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Bsal-driven salamander mortality pre-dates the European index outbreak

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Emerging infectious fungal diseases increasingly become major drivers of global biodiversity loss in the Anthropocene (Daszak et al. 2000, Fisher et al. 2012, Sikes et al. 2018). Amphibians are a textbook example among the deuterostomians. They suffer from fungal skin diseases caused by at least two chytrid fungi (chytridiomycosis), *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). Both are of Asian origin and have spread to other continents – with human mediation playing a key role – causing massive declines of naïve amphibian hosts (Woodhams et al. 2011, van Rooij et al. 2015, Garner et al. 2016, O'Hanlon et al. 2018, Scheele et al. 2019).

Bsal was only discovered and scientifically described after a drastic population breakdown around the year 2010 in a Dutch population of the European fire salamander, *Salamandra salamandra* (Spitzen-van der Sluijs et al. 2013, Martel et al. 2013). Increased monitoring efforts in recent years have revealed that this pathogen is dramatically spreading. It has since been discovered in three additional European countries in the wild, Belgium, Germany and Spain, confronting us with awkward conservation tasks (Martel et al. 2014, 2020, Spitzen-van der Sluijs et al. 2016, Thomas et al. 2019). *Bsal* exclusively affects caudate amphibians (while at least some anurans might act as reservoirs), with salamandrids, especially the genus *Salamandra,* being highly susceptible (Martel et al. 2014, Stegen et al. 2017). European fire salamander populations, once infected, undergo rapid declines demonstrated through both *in situ* observations and epidemiological modelling, and various mass mortality events have been reported (Spitzen-van der Sluijs et al. 2013, 2016, Stegen et al. 2017, Canessa et al. 2018, Dalbeck et al. 2018).

From what we know, more than half of the ca. 80 *Bsal* records in the pathogen's European invasive range are from Germany (Lötters et al. 2020 in this issue). In this country, *Bsal* was first discovered in 2015 (in *S. salamandra terrestris*) in the wild in the northern Eifel region, about 50 km from the Dutch index outbreak (Spitzen-van der Sluijs et al. 2016). Shortly after its first detection in Germany and reports in the local media, a member of the public reported a European fire salamander mass mortality to us that had previously been observed in 2004 at the Vichtbach, Rott near Roetgen (county of StädteRegion Aachen, federal state of North Rhine-Westphalia; 50.676448, 6.198452) (Dalbeck et al. 2018).

From this mortality event, two specimens, found dead on the forest floor and subsequently stored in 10% formalin, have become available for examination. They have been deposited in the UGent collection (numbers 2015/SL/1 and 2015/SL/2). Macroscopic lesions (Fig. 1) in these specimens are consistent with skin ulcerations that characterize *Bsal* chytridiomycosis under experimental conditions and in natural outbreaks (Martel et al. 2013, Stegen et al. 2017). Histopathology demonstrated the presence of epithelial erosions with intracellular fungal thalli in the epithelial cells bordering the lesions, which is indicative of chytridiomycosis caused by *Bsal* (Fig. 2). In contrast, typical lesions of infections with *Bd* consist of epidermal hyperplasia and hyperkeratosis (e.g. van Rooij et al. 2015). Immunohistochemistry (Thomas et al. 2018) resulted in marked brown staining of structures, consistent with chytrid thallus morphology against a hemaluin counterstained background, confirming the presence of *Batrachochytrium* sp. organisms in the epidermal lesions. Applying the diagnostic qPCR for *Bd* (Boyle et al. 2004) and *Bsal* (Blooi et al. 2013, 2016) on skin tissue after using different protocols for DNA extraction did not result in detectable amplification of chytrid DNA from the samples. The latter is problematic since this would be required for a final diagnosis of *Bsal* infection, and is most probably due to the long-time formalin storage of the samples. However, the combination of highly suggestive clinical signs, compatible microscopic epithelial lesions and positive immunohistochemistry from specimens in a region that is confirmed as a 'hotspot' of *Bsal*-associated declines (Lötters et al. 2020 in this issue) provide multiple lines of evidence to support our classification of this case as *Bsal* infection. No other currently known chytrid fungus or any other pathogen produces similar macroscopic and microscopic pathology (cf. Martel et al. 2013). In addition, histopathology has been proven a reliable method to identify both *Bd* and *Bsal* and to distinguish them (Martel et al. 2013, van Rooij et al. 2015, Weldon et al. 2020).

This finding pre-dates the currently assumed initial *Bsal* introduction into Europe by at least six years and identifies Germany as the first country in which a *Bsal* outbreak has been demonstrated. At that time point, the infection may already have been widespread, since Schulz et al. (2020 in this issue) provide a photograph of a European fire salamander found in 2004 in the Ruhr District of Germany that showed skin lesions consistent with *Bsal* infection (which however cannot be confirmed with full reliability as this specimen was not preserved).

It is noteworthy that *Bsal* has been in the northern Eifel for several years, but remained undetected, as here the disease was diagnosed for the first time in 2015 (Spitzen-van der Sluijs et al. 2016). Given the current *Bsal*-induced population declines of *S. salamandra terrestris* in this region, multiple 'silent' declines of this taxon in the years before are plausible. However, systematic data for this time are lacking as adult and larval monitoring efforts started only in 2014 (and at first at a markedly limited number of sites only; Dalbeck et al. 2018, Wagner et al. 2020 in this issue). On the other hand, ongoing monitoring suggests that numerous populations of the European fire salamander declined due to *Bsal* incursions only within the last five years, i.e. until recently salamanders in a population were relatively common and *Bsal* was not detected, then salamanders disappeared, dying specimens were observed and *Bsal* was detected (Dalbeck et al. 2018, Lötters et al. 2020 in this issue).

Observations suggest that *Bsal* dispersal is difficult to understand. This is further enforced by Spitzen-van der Sluijs et al. (2018) who demonstrated that a Dutch population of the European fire salamander apparently has remained unaffected by *Bsal,* although the pathogen has been present for at least about one decade in a population at a distance of < 1 km. Likewise, puzzling is the fact that – perhaps unexpected – at the Vichtbach (at exactly the area where the dead specimens were found in 2004), a vital European fire salamander population has been monitored since 2017 – without any trace of *Bsal* (Lötters et al. 2020 in this issue).

In conclusion, the long-time undetected presence of *Bsal* and its poorly understood modes of dispersal, combined with new remote outbreaks including Southern Eu-

Figure 1. Ventral view of a 10% formalin fixed specimen of *Salamandra salamandra terrestris,* found dead on the forest floor at Vichtbach, northern Eifel region, Germany, in 2004. The circular black spots represent epidermal erosions, typical of *Bsal* infection.

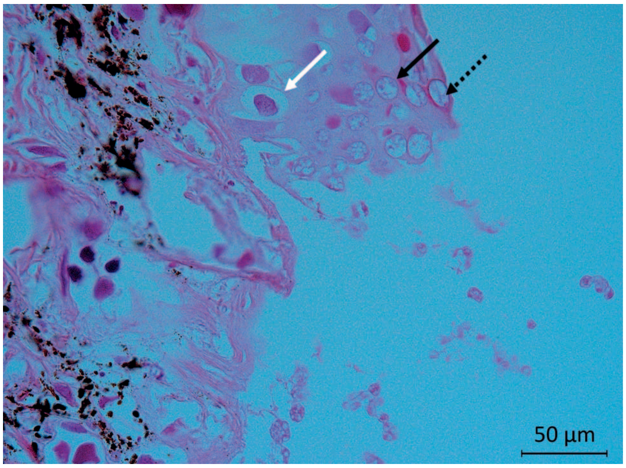

Figure 2. Hematoxylin and eosin staining of a 5 µm thickness skin section (magnification 40×) of a *Salamandra salamandra terrestris,* found dead on the forest floor at Vichtbach, northern Eifel region, Germany, in 2004. Microscopic characteristics are the presence of full thickness necrosis of keratinocytes, which contain (often colonial) thalli that marginate the cell's nucleus. The result is an epidermal erosion. Legend: white arrow – necrotic epithelial cell; black arrow – thallus with spores; stippled arrow – thallus with septae (colonial thallus).

rope, are worrying and prompt installing proper population monitoring and disease surveillance of vulnerable urodele populations in Europe (cf. Martel et al. 2014, 2020, Thomas et al. 2019, Schulz et al. 2020 in this issue).

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References

Blooi, M., F. Pasmans, J. E. Longcore, A. Spitzen-van der Sluijs, F Vercammen & A. Martel (2013): Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – Journal of Clinical Microbiology, **51**: 4173–4177.

Blooi, M., F. Pasmans, J. E. Longcore, A. Spitzen-van der Sluijs, F Vercammen & A. Martel (2016): Correction for Blooi et al., duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – Journal of Clinical Microbiology, **54**: 246.

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. Morgan & A. D. Hyatt (2004): Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. – Diseases of Aquatic Organisms, **60**: 141–148.

Canessa, S., C. Bozzuto, E. H. Campbell Grant, S. S. Cruickshank, M. C. Fisher, J. C. Koella, S. Lötters, A. Martel, F. Pasmans, B. C. Scheele, A. Spitzen-van der Sluijs, S. Steinfartz & B. R. Schmidt (2018): Decision making for mitigating emerging wildlife diseases: from theory to practice. – Journal of Applied Ecology, **55**: 1987–1996.

Dalbeck, L., H. Düssel-Siebert, A. Kerres, K. Kirst, A. Koch, S. Lötters, D. Ohlhoff, J. Sabino-Pinto, K. Preissler, U. Schulte, V. Schulz, S. Steinfartz, M. Veith, M. Vences, N. Wagner & J. Wegge (2018): Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (*Bsal*): aktueller Stand in Deutschland. – Zeitschrift für Feldherpetologie, **25**: 1–22.

Daszak P., A. A. Cunningham & A. D. Hyatt (2000): Emerging infectious diseases of wildlife – threats to biodiversity and human health. – Science, **287**: 443–449.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw & S. J. Gurr (2012): Emerging fungal threats to animal, plant and ecosystem health. – Nature, **282**: 186–194.

Garner, T. W., B. R. Schmidt, A. Martel, F. Pasmans, E. Muths, A. A. Cunningham, C. Weldon, M. C. Fisher & J. Bosch (2016): Mitigating amphibian chytridiomycoses in nature. – Philosophical Transactions of the Royal Society London B, **5**: 371.

Lötters, S., N. Wagner, G. Albaladejo, P. Böning, L. Dalbeck, H. Düssel, S. Feldmeier, M. Guschal, K. Kirst, D. Ohlhoff, K. Preissler, T. Reinhardt, M. Schlüpmann, U. Schulte, V. Schulz, S. Steinfartz, S. Twietmeyer, M. Veith, M. Vences & J. Wegge (2020): The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: past – present – future. – Salamandra, **56**: 173–188 [this issue].

Martel, A., A. Spitzen-van der Sluijs, M. Blooi, W. Bert, R. Ducatelle & M. C. Fisher (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. – Proceedings of the National Academy of Sciences of the USA, **110**: 15325–15329.

Martel, A., M. Blooi, C. Adriaensen, P. van Rooij, W. M. Beukema, M. C. Fisher, R. A. Farrer, B. R. Schmidt, U. Tobler, K. Goka, K. R. Lips, C. Muletz, K. R. Zamudio, J. Bosch, S. Lötters, E. Wombwell, T. W. J. Garner, A. A. Cunningham, A. Spitzen-van der Sluijs, S. Salvidio, R. Ducatelle, K. Nishikawa, T. T. Nguyen, J. E. Kolby, I. van Bocxlaer et al. (2014): Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. – Science, **346**: 630–631.

Martel, A., M. Vila-Escalé, D. Fernández-Giberteau, A. Martinez-Silvestre, S. Canessa, S. van Praet, P. Pannon, K. Chiers, A. Ferran, M. Kelly, M. Picart, D. Piulats, Z. Li, V. Pagone, L. Pérez-Sorribes, C. Molina, A. Tarragó-Guarro, R. Velarde-Nieto, F. Carbonell, E. Obon, D. Martínez-Martínez, D. Guinart, R. Casanovas, S. Carranza & F. Pasmans (2020): Integral chain management of wildlife diseases. – Conservation Letters: e12707.

O'Hanlon, S. J., A. Rieux, R. A. Farrer, G. M. Rosa, B. Waldman, A. Bataille, T. A. Kosch, K. A. Murray, B. Brankovics, M. Fumagalli, M. D. Martin, N. Wales, M. Alvarado-Rybak, K. A. Bates, L. Berger, S. Böll, L. Brookes, F. Clare, E. A. Courtois, A. A. Cunningham, T. M. Doherty-Bone, P. Ghosh, D. J. Gower, W. E. Hintz, J. Höglund et al. (2018): Recent Asian origin of chytrid fungi causing global amphibian declines. – Science, **360**: 621–627.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, I. De la Riva, M. C. Fisher, S. V. Flechas, C. N. Foster, P. Frías-Álvarez, T. W. J. Garner, B. Gratwicke, J. M. Guayasamin, M. Hirschfeld, J. E. Kolby, T. A. Kosch, E. La Marca, D. B. Lindenmayer, K. R. Lips, A. V. Longo, R. Maneyro et al. (2019): Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. – Science, **363**: 1459–1463.

Schulz, V., A. Schulz, M. Klamke, K. Preissler, J. Sabino-Pinto, M. Müsken, M. Schlüpmann, L. Heldt, F. Kamprad, J. Enss, M. Schweinsberg, J. Virgo, H. Rau, M. Veith, S. Lötters, N. Wagner, S. Steinfartz & M. Vences (2020): *Batrachochytrium salamandrivorans* in the Ruhr District, Germany: history, distribution, decline dynamics and disease symptoms of the salamander plague. – Salamandra, **56**: 189–214 [this issue].

Sikes, B. A., J. L. Bufford, P. E. Hulme, J. A. Cooper, P. R. Johnston & R. P. Duncan (2018): Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. – PLoS BIOLOGY, **16**: e2006025.

Spitzen-van der Sluijs, A., F. Spikmans, W. Bosman, M. de Zeeuw, T. van der Meij, E. Goverse, M. J. L. Kik, F. Pasmans & A. Martel (2013): Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. – Amphibia-Reptilia, **34**: 233–239.

Spitzen-van der Sluijs, A., A. Martel, J. Asselberghs, E. K. Bales, W. Beukema, M. C. Bletz, L. Dalbeck, E. Goverse, A. Kerres, T. Kinet, K. Kirst, A. Laudelout, L. F. Marin da Fonte, A. Nöllert, D. Ohlhoff, J. Sabino-Pinto, B. R. Schmidt, J. Speybroeck, F. Spikmans, S. Steinfartz, M. Veith, M. Vences, N. Wagner, F. Pasmans & S. Lötters (2016): Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. – Emerging Infectious Diseases, **22**: 1286–1288.

Spitzen-van der Sluijs, A., G. Stegen, S. Bogaerts, S. Canessa, S. Steinfartz, N. Janssen, W. Bosman, F. Pasmans & A. Martel (2018): Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. – Scientific Reports, **8**: 3800.

Stegen, G., F. Pasmans, B. R. Schmidt, L. O. Rouffaer, S. van Praet, M. Schaub, S. Canessa, A. Laudelout, T. Kinet, C. Adriaensen, F. Haesebrouck, W. Bert, F. Bossuyt & A. Martel (2017): Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. – Nature, **544**: 353–356.

Thomas, V., M. Blooi, P. van Rooij, S. van Praet, E. Verbrugghe, E. Grasselli, M. Lukac, S. Smith, F. Pasmans & A. Martel (2018): Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. – Transboundary and Emerging Diseases, **65**: e478–e488.

Thomas, V., Y. Wang, P. van Rooij, E. Verbrugghe, V. Baláz, J. Bosch, A. A. Cunningham, M. C. Fischer, T. W. J. Garner, M. J. Gilbert, E. Grasselli, T. Kinet, A. Laudelout, S. Lötters, A. Loyau, C. Miaud, S. Salvidio, D. S. Schmeller, B. R. Schmidt, A. Spitzen-van der Sluijs, S. Steinfartz, M. Veith, M. Vences, N. Wagner, S. Canessa, A. Martel & F. Pasmans (2019) Mitigating *Batrachochytrium salamandrivorans* in Europe. – Amphibia-Reptilia, **40**: 265–290.

Van Rooij, P., A. Martel, F. Haesenbrouck & F. Pasmans (2015): Amphibian chytridiomycosis: a review with focus on fungus-host interactions. – Veterinary Research, **46**: 137.

Wagner, N., S. Lötters, L. Dalbeck, H. Düssel, M. Guschal, K. Kirst, D. Ohlhoff, J. Wegge, T. Reinhardt & M. Veith (2020): Long-term monitoring of European fire salamander populations (*Salamandra salamandra*) in the Eifel Mountains (Germany): five years of removal sampling of larvae. – Salamandra, **56**: 243–253 [this issue].

Weldon, C., A. Channing, G. Misinzo & A. A Cunningham (2020): Disease driven extinction in the wild of the Kihansi spray toad, *Nectophrynoides asperginis*. – African Journal of Herpetology, **69**. DOI: 10.1080/21564574.2020.1752313

Woodhams, D. C., J. Bosch, C. J. Briggs, S. Cashins, L. R. Davis, A. Lauer, E. Muths, R. Puschendorf, B. R. Schmidt, B. Sheafor & J. Voyles (2011): Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. – Frontiers in Zoology, **8**: 8.