

## Can protected mountain areas serve as refuges for declining amphibians? Potential threats of climate change and amphibian chytridiomycosis in an alpine amphibian population

Marc Sztatecsny & Walter Hödl

**Keywords:** *alpine habitats, amphibian declines, Bufo bufo, chytridiomycosis, explosive breeding, weather anomalies, isolation mechanism, Ichthyosaura alpestris, Rana temporaria*

### Abstract

In June 2004 and June 2005, we checked 40 and 134 ponds respectively for alive and dead alpine newts, common toads, common frogs, and their clutches on the “Kirchfeld” plateau in the Northern Limestone Alps of Austria. We found several hundred dead common frogs and alpine newts in the two consecutive years and also observed an unusually high number of heterospecific matings between common toads and common frogs in 2004. In order to test whether the observed mortalities were caused by disease, we screened 98 individuals of the three species for chytridiomycosis, a highly infective fungal disease, in 2008. All samples proved to be negative. We therefore assume that the high losses for the common frog population (26% in 2004 and 9% in 2005) during the spring migration, as well as the breakdown of the temporal premating isolation between the common frog and common toad, were caused by temperature anomalies alone, i.e. sudden low temperatures after a warm spring period. The high number of dead alpine newts found in 2005 was negatively correlated to the oxygen saturation of the ponds, frequently covered by ice during the early breeding season. We discuss the possible effects of climate change on phenological timing, breeding isolation and chytridiomycosis in alpine amphibian populations.

### Profile

#### Mountain area

“Kirchfeld” plateau in the Northern Limestone Alps; the study area is part of the EU conservation network

#### Natura 2000

#### Mountain range

#### Alps

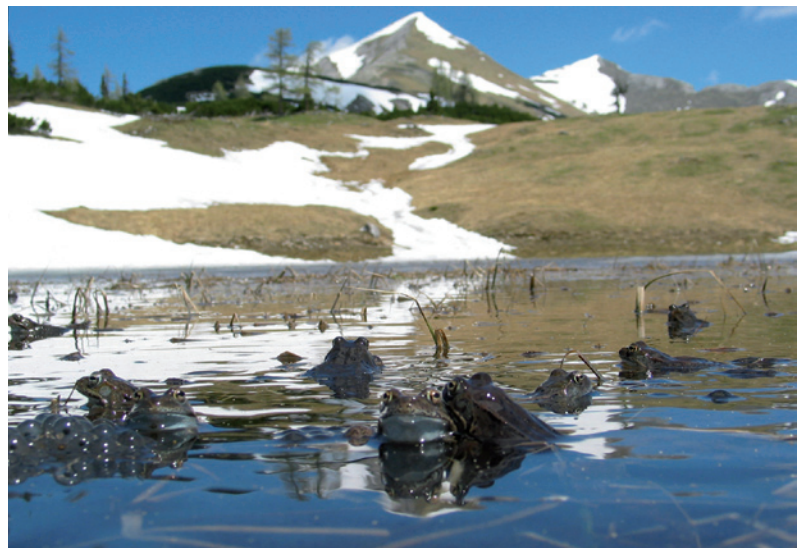
#### Country

#### Austria

### Introduction

Biodiversity is globally under threat, largely as a result of anthropogenic changes to the environment such as habitat loss, climatic changes, and emerging diseases (Gascon 2007). Amphibians seem to be particularly affected by these changes and are considered the most threatened group of vertebrates. Many species of amphibians are especially vulnerable to habitat loss as they need aquatic and terrestrial habitats to complete their life cycle and frequently migrate from one type of habitat to the other (Semlitsch 2003). Habitat deterioration and fragmentation therefore are the prime causes for population declines in densely populated lowland areas such as central Europe (Stuart 2004; Cushman 2006).

Compared to lowlands, our knowledge on threats to alpine amphibian populations is still very limited. The few Austrian populations from montane habitats that have been studied in greater detail seemed not to be declining (Schabetsberger 1995, Schabetsberger et al. 2004; Gutleb 2005). Some of the most worrying amphibian declines, however, have been reported from seemingly pristine mountain areas and partly associated with climate change (e.g. Berger et al. 1998, Pounds et al. 1999, Bosch et al. 2001). The role of climate change in amphibian declines is complex and insufficiently understood as it may affect individuals



*Common frog (Rana temporaria) breeding site at the study area “Kirchfeld” plateau, Northern Limestone Alps, Austria © Walter Hödl*

and populations either directly or indirectly and may be active over long periods (Collins & Storfer 2003). Global warming is causing some amphibian species to breed earlier, which may have consequences for species interactions (Gibbs & Breisch 2001; Tryjanowski et al. 2003; Sparks et al. 2007), and unusual weather events may cause increased mortality (Laurance 1996). The greatest threat associated with environmental

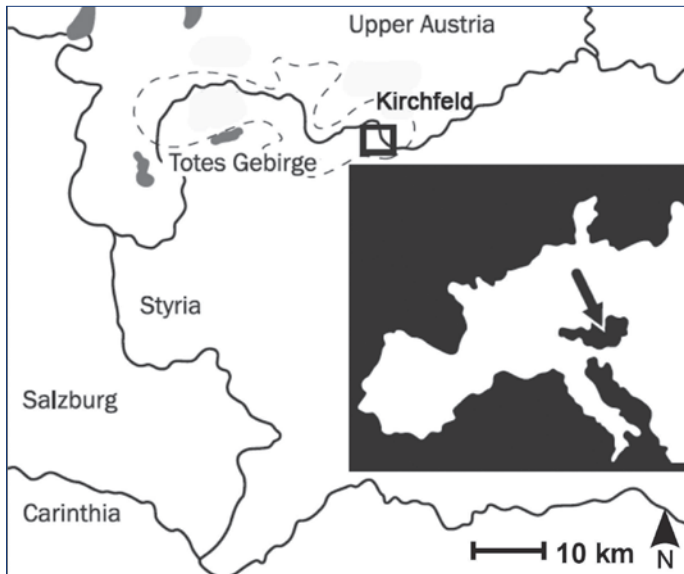


Figure 1 – Location of the study area in the Northern Limestone Alps of Austria (after Grabberr et al. 2009)

change, however, seems to be its influence on disease emergence resulting from effects on host and pathogen physiology (Lips et al. 2008). Outbreaks of amphibian chytridiomycosis, a fungal disease caused by *Batrachochytrium dendrobatidis* (Bd), have been made responsible for die-offs around the world associated with environmental change, particularly in mountain areas (Pounds et al. 2006; Bosch et al. 2007; Lips et al. 2008). Bd is spreading quickly to new regions, probably assisted by commercial amphibian trade (Garner et al. 2006; Picco & Collins 2008), and can cause rapid population declines and disappearances in many amphibian species (Berger et al. 1998; Bosch et al. 2001; Lips et al. 2006).

For the Alps, climatic models predict Bd to be absent from high altitude sites because of low temperatures (Rödder et al. 2009), however, data on the occurrence of chytridiomycosis in alpine amphibians is still lacking. Bd has been detected in lowland amphibian populations of Switzerland and Austria (Schmidt pers. comm; Sztatecsny unpubl.) and, with increasing temperatures, may spread into naïve alpine populations. The warming experienced in the Alps since the early 1980s, while synchronous with global warming, may locally reach a fivefold amplification of the global signal (Beniston et al. 1997).

In June 2004 and 2005, we observed mass mortalities in an alpine amphibian population in the Northern Limestone Alps of Austria. On the basis of these observations, we tested the occurring amphibian species for chytridiomycosis in 2008. We wanted to know if the mortalities were caused by an unusual weather event alone or if the animals might have been infected with Bd. We also found an unusually high number of heterospecific matings between common toads and common frogs in 2004. Breeding in the two species normally does not overlap, as common frogs arrive earlier (Tryjanowski et al. 2003; own obs.). We discuss

this breakdown of temporal premating isolation in relation to unusual weather events and mortality risks.

## Material and Methods

### Study species

Besides the viviparous alpine salamander (*Salamandra atra*), three pond-breeding amphibian species occur in our study area that we focused on: the alpine newt (*Ichthyosaura alpestris*), the common toad (*Bufo bufo*), and the common frog (*Rana temporaria*). The three species are widely distributed throughout Europe and occur from lowlands up to altitudes above 2 000 m (Gasc et al. 1997). Alpine newts stay in the ponds for approx. three months and females lay their eggs continuously over several weeks (Grabberr et al. 2009). The common toad and the common frog are typical explosive breeders in which spawning activity peaks within a few days (Wells 2007, pers. obs.). Males may not distinguish between sex and species and females can be subject to coercion and male-male competition (Marco & Lizana 2002). In our study area, common frogs are the earliest amphibians to arrive at the breeding ponds, sometimes spawning even before the ponds' snow cover has thawed, followed by alpine newts and common toads.

### Field survey

From June 7–12, 2004, and June 12–16, 2005, we surveyed 40 and 134 ponds respectively, on a plateau (“Kirchfeld”) in the Northern Limestone Alps of Austria (14° 9.377' E, 47° 36.503' N, 1800 m, Totes Gebirge, Figure 1) for alive and dead alpine newts (*I. alpestris*), common toads (*B. bufo*), and common frogs (*R. temporaria*) and their egg clutches. The number of egg clutches corresponds to the number of females breeding in a given year. The number of breeding adults can be estimated by doubling that number for the number of males. For alpine newts the number of breeding adults cannot be estimated as easily as they lay their eggs individually. The study area comprised roughly 0.14 km<sup>2</sup> of alpine pasture, interspersed with dwarf pines (*Pinus mugo*), and is part of the EU conservation network Natura 2000. We measured oxygen saturation of 55 of the 134 ponds in 2005 (median size: 17.01 m<sup>2</sup>, median depth: 22 cm, including all ponds studied in 2004) with an Oxy 196 Microprocessor Oxymeter (WTW, Weilheim, Germany). In 2004, we examined the largest pond (surface area 250 m<sup>2</sup>, depth 0.4 m) in greater detail as *B. bufo* and *R. temporaria* were breeding there simultaneously. Six observers crossed the pond, evenly spaced, from one end to the other, recording all *B. bufo* and *R. temporaria* by sex. The procedure was performed twice, on June 10 and 11, 2004, at 17:00. Data on daily mean temperatures and snow cover from April 1 to June 31, 2004, were provided by the Austrian Central Institute for Meteorology and Geodynamics from 1990 to 2004 (weather station Feuerkogel at 1 621 m, 40 km from the study site).



Figure 2 – Common frogs (left) and alpine newts (right) died from late frosts in 2004 and 2005. © Marc Sztatecsny

### Chytrid survey

Based on our observations of dead amphibians at ponds of the Kirchfeld study area, we conducted a survey for Bd in June 2008. To check if the disease was present in the region, we sampled all amphibian species present at three separate sites at different altitudes along an elevational gradient. At 645 m (valley of the river Enns, Wörschach Moos, 14° 11.615' E, 47° 33.344' N), we sampled 20 juvenile common toads at a man-made fish pond. At mid-altitude, we collected 10 female and 10 male alpine newts from a cattle drinking pond (1360 m, 14° 10.78' E, 47° 35.160' N) and at the Kirchfeld study area, we sampled 13 female and 12 male common frogs and 17 female and 17 male alpine newts. All three species are known to get infected by Bd (Garner et al. 2005). Animals were captured by dipnetting and handled with unused latex gloves to reduce the risk of possibly transmitting the disease. Samples were taken by firmly running sterile cotton swabs (Medical Wire & Equipment, MW 100) over the amphibians' ventral surface, flanks, feet and tail (for alpine newts only) as described by Kriger et al. (2006). All animals were released at the point of capture immediately after sampling. Swabs were replaced in their original container and stored at room temperature.

We screened all swabs for the presence of *B. dendrobatidis* by the use of quantitative real-time polymerase chain reaction (qPCR) as described in Boyle et al. (2004). However, we applied the changes suggested by Kriger et al. (2006), ran all samples in duplicate and always added three standard reactions containing DNA from 100, 10, 1, and 0.1 Bd genome equivalents and a negative control. To calculate the probability of freedom from disease for the amphibian populations at the Kirchfeld study area, we used FreeCalc software (Cameron & Baldock 1998), assuming a population prevalence of 5% (as found in other Austrian populations, Sztatecsny unpubl.), and a test sensitivity of 97% (Hyatt et al. 2007).

## Results

### Mortality during spring migration

In 2004, we estimated a total of approx. 250 dead common frogs and 356 common frog egg clutches in 40 survey ponds (many frogs died in dense aggregations

and could not be counted exactly, Figure 2). In 2005, we found 328 dead female and 363 dead male alpine newts, as well as 60 dead common frogs, and 315 egg clutches. Hence, at least 962 common frogs migrated to or arrived at their breeding pond in 2004, and 690 in 2005, of which 26% and 9% respectively died prior to spawning. We did not find any dead common toads in either year. Daily mean air temperature three weeks prior to our observations in 2004 had dropped from 10.2°C on 21 May, 2004, to -2.3°C two days later (Figure 3). The daily mean temperature from May 1 to June 10, 2004, was 2.8°C lower than the average from 1990–2003 (paired t-Test,  $t = 4.35$ ,  $df = 39$ ,  $P < 0.0001$ , Figure 3). The log number of dead alpine newts found in 2005 was negatively correlated to oxygen saturation of the ponds ( $r^2 = 0.16$ ,  $F = 11.59$ ,  $P = 0.001$ ).

### Breakdown of temporal breeding isolation

Low temperatures after May 23, 2004, probably caused high mortality in common frogs and interrupted their breeding activity. When they resumed breeding three weeks later, common toads had arrived at the breeding site as well. On June 10, we counted 206 freshly laid frog egg clutches and 32 common frog males in the largest of our study ponds, whereas all female frogs had left the spawning site. The ratio between common toad and frog males was 1:1.45 and of the 23 toad females in amplexus, 12 (52.2%) were clasped by male frogs (Figure 4). We observed five female toads spawning while in amplexus with a heterospecific male. On the following day, common toad males outnumbered

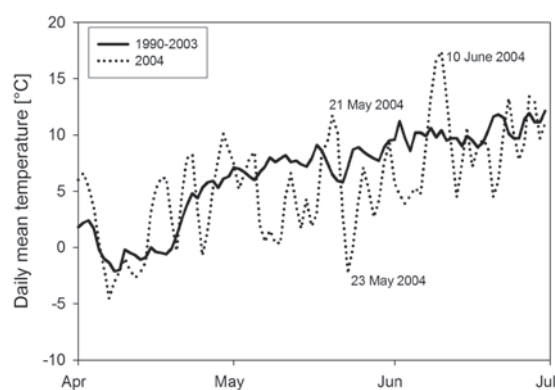


Figure 3 – Daily mean temperature from April 1 to June 30 in 2004 and averaged for the same time period from 1990–2003



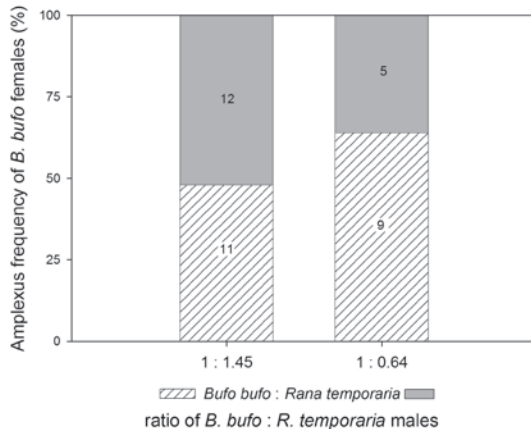


Figure 4 – Frequency of amplexus of *B. bufo* females with conspecific and heterospecific *R. temporaria* males

frog males by 1:0.64 and 35.7% of female toads were amplexed by a frog male.

### Chytridiomycosis

*Batrachochytrium dendrobatidis* was detected in none of the 98 samples analysed. The calculated confidence level for Bd to be absent from the Kirchfeld study area at an assumed prevalence of 5% was 96.48%.

### Discussion

We found several hundred dead common frogs and alpine newts in two consecutive years and daily mean temperature data from one year suggested that colder than average temperatures in spring may have been responsible for the amphibians' death. No samples were positive for Bd in the populations, suggesting that chytridiomycosis was not a cause for the die-offs. The low temperatures in May 2004 may have caused the breakdown of temporal separation in breeding of syntopic common frogs and toads.

In case of amphibian mortality events in high elevation sites, it remains difficult to determine whether death was caused by weather anomalies alone or if other factors played a role as well. The rapid drop of temperature around May 23 may have happened too quickly for the migrating or spawning frogs to seek shelter. The dead alpine newts found in 2005 had reached their breeding sites and probably suffocated when ice formed on the ponds during cold weather. Common toads remained unaffected by the cold weather as they breed later (Tryjanowski et al. 2003; own obs.). Seasonally early breeding should be favoured by natural selection as it increases the time for juvenile development and energy allocation for hibernation. Extending the activity period towards spring is particularly advantageous at high altitudes, as summers are short. The disadvantage of early arrival at the breeding site, however, is an increased susceptibility to adverse spring weather conditions (Morbey & Ydenberg 2001).

In 2004, up to 50% of common toad females in our study pond may have spawned with frog males at a given time. Frequent interspecific spawning between

the two species occurs only in case of syntopy and unusually cold breeding seasons (Reading 1984). Reading (1984), however, made his observations at an artificial pond where both species had been deliberately introduced. Here we could show that similar interactions may happen in natural populations as a consequence of a temperature anomaly alone. Due to low temperatures in May and the lasting ice cover on the pond, common frogs could not start breeding before the ice had thawed. At that time, toads were also ready to start their spawning migration.

The size of amphibian populations is known to fluctuate significantly over time (e.g. Meyer et al. 1998), however, losses of 9–25% of one year's breeding adults may have dramatic effects on a population if such events occur repeatedly. Amphibians from high elevation populations in the Alps need up to ten years to reach maturity and can reach an age of over 20 years (Schabetsberger & Goldschmid 1994; Miaud & Merilä 2000; Gutleb 2005). Considering the high age of mature adults, premating mortality might have more severe effects on high altitude compared to lowland populations.

To what extent chytridiomycosis will affect alpine amphibian populations in the future remains largely unknown. Climate models predict high elevation sites in the Austrian Alps to be too cold for Bd to occur (Rödler et al. 2009), however, infected *I. alpestris* have recently been found at an altitude of 1630m in western Austria (Glaser & Sztatecsny unpubl.). The spread of Bd to higher elevation sites may just be a matter of time and could be accelerated by increasing temperatures. The disease exhibited the most devastating effects in Europe in montane amphibian populations of Spain (Bosch et al. 2001; Bosch & Martinez-Solano 2006). Climate change seemed to play a role in the outbreaks (Bosch et al. 2007), but it cannot be ruled out that high-altitude amphibian populations may also be more susceptible to the disease than lowland populations. There is some evidence for local adaptation in populations from different altitudes in the Alps and this may cause different sensitivity to environmental stressors (Miaud & Merilä 2001; Ficetola & De Bernardi 2005).

The Alps are more affected by global change than lowland areas and the changes observed so far may have significant effects on mountain hydrology, snow cover, and mountain vegetation (Beniston & Junco 2002). Our observations demonstrate the reliance of amphibians on temperature for phenological timing and breeding isolation. In addition to an increase in temperature anomalies, climate warming is predicted to cause habitat range extensions for amphibians in many parts of Europe (Araujo et al. 2006). Except for the alpine salamander, all amphibian species occurring in the Austrian Alps are not restricted to certain elevations (Gasc et al. 1997) and should be able to extend their range upwards. The few studies on alpine amphibian populations in the Austrian Alps (see intro-

duction) may become important for monitoring possible changes in species distribution and abundance in the years to come. Whether high altitudes in the Alps will remain free of Bd and can serve as climatic refuges for amphibians as proposed for certain lowland areas of Costa Rica (Puschendorf et al. 2009) is an important issue of future research.

### Acknowledgements

We wish to thank Frances Clare and Trent Garner for their support during the analysis of chytrid samples and two anonymous referees for their valuable comments. Helmut Faber, A. Hille, M. Hable, A. Kleewein, I. Kohl, W. Prunner, R. Schabetsberger, M. Sauberer, A. Vidic, and S.P. Vijayakumar helped in the field. The Austrian Central Institute for Meteorology and Geodynamics provided the weather data. MS was supported by the Hochschuljubiläumsstiftung der Stadt Wien and permits came from the provincial government of Styria (FA13C-53S7/59-2008).

### References

- Araujo, M.B., W. Thuiller & R.G. Pearson 2006. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* 33: 1712-1728.
- Beniston, M. & P. Junco 2002. Shifts in the distributions of pressure, temperature and moisture in the alpine region in response to the behavior of the North Atlantic Oscillation. *Theoretical and Applied Climatology*: 29-42.
- Berger, L., R. Speare, P. Daszak, D. Green, A. Cunningham, C. Goggin, R. Slocombe, M. Ragan, A. Hyatt, K. McDonald, H. Hines, K. Lips, G. Marantelli & H. Parkes 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95: 9031-9036.
- Bosch, J., I. Martinez-Solano & M. Garcia-Paris 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of Central Spain. *Biological Conservation* 97: 331-337.
- Bosch, J. & I. Martinez-Solano 2006. Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Penalara Natural Park, Spain. *Oryx* 40: 84-89.
- Bosch, J., L.M. Carrascal, L. Duran, S. Walker & M.C. Fisher. 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proceedings of the Royal Society Biological Sciences Series B* 274: 253-260.
- Boyle, D.G., Boyle D.B., Olsen V., Morgan J.A.T., Hyatt A.D. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 16: 141-148.
- Cameron, A.R. & F.C. Baldock 1998. A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine* 34: 1-17.
- Collins, J.P. & A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. *Diversity & Distributions* 9: 89-98.
- Cushman, S.A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128: 231-240.
- Ficetola, G.F. & F. De Bernardi 2005. Supplementation or in situ conservation? Evidence of local adaptation in the Italian agile frog *Rana latastei* and consequences for the management of populations. *Animal Conservation* 8: 33-40.
- Garner, T.W.J., S. Walker, J. Bosch, A.D. Hyatt, A.A. Cunningham & M.C. Fisher. 2005. Chytrid Fungus in Europe. *Emerging Infectious Diseases* 11: 1639-1642.
- Garner, T.W.J., M.W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A.A. Cunningham & M.C. Fisher 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biology Letters* 2: 455-459.
- Gasc, J.-P., A. Cabela, J. Crnobrnja-Isailovi, D. Dolmen, K. Grossenbacher, P. Haffner, J. Lescure, H. Martens, J.P. Martinez Rica, H. Maurin, M.E. Oliveira, T.S. Sofianidou, M. Veith & A. Zuiderwijk 1997. *Atlas of Amphibians and Reptiles in Europe*. Paris.
- Gascon, C., J.P. Collins, R.D. Moore, D.R. Church, J.E. McKay & J.R. Mendelson (eds.) 2007. *Amphibian Conservation Action Plan*. IUCN/SSC Amphibian Specialist Group, Gland, Switzerland and Cambridge, UK.
- Gibbs, J.P. & A.R. Breisch 2001. Climate warming and calling phenology of frogs near Ithaca, New York, 1900-1999. *Conservation Biology* 15: 1175-1178.
- Grabherr, H., R. Schabetsberger & M. Sztatecsny 2009. Are sex ratios of larval alpine newts (*Mesotriton alpestris*) biased in high-altitude spawning sites with different temperature regimes? *Amphibia-Reptilia* 30: 389-399.
- Gutleb, B. 2005. *Populationsökologische Langzeitstudie über den Bergmolch (Triturus alpestris) am Firstmoor (1920 m) im Nationalpark Nockberge*. Unpublished report. Institut für Wildtierforschung und Naturschutz Glanegg, January 2005.
- Hyatt, A.D., D.G. Boyle, V. Olsen, D.B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason & A. Colling 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases Of Aquatic Organisms* 73: 175-192.
- Kruger, K.M., H.B. Hines, A.D. Hyatt, D.G. Boyle & J.M. Hero. 2006. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. *Diseases of Aquatic Organisms*: 141-148.
- Laurance, W.F. 1996. Catastrophic declines of Australian rainforest frogs: is unusual weather responsible? *Biological Conservation* 77: 203-212.

- Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, R.A. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pessier & J.P. Collins 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3165-3170.
- Lips, K.R., J. Diffendorfer, J.R. Mendelson & M.W. Sears. 2008. Riding the Wave: Reconciling the Roles of Disease and Climate Change in Amphibian Declines. *PLoS Biology* 6: e72.
- Marco, A. & M. Lizana 2002. The absence of species and sex recognition during mate search by male common toads, *Bufo bufo*. *Ethology Ecology and Evolution* 14: 1-8.
- Meyer, A.H., B.R. Schmidt & K. Grossenbacher 1998. Analysis of three amphibian populations with quarter-century long time-series. *Proceedings Of The Royal Society B-Biological Sciences* 265: 523-528.
- Miaud, C. & J. Merilä. 2001. Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. *Biota* 2: 31-50.
- Morbey, Y. & R. Ydenberg 2001. Protandrous arrival timing to breeding areas: a review. *Ecology Letters*, 4: 663-667.
- Picco, A.M. & J.P. Collins 2008. Amphibian Commerce as a Likely Source of Pathogen Pollution. *Conservation Biology* 22: 1582-1589.
- Pounds, J.A., M.P.L. Fogden & J.H. Campbell 1999. Biological response to climate change on a tropical mountain. *Nature* 398: 611-614.
- Pounds, J.A., M.R. Bustamante, L.A. Coloma, J.A. Consuegra, M.P.L. Fogden, P.N. Foster, E. La Marca, K.L. Masters, A. Merino-Viteri, R. Puschendorf, S.R. Ron, G.A. Sanchez-Azofeifa, C.J. Still & B.E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439: 161-167.
- Puschendorf, R., A.C. Carnaval, J. VanDerWal, H. Zumbado-Ulate, G. Chaves, F. Bolaños & R.A. Alford 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* 15: 401-408.
- Reading, C. J. 1984. Interspecific spawning between common frogs (*Rana temporaria*) and common toads (*Bufo bufo*). *Journal of Zoology* 203: 95-101.
- Rödger, D., J. Kielgast, J. Bielby, S. Schmidlein, J. Bosch, T.W.J. Garner, M. Veith, S. Walker, M.C. Fisher & S. Lötters 2009. Global Amphibian Extinction Risk Assessment for the Panzootic Chytrid Fungus. *Diversity* 1: 52-66.
- Schabetsberger, R. & A. Goldschmid 1994. Age structure and survival rate in Alpine newts (*Triturus alpestris*) at high altitude. *Alytes (Paris)* 12: 41-47.
- Schabetsberger, R., R. Jehle, A. Maletzky, J. Pesta & M. Sztatecsny 2004. Delineation of terrestrial reserves for amphibians: post-breeding migrations of Italian crested newts (*Triturus c. carnifex*) at high altitude. *Biological Conservation* 117: 95-104.
- Semlitsch, R.D. 2003. General threats to amphibians. In: Semlitsch, R.D. (ed.), *Amphibian Conservation*: 1-7. Washington, DC.
- Smith, K.F., D.F. Sax & K.D. Lafferty. 2006. Evidence for the role of infectious disease in species extinction. *Conservation Biology* 20: 1349-1357.
- Sparks, T., P. Tryjanowski, A. Cooke, H. Crick & S. Kuzniak 2007. Vertebrate phenology at similar latitudes: temperature responses differ between Poland and the United Kingdom. *Climate Research* 34: 93-98.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman & R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.
- Tryjanowski, P., R. Mariusz & T. Sparks 2003. Changes in spawning dates of common frogs and common toads in western Poland in 1978–2002. *Annales Zoologica Fennici* 40: 459-464.
- Wells, K.D. 2007. *The ecology and behavior of amphibians*. Chicago.

## Authors

### Marc Sztatecsny

Marc Sztatecsny is a research scientist with the Department of Evolutionary Biology at the University of Vienna. His work focuses on ecology, conservation, and mating behaviour of European amphibians and he is presently screening Austrian amphibians for the fungal disease chytridiomycosis.  
marc.sztatecsny@univie.ac.at

### Walter Hödl

Walter Hödl is professor of biology at the Department of Evolutionary Biology at the University of Vienna and has a long history of research on amphibian ecology, behaviour and conservation. His main interest is acoustic and visual communication in anurans with an emphasis on tropical species.  
walter.hoedl@univie.ac.at

Department of Evolutionary Biology  
University of Vienna  
Althanstrasse 14  
1090 Vienna