochytrium dendrobatidis* and ranavirus FV3 in amphibian populations, and the study of developmental instability in green frogs (*Rana clamitans*) in relation to the same pathogens. Upcoming studies will possibly concentrate on the consequence of habitat fragmentation on outbreaks of infectious diseases in amphibian populations in Ontario. Associations between EIDs and habitat fragmentation have already been observed in fruit bats and in

humans in previous scientific articles, emphasizing underlying and provident ecological relationships between animals and their environment. As amphibians are particularly sensitive to environmental changes (in water and terrestrial habitats), understanding the consequences of current anthropological infrastructures on amphibian populations will accentuate the importance of habitat restoration and conservation to animal health.

**First report of ranavirus in the common midwife toad (*Alytes obstetricans*) in Spain**

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We describe the pathology, isolation and characterization of a novel systemic haemorrhagic ranavirus responsible for a disease outbreak with a high mortality rate affecting tadpoles of the common midwife toad (CMT) (*Alytes obstetricans*) in the National Park of the "Picos de Europa" in Northern Spain. Macroscopically, systemic haemorrhages were observed. Microscopic lesions were similar to those described for the systemic haemorrhagic form of ranavirus disease in frogs and other amphibians, consisting primarily of variably sized foci of necrosis throughout most organ systems. Round, intracytoplasmic, basophilic inclusions consistent with ranaviral inclusions were present in the skin, liver, kidney and gastrointestinal tract. Virus was isolated from total homogenates of diseased toad tadpoles following inoculation of epithelioma papillosum cyprini cells. Molecular characterization of the virus, including partial sequence analysis of the DNA polymerase and major capsid protein genes, confirmed that the isolated virus was a ranavirus with marked sequence identity to EHNV, FV3, TFV, ATV and other members of the genus *Ranavirus* in the Iridoviridae family. We have provisionally named the virus "common midwife toad virus" (CMTV). A rabbit anti-serum raised against purified virions was prepared and used to definitively demonstrate systemic distribution of the virus. Immunolabelling for CMTV was observed within the skin, liver, kidney and gastrointestinal tract. The mechanism of CMTV emergence in the CMT is unknown. It is important to remain vigilant elsewhere in Spain in order to detect new outbreaks of ranavirus disease, to monitor the possible spread of this pathogen and its possible impact on wildlife biodiversity.

**Elimination of the amphibian chytrid fungus by Archey's frog, a critically endangered New Zealand endemic species and the no.1 EDGE amphibian**

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Archey's frog, *Leiopelma archeyi*, is a critically endangered New Zealand endemic species. Twelve wild-caught Archey's frogs naturally infected with the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, were monitored in captivity by clinical signs, weight gain and repeated PCR tests. Eight frogs were treated with topical chloramphenicol without knowledge of PCR results for *B. dendrobatidis* at day of entry into trial. Eleven of the 12 frogs (92%) were clear of infection within 3 months of capture,

even though they were held at 15°C and in high humidity, conditions ideal for the survival and propagation of *B. dendrobatidis*. *Batrachochytrium dendrobatidis* in the remaining positive frog was eliminated after treatment with topical chloramphenicol. Archey's frog appears to have a low level of susceptibility to the clinical effects of chytridiomycosis. Individual frogs can eliminate *B. dendrobatidis* and Archey's frog can apparently be treated with topical chloramphenicol with no apparent acute adverse reactions. During re-infection experiments Archey's frogs were able to self-cure, without any treatment, within 5 weeks. The significance of the amphibian chytrid fungus for wild populations of Archey's frog needs to be determined by a longitudinal study in an infected wild population to correlate the presence of *B. dendrobatidis* in individual frogs over at least 3 years with clinical assessment, survival, growth and body condition parameters.

### **Investigating amphibian responses to habitat change in northern Thailand: a pilot study using predictive models**

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Habitat loss, and particularly tropical deforestation, is recognised as the leading cause of global amphibian declines. Accelerating rates of habitat loss and a lack of connectivity between existing reserves demand new approaches to promoting the persistence of amphibians in landscapes dominated by secondary and degraded habitats. Critical to the success of these efforts is improving our ability to understand how species differ in their responses and susceptibility to habitat modification, the basis for assessing extinction risk.

Investigating associations between species occurrence and microhabitat features, and using these to construct predictive models, is a potentially powerful tool for clarifying our understanding of factors that underlie species-specific responses to disturbance. At present, little data are available regarding habitat associations in tropical frogs, particularly in understudied regions such as Southeast Asia.

Making use of a sequence of artificial pools at a site in north-eastern Thailand, I investigated the occurrence of four frog species along a natural habitat gradient from moist evergreen to dry deciduous forest. I hypothesised that amphibian distributions are most strongly limited by local characteristics of their reproductive habitat, and that differences in community structure and species occurrence would therefore be related to microhabitat-scale differences in breeding-site characteristics.

The results support this hypothesis for single species, although community structure as a whole could not be related to any measured variable. The success of predictive models in recovering the observed distributions of three species from a small number of variables provides encouraging support for this approach to investigating species-habitat associations.

### **Reintroducing the northern clade pool frog to England**

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The pool frog (*Pelophylax lessonae*) is one of the widely-distributed European 'green frogs'. British records date back to the late 1700s and there are documented introductions from this period. When the status of a small, and apparently declining, population of pool frogs in Norfolk was reviewed during the early 1990s, the conservation community was faced with some very basic questions: was the population still extant; what species was it and was it native to the UK?

Surveys determined that the frog was extinct at the site, with just a single male left in captivity. Archive studies confirmed the species to be *P. lessonae*, and that there had been a debate since the 1770s about its native status. Genetic analyses were carried out on the surviving male and on museum specimens, and an analysis of calls was conducted. These techniques confirmed that the Norfolk frogs were closely related to pool frogs from Scandinavia (the 'northern clade') but different from those

originating from documented introductions. Examination of material from archaeological sites in the East Anglian Fens identified two pool frog bones pre-dating these introductions.

Evidence from the different lines of research all indicated native status, so a reintroduction strategy was developed, assessing ecological needs and identifying a suitable release site. Habitat management was initiated at the chosen site and, with appropriate licensing and veterinary checks, the species was reintroduced, with tadpoles, juveniles and adult frogs being released over four years (2005–2008). All life stages appear to have fared well and a monitoring programme is in place to determine whether the reintroduction is a success in the long term.

### **Ghost frogs – Living on the EDGE**

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In 2004 the Global Amphibian Assessment indicated that 32% of the world's amphibian species are threatened. Of the South African frog species, 15% are listed as threatened in various categories of intensity. Two of the four critically endangered frogs in South Africa are ghost frog species and they are high on the local EDGE species list for conservation research. Ghost frogs belong to the Heleophryniidae family, endemic to South Africa, and are restricted to swift-flowing, permanent mountain streams. These species are extremely well adapted to this environment: they are dorsoventrally flattened so that they can hide in deep crevices; extensive webbing on the feet makes them excellent swimmers; well-developed discs on the toes ensures a firm grip on rocks in fast-flowing water; limbs are long and fingertips and toe tips are expanded to improve grip on wet rocks.

*Heleophryne hewitti* (Hewitt's ghost frog) is restricted to four small streams engulfed in pine plantations and *Heleophryne rosei* (Table Mountain ghost frog) is restricted to a few streams on the northern face of Table Mountain. We re-evaluate all threats, including disease, and perform risk assessments. The final outcome will be to compile a Conservation Action Plan for both species. To put this conservation plan into action, we have initiated long-term population and disease monitoring, and intend to supply all local stakeholders from the private, conservation and scientific research sectors with species-specific conservation guidelines (e.g. through interactive workshops). Throughout the project, public awareness of amphibian conservation will be heightened through printed, audio, and visual media as well as through online blogging on the EDGE website ([www.edgeofexistence.com](http://www.edgeofexistence.com)).

### **No evidence of population decline associated with chytridiomycosis in the Mallorcan midwife toad**

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Amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), is now known to be one of the most potent threats to biodiversity yet discovered. In recent decades, over 43% of amphibian species have gone into steep decline and the fungus has been found to be a principal driver in many of these declines. Recently, Walker *et al.* (2008) have reported that native island populations of the IUCN red-listed Mallorcan midwife toad *Alytes muletensis* are infected by *Bd* and suffering from chytridiomycosis. However, analysis of 33 time series of *A. muletensis* tadpole populations shows that overall *A. muletensis* populations are increasing, and that there is no evidence that chytridiomycosis is

associated with population decline in this species. Possible causes for the lack of declines have been investigated using both experimental and modelling techniques and results will be presented.

Walker, S. F., Bosch, J., James, T. Y., Litvintseva, A. P., Valls, J. A. O., Piña, S., García, G., Rosa, G. A., Cunningham, A. A., Hole, S., Griffiths, R. & Fisher, M. C. (2008): Invasive pathogens threaten species recovery programs. *Current Biology* **18**: R853-R854.

### **Ranavirus infections in UK amphibian species**

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Amphibians are declining on a global scale. There are many different factors contributing to these declines and disease is thought to be a major contributing group. The ranaviruses are a group of emerging infectious diseases in amphibian populations and have been implicated in mortality events in North America, Asia and the UK.

The ranaviruses are a group of DNA viruses from the viral family Iridoviridae and affect cold blooded vertebrates. Infection with a ranavirus usually results in a high mortality rate and the presence of a stereotypical set of clinical signs such as ulcerations, emaciation, and loss of digits.

In the late 1980s, the ranavirus was introduced into the UK and has been spreading ever since. Mortality events occur predominantly in adult common frog (*Rana temporaria*) populations, but infections are also found in common toads (*Bufo bufo*) and now in common newts (*Triturus vulgaris*).

Current investigations into the ecology of the ranavirus in the UK have revealed that the virus present here acts differently from those from other areas. In this study tadpoles that were screened for the presence of the ranavirus genome revealed no infections. This is quite unexpected as ranaviruses are known to infect both adults and tadpoles in most species (e.g. wood frogs (*Rana sylvatica*) from North America). Therefore, more investigation into the ecology of the ranavirus to determine how the virus is maintained in the populations of affected amphibians is needed in the UK.

### **Not forgetting the fundamentals: reproductive behavioural research in amphibian conservation**

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It is clear from the 2004 Global Amphibian Assessment and preceding research that many amphibian species are in decline. A series of initiatives has been trying to pinpoint the causes and synthesize strategies to halt these declines. However, we should not to lose sight of the importance of fundamental research and, in particular, the vital contribution that a better understanding of behaviour can make to conservation. In recent years there has been a lack of focus on reproductive behaviour of amphibians, including, crucially, female oviposition strategies. Our research on one of the best-studied urodele groups (*Triturus* newts) demonstrates that there is still much to learn about how females select oviposition sites. Female newts wrap eggs individually in a complex sequence of behaviours. We quantified key elements of this sequence in detail for each of three species of *Triturus* on different substrates and illustrate two of these behaviours here. 'Sniffing' (part of the leaf selection process) differs in the same way for each newt species across the three substrates but 'flexing' (involved with leaf preparation for oviposition) does not. The conservation implications of this will be discussed as an example of the importance of fully understanding reproductive strategies and, particularly, how they may differ for even very closely related species. With many of the world's species considerably less well-

known than this group, there remains a crucial role for fundamental research, which should not be lost alongside the equally vital 'big picture' strategies.

### **Chytrid fungus *Batrachochytrium dendrobatidis* differentially affects amphibian species distribution models in the Maya Mountains of Belize**

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Amphibian populations in the Maya Mountains of Belize remain poorly studied. Few baseline data exist, and while local capacity is increasing, currently there are no monitoring studies being carried out on a landscape-scale. Recent surveys for *Batrachochytrium dendrobatidis* (*Bd*) have shown varying prevalence across survey sites in the Maya Mountains. We tested the hypothesis that *Bd* affected amphibian species distributions. We collected *Bd* DNA swabs at a variety of point localities in the protected areas of the Maya Mountains from 2006–2007 and analyzed them using rt-RPCR. We used ArcMap spatial analyst to interpolate *Bd* prevalence for a section of the Maya Mountains. To model amphibian species distributions, we combined georeferenced field survey and published amphibian observation data from the area. We included 25 climatic and habitat remotely sensed layers, as well as *Bd* presence in Maxent models for species distributions. Maxent outputs allow determination of how each variable affects species distributions. Preliminary models suggest that different species' distributions are affected by different factors: some species appear to be unaffected by the presence of this pathogen, while other species' distributions seem to be driven at least in part by the presence of *Bd*, perhaps even more so than by other climatic variables. Future implementations will include increased chytrid and species location data from a larger area of the region, allowing for more geographically inclusive models.

### **Climate Change: a growing synergistic threat for amphibians**

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Amphibians face a multitude of threats, including more recently climate change. Climate change has the potential to work synergistically with already prevalent threats, increasing the impacts on amphibians and increasing their overall risk of extinction.

Climate change is already affecting species globally with studies showing amphibians to be some of the most vulnerable species. Impacts on physiology, phenology, reproductive success as well as range shifts are all being reported. More hotly debated is the potential ability for climate change to interact with other threat processes such as disease spread, invasive species, habitat fragmentation and ecosystem degradation. Building resilience in ecosystems and reducing other stressors and threats to amphibians is seen to be a potential means of increasing the capacity of these species to adapt to climatic changes alongside rapid measures to reduce greenhouse gas emissions.

Levels of CO<sub>2</sub> in the atmosphere have already reached 385 ppm, with pre-industrial levels being 278ppm. At 450 ppm of CO<sub>2</sub> we are considered to be committed to a 2°C rise in temperature. With present policy measures it is unlikely we will stop increases in carbon emission before we hit this concentration. The time delay between CO<sub>2</sub> emissions and climatic changes means that we are committing ourselves to altered environmental states in the future without fully appreciating the consequences. Because of its wide-ranging biological impacts, climate change is expected to accelerate

extinction rates for populations and species. Climate models show rapid and wide ranging environmental changes if the present emission rates are not curbed.

We urge that immediate conservation action be taken to prevent accelerated and rapid ecosystem change and biodiversity collapse.

### **Great crested newts in Scotland: A translocation case study**

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Great crested newts (GCN) (*Triturus cristatus*) are widespread but localised in mainland Britain. Populations are scarce in the uplands and Scotland. The combination of being both widespread and highly protected has led to conflict between conservationists and developers. Translocation has become increasingly popular as a method of resolution despite a lack of definitive evidence that it is a successful conservation tool.

Such a conflict arose when development of the former steelworks site in Gartcosh, North Lanarkshire, was proposed. In 2003 the Scottish Executive granted a licence to allow the population of GCN, thought to be one of the largest in Scotland, to be translocated to a purpose-built reserve on the periphery of the development site. This began in 2004 and is the largest translocation project in Scotland.

The GCN is an internationally important species, protected in the UK under Schedule 2 of the Conservation (*Natural Habitats etc.*) Regulations, 1994 and the *Wildlife and Countryside Act, 1981*. Yet the ability to protect this species in Scotland is hampered by knowledge gaps in the basic ecology within the Scottish context.

Gaps identified:

- Scottish population estimate and distribution.
- Is translocation an effective method of mitigation?
- Ecological differences compared with other UK populations.

### **Amphibians on the EDGE: combining phylogeny and threat**

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### **Does being dull really matter?: Mating success and colour variation in a wild population of *Agalychnis moreletii* frogs**

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Colour degradation is common in captive anurans and may be an indication of poor health resulting from nutritional deficiencies in diets. Colour may also play a role in antipredator and/or a sexual signalling, therefore frogs with reduced colour may be unsuitable for reintroduction. Yellow and red colouration in anurans is often carotenoid based. Carotenoids are pigments that function in the immune and antioxidant systems of vertebrates, although research with frogs is very limited. Carotenoids are obtainable through the diet alone since animals are incapable of *de novo* synthesis. Inadequate access to carotenoids in captivity may therefore be responsible for colour degradation. By studying wild and ex

*situ* populations we aim to assess the importance of carotenoid availability and skin colour on the health and behaviour of anurans. Here we assessed orange colour variation in a wild population of *Agalychnis moreletii* frogs, and related colour to mating behaviour. Males had significantly redder legs than females, and amplexant males had redder legs relative to all males. This is clear evidence of sexual selection for male leg colour, and suggests that female mate choice is influenced by leg colour. There was a positive correlation in leg colour within amplexant pairs, which may have evolved to prevent wasted breeding effort with a similar sympatric species (*A. callidryas*). This is the first study to indicate that colour may function in mating behaviour in this species. We are currently working with captive *A. moreletii* frogs to explore the importance of carotenoids in health and colour expression.

### **The changing status of Britain's frogs and toads: Agricultural impact on toads**

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The native anuran species in Britain are the natterjack toad (*Bufo calamita*), the common toad (*Bufo bufo*) and the common frog (*Rana temporaria*). The habitat of these species differs markedly and consequently they have been differentially impacted by agricultural intensification and urbanisation. The natterjack toad is found in heathland and coastal dunes/saltmarshes. It has been most severely impacted and is classed as endangered, which is probably due to destruction/modification of its specific habitat. The common toad is found in large old pools and surrounding woodland. It has also been heavily impacted due to the disappearance of historical ponds from villages and farms as living conditions and agriculture have been modernised. The common frog is found in and around garden ponds and has fared better by monopolising this habitat, although there are concerns about genetic inbreeding due to the isolation of these populations. The common toad breeds primarily in and around farmland and we investigated the potential effects of agricultural chemicals on growth and development. We conducted a field to laboratory study, whereby collected toad spawn was reared in the laboratory and in cages at field sites simultaneously. We found that survivorship differed between sites, that origin of spawn had a bigger impact than the experimental condition and that intersex individuals were common from one intensely agricultural site (20–30%). Further research on population status, habitat requirements and the effects of the agricultural environment on anurans are warranted.

### **Agnihotra, the traditional knowledge method as a powerful and cost-effective tool in amphibian conservation**

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The role of traditional knowledge in nature conservation is emphasized by the Convention on Biological Diversity. However, in conservation practice traditional nature management methods are still rarely employed.

Agnihotra is a Vedic fire ritual, recently gaining a growing popularity in many regions owing to its often 'miraculous' effects on improving agricultural crops and the health of domestic animals and humans. It is believed that Agnihotra acts in two ways, through improving local atmosphere and through ash, changing the properties of soil or water treated.

Earlier experiments had shown significant effects of Agnihotra ash treatment on the structure of algal/macrophyte/invertebrate communities in aquatic microcosms. To assess Agnihotra effects on survival and growth of *Rana temporaria* tadpoles, freshly hatched tadpoles were placed in water containers with algae, macrophytes and decaying plant debris, with addition of Agnihotra or non-ritual ashes of the same substrate against control tanks, placed each in triplicates at Agnihotra and organic farms in Southern Poland.

Agnihotra ash treatment significantly increased growth of tadpoles by 17-32% and reduced their mortality. With non-Agnihotra ash the mortality was even higher than in controls. Significant differences

in final yield of diatoms, filamentous algae, plants and in decomposition of plant debris were also found. Agnihotra atmosphere may be responsible for faster development and emergence of frogs. This is consistent with observations of Agnihotra farmers who claim their crops ripe earlier and more simultaneously than in conventional neighbours' farms.

Although the mechanisms of Agnihotra effects on biota are not fully known, some explanations are proposed. Further studies are needed on more endangered species, and on treatment of chytridiomycosis, as Agnihotra ash is often considered an effective remedy against fungal diseases. As many Agnihotra farms are located in or near global biodiversity hotspots, they may contribute to the conservation of endangered amphibian populations living there.

### **Working with the Mexican axolotl (*Ambystoma mexicanum*) in the Chapultepec Zoo, Mexico City**

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The Mexican axolotl (*Ambystoma mexicanum*), a urodele amphibian, is an endemic species of Lake Xochimilco in Mexico City. It has the amazing ability to regenerate limbs and internal organs, such as the nervous system, heart and liver. The species is currently considered to be critically endangered.

In 2000, with the support of the UAM (Metropolitan Autonomous University), an axolotl colony was established at the Chapultepec Zoo and in the last eight years we have learned much about these extraordinary animals. We have also had the opportunity to trial a number of reproductive techniques, including semi-captive conditions, with success after one year. We intend to carry out further research to improve our understanding of axolotl breeding techniques, which can be applied to *in situ* and *ex situ* conservation initiatives.

In the veterinary field, our research is focused on the diagnosis of diseases that affect axolotls in the Chapultepec Zoo colony and other captivity colonies in Mexico City. We have carried out research on skin samples to detect fungus, parasites and bacterial diseases, and have developed X-ray and ultrasound techniques, and chemical restraint methods, with good results. We are also working on improving blood-sampling techniques.

As one of the most visited zoos in Mexico, the Chapultepec Zoo is in a good position to educate visitors on the biology and conservation of axolotls, and the educational department has developed a number of activities to raise awareness about axolotls and conservation of their habitat.

### **The Belantikan Conservation Programme**

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The Belantikan Watershed (Central Kalimantan, Indonesian Borneo) consists of an area of hills and valleys of lowland and highland Dipterocarp forests of high biodiversity. Previous studies have highlighted a large population of orangutan (6000 individuals) and 32 species of anuran of which seven are endemic to Borneo, including a new population of the little known *Barbourula kalimantanensis*. Consisting of the largest tracts of lowland forest in Borneo, the area is also subject to production forest logging concessions. The BWCP aims to undertake the following actions: (1) to evaluate the role of climate in chytrid fungus outbreaks via the systematic sampling of amphibian communities at different altitudes (up to bukit Belah Hulu, 1019m) for chytrid occurrence, and the analysis of long-term temperature gradients for trends that could encourage fungal outbreaks; (2) to further survey the area from the lowlands to the Schwaner mountains (1000m altitude) for amphibian species including those from the more elusive leaf-litter (e.g. Microhylidae) and forest canopy (e.g. Rhacophoridae) groups, with particular reference to obtaining abundance and distributional data for *B. kalimantanensis*. Montane

regions often contain a high number of endemic species, and loss of these communities, whether to disease or habitat destruction, would represent a significant loss to global amphibian biodiversity. The goals of the BWCP are to inventory the amphibian biodiversity of the area, investigate how the presence of chytrid fungus may affect these communities and provide information for the designation of the Belantikan area as a protected habitat.

### **2008: Year of the Frog, also at Delft University of Technology**

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Parallel to the 'Amphibian Ark' programme, alternatives to help maintain our planet's amphibian biodiversity should be explored and exploited. Long-term strategies need to be developed in order to generate the substantial financial support and public interest needed to conserve these wonderful creatures. We adopt the following strategy. We scrutinize selected species for molecules with extraordinary pharmaceutical activities. In this way, we hope to trigger the interest of pharmaceutical/biotechnology companies to take part in the global attempts to protect and conserve these species and their local habitats.

Bioactive compounds are non-invasively obtained by mild electrical stimulation of the amphibian tegument, one of the richest sources of such substances known. Besides small metabolites, many of these biochemicals are encoded in the genome as small proteins (peptides). Millions of years of evolution resulted into a unique 'peptidome' for every species in harmony with its environmental challenges/predator species/ecological niche.

We use over forty bioassays to assess chromatographically fractionated peptides from crude venoms for interesting biological activities. Tandem mass spectrometry yields sequence information for hundreds of peptides per species. These data are represented in two-dimensional maps (retention time versus m/z ratio) and interpreted in the light of mRNA sequence data of secretory peptide genes cloned '*in situ*' from the very same samples. Indeed, frog venom nucleic acid sequences can be amplified directly from the lyophilized secretion, on the basis of primers designed from conserved secretory peptide leader sequences identified from earlier amphibian skin tissue cloning work. The obtained specific transcriptomic sequence information is invaluable for the biochemical identification of the actually secreted peptides. Together these data yield unequivocal bioactive peptide identifications, including their posttranslational modifications.

If we allow frog species to become extinct before we can study their unique libraries of biologically active peptides, we will lose this information completely. It would be like throwing away books from a library without having read them.

Our aim is to interest pharma or other entrepreneurs in research projects that *require* frog conservation to gain financial support for conservation initiatives.

**The distribution of *Batrachochytrium dendrobatidis* and fatal outbreaks of chytridiomycosis among populations of common midwife toads in the Pyrenees**

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Amphibian chytridiomycosis, the disease caused by the chytridiomycete fungus, *Batrachochytrium dendrobatidis* (*Bd*), was described only in 1998. In the space of ten years the pathogen has been reported from over 350 species of amphibian and across five continents. However, the spatial distribution and effects that *Bd* is having on European amphibian biodiversity are almost completely unknown. Our research presents the first systematic description of patterns of *Bd* prevalence in the Pyrenees. We show using field-surveillance over the period 2002–2008 that infection is patchy with an apparent declining west-east trend. We further show that mass mortalities are occurring in high-altitude settings. We use Bayesian zero-inflated binomial models and other statistical analyses to investigate the role of environmental drivers in determining the distribution of *Bd* and the potential risk of fatal chytridiomycosis to populations of *Alytes obstetricans*.