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Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*

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The recent arrival of *Batrachochytrium salamandrivorans* in Europe was followed by rapid expansion of its geographical distribution and host range, confirming the unprecedented threat that this chytrid fungus poses to western Palaearctic amphibians[1,2]. Mitigating this hazard requires a thorough understanding of the pathogen's disease ecology that is driving the extinction process. Here, we monitored infection, disease and host population dynamics in a Belgian fire salamander (*Salamandra salamandra*) population for two years immediately after the first signs of infection. We show that arrival of this chytrid is associated with rapid population collapse without any sign of recovery, largely due to lack of increased resistance in the surviving salamanders and a demographic shift that prevents compensation for mortality. The pathogen adopts a dual transmission strategy, with environmentally resistant non-motile spores in addition to the motile spores identified in its sister species *B. dendrobatidis*. The fungus retains its virulence not only in water and soil, but also in anurans and less susceptible urodelan species that function as infection reservoirs. The combined characteristics of the disease ecology suggest that further expansion of this fungus will behave as a 'perfect storm' that is able to rapidly extirpate highly susceptible salamander populations across Europe.

The past two decades have seen the emergence of novel fungal diseases that globally affect biodiversity, leading to the potential extinction of animal and plant species[3–8]. When fungal pathogens are vectored into naive ecosystems, firm pathogen establishment and extensive host population decline typically precede elucidation of the disease ecology, which is required for the development of threat abatement plans[3,9]. The chytrid fungus *Batrachochytrium salamandrivorans* is a prime example of an emerging infectious disease that has recently become a threat in Europe, where it causes massive decline of salamander populations and poses an unprecedented threat to Western Palaearctic amphibian diversity[1,4,10]. Here we unravel the fundamental mechanisms of amphibian extirpation mediated by the recent arrival of *B. salamandrivorans*. Immediately after the discovery of the first signs of disease (April, 2014) in a population of fire salamanders in Robertville, Belgium, 57 km from the *B. salamandrivorans* index site in the Netherlands[4], we began to continuously monitor infection, disease and host population dynamics for two years. Our study demonstrates how the combined characteristics of host susceptibility, pathogen virulence and environmental persistence create a 'perfect storm' with high probability of extirpation after pathogen arrival in a susceptible host population.

Our monitoring revealed that introduction of *B. salamandrivorans* leads to a fast host population collapse, without any sign of recovery, owing to sustained and disproportionate mortality of adults, which leads to a demographic shift in the population (Fig. 1a, b). Across ten-day intervals, we found a probability of infection of 0.33 (95% credible interval (CRI) = 0.169–0.512). Infection resulted in a sixfold difference in survival rate (mean survival in infected versus non-infected animals (0.13 ± 0.11 s.d., 95% CRI = 0.004–0.403) versus (0.84 ± 0.10, 95% CRI = 0.63–0.99)).

In a series of infection trials, we studied the host–pathogen interaction underpinning the susceptibility of fire salamanders to infection with *B. salamandrivorans*. We demonstrate that the outcome of the disease in this species is dose- and temperature-independent and that infected animals do not mount any protective immune response. Experimental inoculation of fire salamanders with a high or low dose of four different *B. salamandrivorans* isolates resulted in lethal disease in all animals, despite a slower build-up of infection load at the low dose (Fig. 2a, b). When comparing infection dynamics of *B. salamandrivorans* at the fungus' optimal temperature (15 °C)[4] with those at 4 °C, all animals again developed lethal infection, with slower build-up of infection at 4 °C (Fig. 2c, d), indicating that the pathogen is able to infect and kill amphibians over a broad temperature range. In a final experiment, we assessed build-up of protection in salamanders after five cycles of exposure treatment. Contrary to the theory that non-lethal exposure to the pathogen could provide opportunities to mount a protective immune response[11], this experiment confirmed that resistance against infection did not increase (Fig. 2e, f). The inability of salamanders to mount resistance against *B. salamandrivorans* infection largely excludes vaccination as a mitigation measure for susceptible salamander species and could preclude build-up of population immunity. Indeed, the few salamanders that were still present at the outbreak site after two years were still highly susceptible to infection with the local *B. salamandrivorans* isolate, showing a 100% mortality rate after exposure.

Given the continuous high mortality and high transmission rate, persistence of any susceptible European host population after infection would only be likely when compensated by elevated recruitment[12]. However, we found that *B. salamandrivorans* disproportionately infects and kills sexually mature animals (Fig. 1b), quickly resulting in a demographic shift that massively decreases the recruitment potential of the population that would be necessary to compensate for adult mortality[13]. Increased infection probability and subsequent mortality of adult fire salamanders compared to juveniles can be explained by intimate contact with infected adult conspecifics during territorial displays and reproduction (Fig. 1c), and back-and-forth migration of females to the streams where they give birth to aquatic larvae. Juveniles show less surface activity and interactions with conspecifics[14], with reduced probability of becoming infected.

Our analyses indicate that the rapid population decline can be largely associated with two fungal pathogen determinants: sustained fungal

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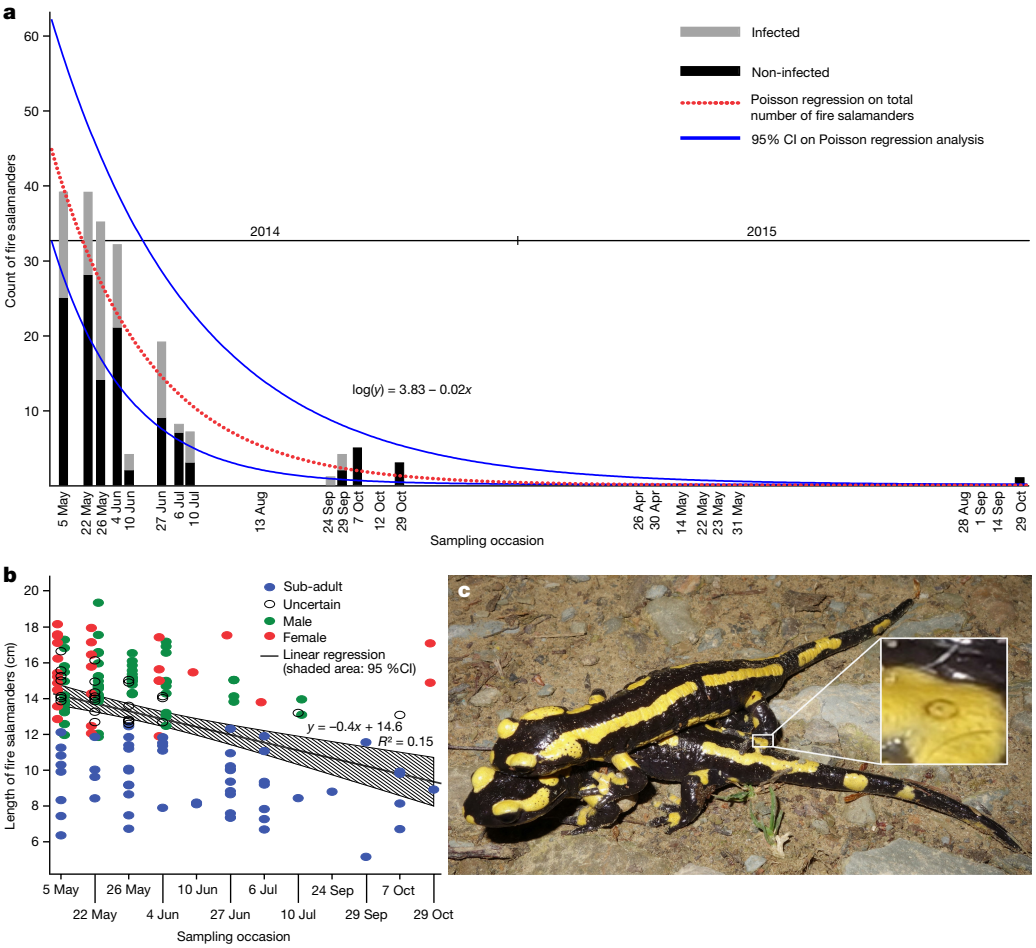

Figure 1 | *B. salamandrivorans* leads to fire salamander population extirpation. a, **b**, An estimated 90% decline within 6 months (**a**) coincides with a decrease of the average length and a demographic shift (**b**). CI, confidence interval. **c**, Contact between an infected (on top; skin lesion shown in insert) salamander with an un-infected one during courtship.

virulence and the presence of an environmentally resistant encysted spore. Sustained virulence of the fungus in its novel susceptible host species was demonstrated by the persistence of highly virulent *B. salamandrivorans* two years after the outbreak in our study site, despite almost complete depletion of the host population. Indeed, isolates cultured from infected animals at the end of our two-year monitoring period were equally capable of killing 100% of the experimentally inoculated salamanders as an initial isolate. The sustained virulence is assisted by the fact that, in addition to motile spores as in its closest sister species *B. dendrobatidis*, *B. salamandrivorans* produces a second type of infectious encysted spores both *in vitro* and *in vivo* in salamander skin, with a distinct

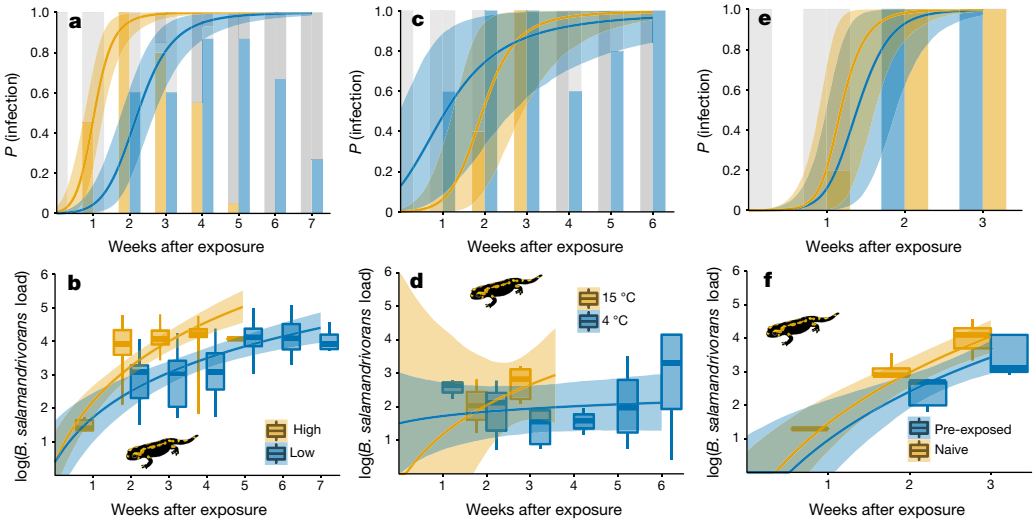

Figure 2 | Effect of different variables on infection dynamics of *B. salamandrivorans* in fire salamanders. a, **b**, A lower dose results in slower build-up of infection and delayed mortality. **c**, **d**, At lower temperatures, similar infection intensities are reached more slowly. **e**, **f**, Previous exposure to *B. salamandrivorans* does not protect against re-infection. Bars (**a**, **c**, **e**) indicate the proportion of alive and infected (coloured), alive and uninfected (light grey) and dead (dark grey) individuals; curves indicate the estimated treatment-specific probability of infection (shading: 95% CRI). Box plots (**b**, **d**, **f**) indicate infection loads in genomic equivalents per swab (bars indicate 2.5th and 97.5th percentiles); curves indicate the estimated treatment-specific load (shading: 95% CRI).

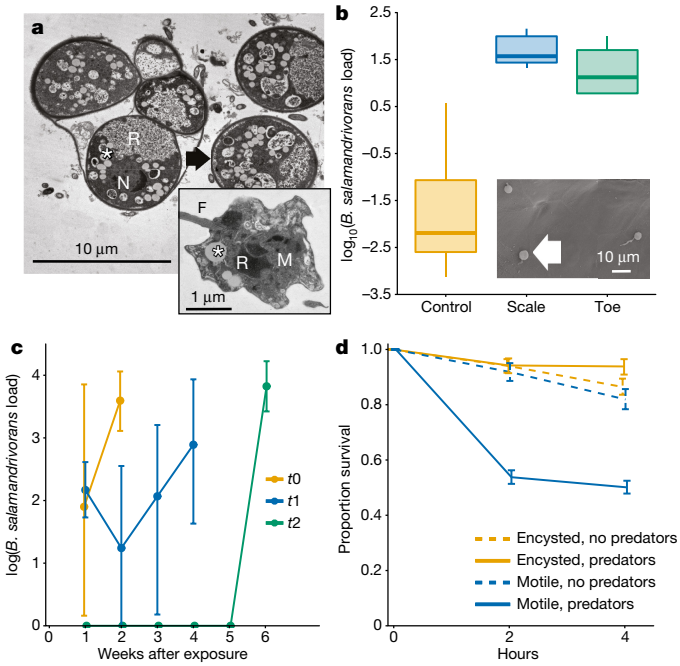

Figure 3 | *B. salamandrivorans* encysted spores avoid predation and infect fire salamanders. a, Transmission electron microscopic picture of a sporangium containing encysted spores and free encysted spores in the superficial epidermis and a zoospore (insert). *, lipid globule; arrow, cell wall; F, flagellum; M, mitochondrion; N, nucleus; R, ribosomal mass. **b**, Adhesion of encysted spores to salamander skin and scales from goose feet (control: skin dipped in culture supernatant; insert: scanning electron microscopic image of attachment to salamander skin). **c**, *B. salamandrivorans* infection loads in salamanders exposed to encysted spores that were either freshly collected (*t*0, with $n = 6$) or were incubated in pond water for 14 or 31 days (*t*1 and *t*2, with $n = 4$ and $n = 2$). **d**, Estimated survival of motile and encysted spores after exposure to aquatic micropredators. Bars indicate the 2.5th and 97.5th percentiles in **b**, **c** and 95% CRI in **d**. *B. salamandrivorans* loads (**b**, **c**) are expressed in genomic equivalents per swab.

fungal infection, dissemination and persistence strategy (Fig. 3a). Whereas zoospores actively swim to their host[15], encysted spores float at the water–air interface (Supplementary Video 1) and are capable of quickly adhering to salamander skin and to scales of the feet of waterfowl (Fig. 3b). This passive adherence to inert matrices may promote fungal spread over large spatial distances. Encysted

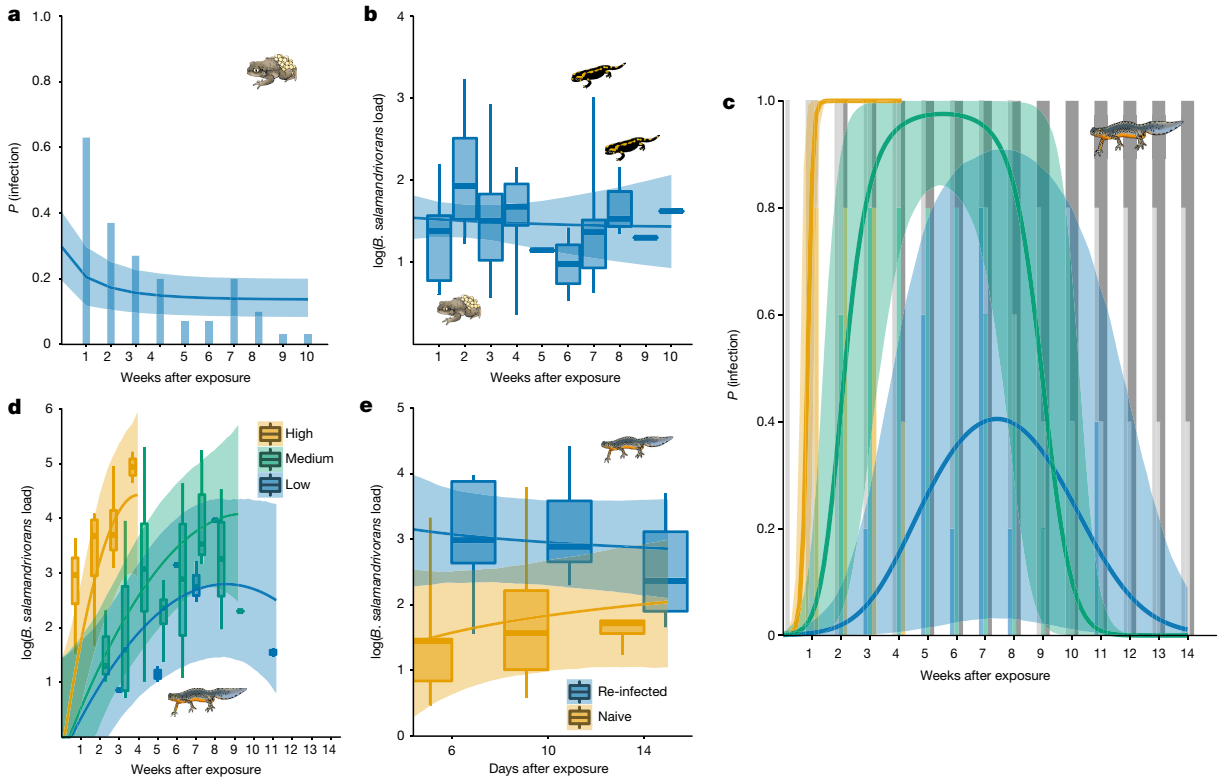

Figure 4 | Anuran and urodelan reservoirs promote *B. salamandrivorans* sustenance. a, **b**, Probability of infection and infection load in midwife toads. **a**, Bars indicate the proportion of infected individuals (no mortality observed) and the curve indicates the estimated probability of infection. **b**, Box plots and curve indicate, respectively, observed and estimated infection load of *B. salamandrivorans*-positive individuals; salamander icons indicate infection in fire salamanders that had been co-housed with the toads from the second week post exposure onwards. **c**, **d**, Probability of infection and infection load in Alpine newts after exposure to 10,000 (orange), 1,000 (green) or 100 (blue) spores. **c**, Bars indicate the proportion of alive and infected (coloured), alive and uninfected (light grey) and dead individuals (dark grey); curves indicate the probability of infection. **d**, Box plots and curves indicate, respectively, observed and estimated infection load of *B. salamandrivorans*-positive individuals. **e**, Infection loads for naive or previously infected Alpine newts; box plots and curves indicate, respectively, observed and estimated infection load of *B. salamandrivorans*-positive individuals. *B. salamandrivorans* loads (**b**, **d**, **e**) are expressed in genomic equivalents per swab. In all plots, shading around curves indicates 95% CRI; box plot bars indicate 2.5th and 97.5th percentiles.

spores survived and remained infective for fire salamanders for at least 31 days in filtered pond water (Fig. 3c) and were more resistant than zoospores to predation by zooplankton (Fig. 3d), highlighting their potential to persist in an aquatic environment.

Long-term persistence of *B. salamandrivorans* is further promoted by its presence on less-susceptible amphibian pathogen reservoirs, as we demonstrated by experimental infection of anuran and urodelan hosts. A proportion of the four used *B. salamandrivorans* isolates was capable of infecting anuran hosts (midwife toads, *Alytes obstetricans*) at low intensities for several weeks after experimental inoculation. Whereas the toads showed no sign of disease, their colonization with the pathogen was sufficient to transmit *B. salamandrivorans* to susceptible salamanders (Fig. 4a, b, Extended Data Table 1). In urodelans, experimental infection of Alpine newts (*Ichthyosaura alpestris*), a species that co-occurs syntopically with fire salamanders, showed a dose-dependent disease course. Whereas infection with a high dose resulted in disease and death after an average of three weeks, exposure to a low dose resulted in significant *B. salamandrivorans* shedding for several months with eventual fungal clearing and clinical cure (Fig. 4c, d). However, previous infection was shown to provide the newts with no protection against re-infection and mortality (Fig. 4e). These newts thus meet the criteria for a pathogen reservoir. Indirect transmission would also favour pathogen maintenance in host populations; therefore, in a final infection experiment we demonstrated the potential for pathogen transmission via contaminated forest soil. Infected salamanders were shown to contaminate the forest soil, in which the fungal DNA could be detected even after 200 days. Actual transmission through contaminated forest soil was demonstrated up to 48 h after the soil had been in contact with an infected animal (Extended Data Figs 1, 2, Extended Data Tables 2, 3). Altogether, the presence of a resistant spore with the ability to persist environmentally and to transmit through contaminated water and soil, combined with the occurrence of long-term-infected and pathogen-shedding amphibian hosts, creates the potential for extensive environmental reservoirs and hampers any effort to eradicate *B. salamandrivorans* from an infected ecosystem.

Our study reveals that the multifaceted ecology of this expanding fungal disease is likely to result in fast extirpation of highly susceptible salamanders, with no available options to halt the spread or to mitigate the disease *in situ*. Although several potential measures to counteract the effect of chytrid fungi on amphibian communities have been proposed[9], *ex situ* conservation programmes are currently the only intervention available that will effectively avert loss of susceptible urodelan populations upon *B. salamandrivorans* arrival. Given the continuous range expansion of the disease and the speed of its effects, the development of a pan-European early warning system to monitor the fungal invasive front and the enforcement of emergency action plans that allow fast implementation of *ex situ* conservation in acutely threatened urodelan species are urgently needed. A thorough understanding of the host, pathogen and environment determinants underpinning susceptibility to *B. salamandrivorans* may yield the tools required for risk analysis of the actual threat of the fungal disease to western Palaearctic urodelans, which will guide prioritization of conservation efforts. Indeed, although most western Palaearctic urodelan taxa were shown to be susceptible to *B. salamandrivorans* in laboratory trials[1], we demonstrate marked interspecific differences. Fire salamanders represent a hyper-susceptible species in which *B. salamandrivorans* causes acute, dose-independent mortality and population extirpation. The rapid and consistent mortality at least in species of the genera *Salamandra*, *Euproctus, Neurergus, Pleurodeles* and in *Lissotriton italicus* after exposure to *B. salamandrivorans*[1,16] suggests a similar risk of disease driven extirpation for many other species of western Palaearctic urodelans. As eradication of *B. salamandrivorans* from the European continent is highly unlikely, long-term sustainable mitigation should aim at host–pathogen co-existence, which implies the development of intervention strategies that permanently increase resistance of susceptible species against *B. salamandrivorans*[9]. For regions that are currently considered free of *B. salamandrivorans*, such as the Americas, prevention of introduction in naive environments should be considered the sole effective control measure available. This will require knowledge of all introduction pathways that, besides amphibians in trade[1,17,18], may also include non-amphibian sources carrying resistant spores.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Martel, A. *et al.* Wildlife disease. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* **346,** 630–631 (2014).
2. Spitzen-van der Sluijs, A. *et al.* Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. *Emerg. Infect. Dis.* **22,** 1286–1288 (2016).
3. Fisher, M. C. *et al.* Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484,** 186–194 (2012).
4. Martel, A. *et al. Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proc. Natl Acad. Sci. USA* **110,** 15325–15329 (2013).
5. Cheng, T. L., Rovito, S. M., Wake, D. B. & Vredenburg, V. T. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proc. Natl Acad. Sci. USA* **108,** 9502–9507 (2011).
6. Kim, K. & Harvell, C. D. The rise and fall of a six-year coral-fungal epizootic. *Am. Nat.* **164** (Suppl 5), S52–S63 (2004).
7. Frick, W. F. *et al.* An emerging disease causes regional population collapse of a common North American bat species. *Science* **329,** 679–682 (2010).
8. Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. & Sieber, T. N. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Mol. Plant Pathol.* **15,** 5–21 (2014).
9. Garner, T. W. J. *et al.* Mitigating amphibian chytridiomycosis in nature. *Phil. Trans. R. Soc. B* **317,** 20160207 (2016).
10. Spitzen-van der Sluijs, A. *et al.* Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. *Amph. Rept.* **34,** 233–239 (2013).
11. McMahon, T. A. *et al.* Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature* **511,** 224–227 (2014).
12. Muths, E., Scherer, R. D. & Pilliod, S. D. Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *J. Appl. Ecol.* **48,** 873–879 (2011).
13. Schmidt, B. R., Feldmann, R. & Schaub, M. Demographic processes underlying population growth and decline in *Salamandra salamandra*. *Conserv. Biol.* **19,** 1149–1156 (2005).
14. Seifert, D. Untersuchungen an einer ostthüringischen Population des Feuersalamanders (*Salamandra salamandra*). *Artenschutzreport* **1,** 1–16 (1991).
15. Rosenblum, E. B., Voyles, J., Poorten, T. J. & Stajich, J. E. The deadly chytrid fungus: a story of an emerging pathogen. *PLoS Pathog.* **6,** e1000550 (2010).
16. Sabino-Pinto, J. *et al.* First detection of the emerging fungal pathogen *Batrachochytrium salamandrivorans* in Germany. *Amph. Rept.* **36,** 411–416 (2015).
17. Yap, T. A., Koo, M. S., Ambrose, R. F., Wake, D. B. & Vredenburg, V. T. Biodiversity. Averting a North American biodiversity crisis. *Science* **349,** 481–482 (2015).
18. Price, S. J., Garner, T. W. J., Cunningham, A. A., Langton, T. E. S. & Nichols, R. A. Reconstructing the emergence of a lethal infectious disease of wildlife supports a key role for spread through translocations by humans. *Proc. R. Soc. Lond. B* **283,** 20160952 (2016).

Supplementary Information is available in the online version of the paper.

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METHODS

***Batrachochytrium salamandrivorans* isolates and culture conditions.** Four *B. salamandrivorans* isolates were isolated from wild fire salamander populations that are declining owing to a *B. salamandrivorans* outbreak in the Netherlands (AMFP13/1)[4] and Belgium (AMFP14/1 (Robertville, 2014); AMFP 15/3 (Robertville, 2015) and AMFP14/2 (Luik)[2]. One isolate originated from a captive population suffering from a *B. salamandrivorans* disease outbreak in Germany (AMFP15/1)[16]. All isolates were grown in a 1.6% tryptone, 0.4% gelatinehydrolysate and 0.2% lactose monohydrate liquid medium at 15 °C. Motile or encysted spores were collected in distilled water after 5–7 days of growth. Zoospores were obtained by washing the culture flasks with filtered pond water (0.2-μm filter). Floating encysted spores were collected from the water surface using a 10-μl inoculation loop. Purity of the spore suspension was assessed using inverted microscopy. Encysted spore suspensions were only used in experiments when motile spores were absent. No statistical methods were used to predetermine sample size.

DNA extraction and *B. salamandrivorans* qPCR. DNA extraction of skin swabs and *B. salamandrivorans* qPCR were performed as described in refs 19, 20. Animals were considered positive for *B.-salamandrivorans* infection when the following conditions were fulfilled: (1) the qPCR sample quantity was above the detection limit of 0.1 genomic equivalent (GE)/qPCR reaction for both replicates, (2) the mean starting quantity value of each sample was higher than the standard deviation of its starting quantity, and (3) the amplification curve from each replicate was logarithmic.

Infection trials. For all experimental replicates, all fungal cultures were grown independently. All animals were housed individually in terraria at the fungus thermal preference of 15 °C[4] (unless otherwise stated) on moist tissue with access to a hiding place. All animals (males/females) were captive bred, clinically healthy and free of *B. dendrobatidis*, *B. salamandrivorans* and Ranavirus as assessed by sampling the skin using cotton-tipped swabs and subsequently performing qPCR[19,20] or PCR[21]. Infection experiments were carried out as described in ref. 1. Individuals were randomly assigned to treatments. All animals were clinically inspected daily. Skin sampling was done weekly and the swabs were analysed for the presence of *B. salamandrivorans* using qPCR described in refs 19, 20. Investigators were blinded to allocation during experiments and outcome assessment.

Hygiene and biosafety protocols. The animal experiments were performed under strict BSL2 conditions. During the fieldwork, each individual was handled with a new pair of nitrile gloves. At the end of each field visit, boots and other equipment which came into contact with the environment were disinfected with a 1% Virkon solution for at least 5 min.

Ethics statement. The animal experiments were performed with the approval of the ethical committee of the Faculty of Veterinary Medicine (Ghent University EC2013/10; EC2014/170; EC2015/29; EC2015/42; EC2016/87). The capture, handling, sampling and transport of wild salamanders and access to the sampling site were permitted by the Wallonian Department of Nature and Forests (Département de la Nature et des Forêsts) (reference 2014/RS/n°23).

Infection and disease dynamics during a *B. salamandrivorans* outbreak in fire salamanders: a 2-year follow-up study. In May 2014, a *B. salamandrivorans* outbreak with mass mortality was identified in a population of fire salamanders (*Salamandra salamandra terrestris*) in the forest of Robertville (50° 27′ 10″ N, 06° 06′ 10″ E), Belgium. Over the course of 2 years, the population was monitored during the activity periods of the salamanders: (1) May to October 2014 and (2) April to September 2015. Over a fixed 475-m-long transect, salamanders were detected using a visual encounter survey and sampled by collecting skin swabs. To investigate whether *B. salamandrivorans* can be detected in terrestrial environments in the wild, soil samples were taken in the close vicinity of animals with chytridiomycosis (Extended Data Fig. 1). The animals that were found during the second year, one on the study transect and 11 outside the transect, were brought to our quarantine facilities to test their susceptibility to chytridiomycosis. None of these tested positive for *B. salamandrivorans* when sampled at location. However, the animals developed severe to lethal signs of chytridiomycosis within 3 weeks after exposure to the fungus.

In total, 24 visits resulted in 197 captures of fire salamanders. Individual salamanders were identified by their conspicuous yellow marks; from each captured individual, dorsal and lateral photographs were taken. On the basis of the capture-mark-recapture data from the first two visits between which there were confirmed recaptures, the population of fire salamanders on the study-transect was estimated to consist, at the beginning of the study, of 239 (95% confidence interval, 112–459) individuals, using the Lincoln–Petersen index[22].

Host population dynamics analysis was performed on the basis of a multistate capture-mark-recapture model[23]. On the basis of the qPCR results from the skin swabs, each individual was classified as either infected or non-infected. We constructed a Bayesian multistate capture–recapture model[24] to estimate survival probabilities of non-infected and infected individuals as well as transition probabilities between these two states. The transition probability from a non-infected to infected state is the infection probability, the transition probability from infected to non-infected is the recovery probability. Because the population was declining rapidly and no recovered individuals were observed, all parameters were set constant through time, reencounter probability of infected individuals was assumed to be equal to reencounter probability of uninfected salamanders. The interval between two sampling periods was highly influenced by weather conditions and activity periods of salamanders. To adjust for the unequal time intervals, dummy sampling occasions were added to the dataset to equalize the intervals between two sampling periods to approximately 10-days. The reencounter probability was set to 0 at the dummy occasions. The estimated survival and transition probabilities refer therefore to 10 day intervals. Analyses were performed for the data collected during the first period only (sampling occasions 1–14). The multistate model was fitted in JAGS[25] through package jagsUI in R[24]. Vague priors were chosen for all the parameters (uniform between 0 and 1) and convergence was inferred by $\hat{R}$ values <1.1.

Drivers of *B. salamandrivorans* dynamics in susceptible fire salamanders. *Dose dependency of* B. salamandrivorans *infection and disease dynamics.* Four *B. salamandrivorans* isolates (AMFP13/1, AMFP14/1, AMFP14/2, and AMFP15/1) were used to expose 40 juvenile fire salamanders. Animals were infected with one isolate. For each isolate two doses were used: either 10^4 spores (high dose) or 100 spores (low dose). For each dose-by-isolate combination, 5 animals were inoculated per isolate and each animal was housed individually. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 8 weeks (Fig. 2a, b).

Temperature dependency of B. salamandrivorans *infection dynamics.* In this experiment, the infectivity of *B. salamandrivorans* after incubation in water for 4 weeks was evaluated. A suspension of 1.3 10^4 GE *B. salamandrivorans* per ml environmental water was incubated for 4 weeks at both 4 °C and 15 °C. After 4 weeks, the concentration of *B. salamandrivorans* was 2.6×10^4 GE per ml water (4 °C) and 1.3×10^4 GE per ml water (15 °C). This water was used to inoculate 10 salamanders as described in ref. 1. Water containing *B. salamandrivorans* incubated at 4 °C was used to infect salamanders which were later housed individually at 4 °C. Water containing *B. salamandrivorans* incubated at 15 °C was used to infect salamanders which were later housed individually at 15 °C. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 10 weeks (Fig. 2c, d).

Effect of previous infection on infection and disease dynamics of B. salamandrivorans. Five fire salamanders were infected with 10^3 spores of AMFP13/1. After increase of the GE load in two subsequent swabs (weekly sampling), animals were treated at 25 °C for 10 days as described in ref. 26. One month after finishing the treatment, the animals were re-infected. This exposure–treatment cycle was repeated five times. For the subsequent challenge experiment, infection dynamics of *B. salamandrivorans* in these five pre-exposed salamanders were compared their initial infection dynamics (Fig. 2e, f).

***B. salamandrivorans* produces infectious, encysted and environmentally protected spores.** *Production of encysted spores by* B. salamandrivorans. Using microscopy and transmission electron microscopy, we identified two types of spores in *B. salamandrivorans*: a motile (zoo)spore and a non-motile, encysted spore with a cell wall. The ultrastructure of the different spores was investigated in *in vitro* cultures of *B. salamandrivorans* and in skin samples of infected fire salamanders. Transmission and scanning electron microscopy was performed as described in ref. 3 (Fig. 3a, insert Fig. 3b).

Brief contact with floating, encysted spores results in adherence of B. salamandrivorans *to salamander skin and scales from goose feet.* Encysted spores were collected and inoculated in filtered (0.2-μm filter) environmental water at a concentration of 10^6 spores per ml water. A salamander toe and scale from goose feet were dipped in the suspension for 1 s. Controls to quantify *B. salamandrivorans* DNA contamination consisted of toes, dipped in filtered (0.5-μm filter) culture supernatant. *B. salamandrivorans* load in all samples was determined using qPCR[19,20]. Two independent experiments were performed in triplicate. Results are expressed as mean number of genomic equivalents per mm^2 + standard deviation of the respective tissue (Fig. 3b).

Survival in the aquatic environment and infectivity of encysted spores. Encysted spores were collected and inoculated in filtered environmental water at a concentration of 10^8 spores per ml water. At time points 0 (immediately after collection), 1 (15 days after collection) and 2 (31 days after collection) fire salamanders ($n = 6$ at $t0$, $n = 4$ at $t1$ and $n = 2$ at $t2$) were inoculated by dropping 100 μl of this suspension on the salamanders' dorsum. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 10 weeks. Infection loads were determined by quantifying *B. salamandrivorans* DNA in skin swabs using qPCR[19,20]. Results are presented as average infection loads ± standard deviation (Fig. 3c).

Predation of motile and encysted spores. Predation of motile and encysted spores by micropredators present in pond water was tested as described in ref. 27. Briefly, 10^6 motile or encysted spores were incubated with 1 ml of pond water containing 456 zooplanktonic organisms per ml, for four hours in 24-well plates at 15 °C. The water contained copepods (30 per ml), ciliates (paramecium (338 per ml) and peritrich ciliates (18 per ml)), rotifers (16 per ml), ostracods (35 per ml), heliozoans (1 per ml) and water fleas (18 per ml) as determined by counting the total content in 1 ml of pond water using light microscopy. For comparison, zoospores and encysted spores were incubated in pond water that was filtered using a 5-μm filter. After 4 h incubation the number of remaining spores was counted using a Bürker counting chamber. Removal of spores from the aquatic environment was quantified as proxy for spore ingestion. Ingestion was calculated as the proportion of remaining encysted or zoospores at a given time point compared to the number of spores recovered in the wells with filtered pond water at that time point. Three independent experiments were carried out in triplicate. Results shown are experimental means with standard error of the mean (Fig. 3d).

Vectors and potential reservoirs of *B. salamandrivorans*. *Anuran reservoirs of* B. salamandrivorans. Four *B. salamandrivorans* isolates (AMFP13/1, AMFP14/1, AMFP14/2, and AMFP15/1) were used to expose 32 juvenile midwife toads (*Alytes obstetricans*) to 10^5 spores. Eight animals were inoculated per isolate. To assess whether infected midwife toads are capable of transmitting *B. salamandrivorans* to susceptible fire salamanders, from 14 days after inoculation, five randomly selected midwife toads per isolate were selected and each toad was housed together with a juvenile fire salamander in a new terrarium. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 10 weeks (Fig. 4a, b). Two fire salamanders developed infection and clinical disease. They were taken out of the experiment and treated as described in ref. 26.

Urodelan reservoirs of B. salamandrivorans. The holotype isolate AMFP13/1 was used to inoculate 20 Alpine newts (*Ichthyosaura alpestris*). Four different infection doses (5 animals per infection dose) were used: 10^4, 10^3, 10^2 and 10 spores. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 14 weeks (Fig. 4c, d). As exposure to 10 spores resulted in only 2 out of 5 animals becoming infected, results were omitted from further analyses.

Previous infection does not protect Alpine newts against B. salamandrivorans *re-infection.* In this study, we assessed whether Alpine newts that had been chronically infected by *B. salamandrivorans* are protected against re-infection with *B. salamandrivorans*. From previous experiments in which Alpine newts were exposed to a low (10^3 zoospores) dose of *B. salamandrivorans* (see urodelan reservoirs of *B. salamandrivorans*), nine animals developed chronic infection without subsequent mortality. Skin swabs from these animals were positive for *B. salamandrivorans* between 28 and 175 days after exposure to *B. salamandrivorans* (average ± s.d. = 95 ± 45 days), with average *B. salamandrivorans* counts per sample of $\log_{10}(2.02 \pm 0.54)$. All except one animal cleared *B. salamandrivorans* infection before experimental re-infection. Of the animals that cleared infection, the time between the last positive skin sample and the experimental re-infection ranged between 54 and 567 days (average ± s.d. = 278 ± 218 days). Re-infection with 10^6 zoospores of *B. salamandrivorans* was performed as described before and animals were followed up by determining *B. salamandrivorans* infection loads in skin swabs using qPCR (Fig. 4e). For comparison, five negative control animals were included that had never been exposed to *B. salamandrivorans*.

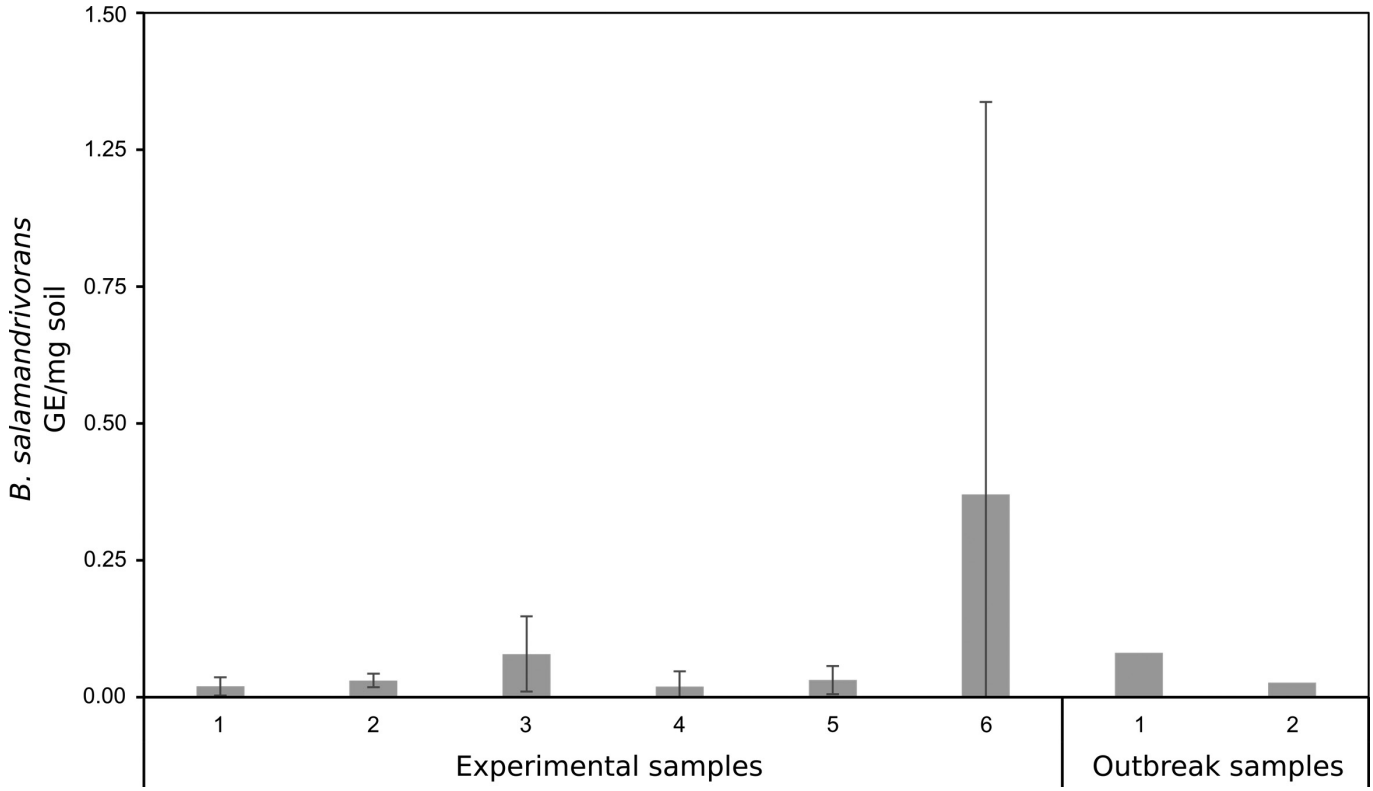
Forest soil as a vector for B. salamandrivorans *transmission.* 15 g of forest soil (moisture content: 47.24 ± 0.07%) was moistened with 10 ml of distilled water and inoculated with 1 ml of *B. salamandrivorans* suspension containing 6.0×10^6 GE. A fire salamander was exposed to 1 g of this soil for 24 h either immediately after inoculating the soil, after 8 h incubation, after 24 h incubation, after 2 days incubation, after 4 days incubation or after one week incubation. At the different time points, the amount of *B. salamandrivorans* in soil was determined with qPCR after DNA extraction using the Powerlyzer Powersoil DNA Isolation Kit (MO BIO Laboratories Inc.). This experiment was done in triplicate at 15 °C and 4 °C. (Extended Data Fig. 2, Extended Data Table 2).

Fourteen infected salamanders (mean infection load $3.2 \times 10^3 \pm 4.4$) were housed individually on forest soil for 24 h. Afterwards the animals were removed and were replaced by a non-infected individual. In one group (7 animals), the replacement was done immediately after removal of the infected animal. In the other group, animals were replaced with a non-infected individual 24 h after removal of the infected animal. The course of disease was followed up by daily clinical inspection and weekly sampling of the animals for 4 weeks (Extended Data Table 3). An animal was considered to be infected after two positive skin swabs in two subsequent weeks.

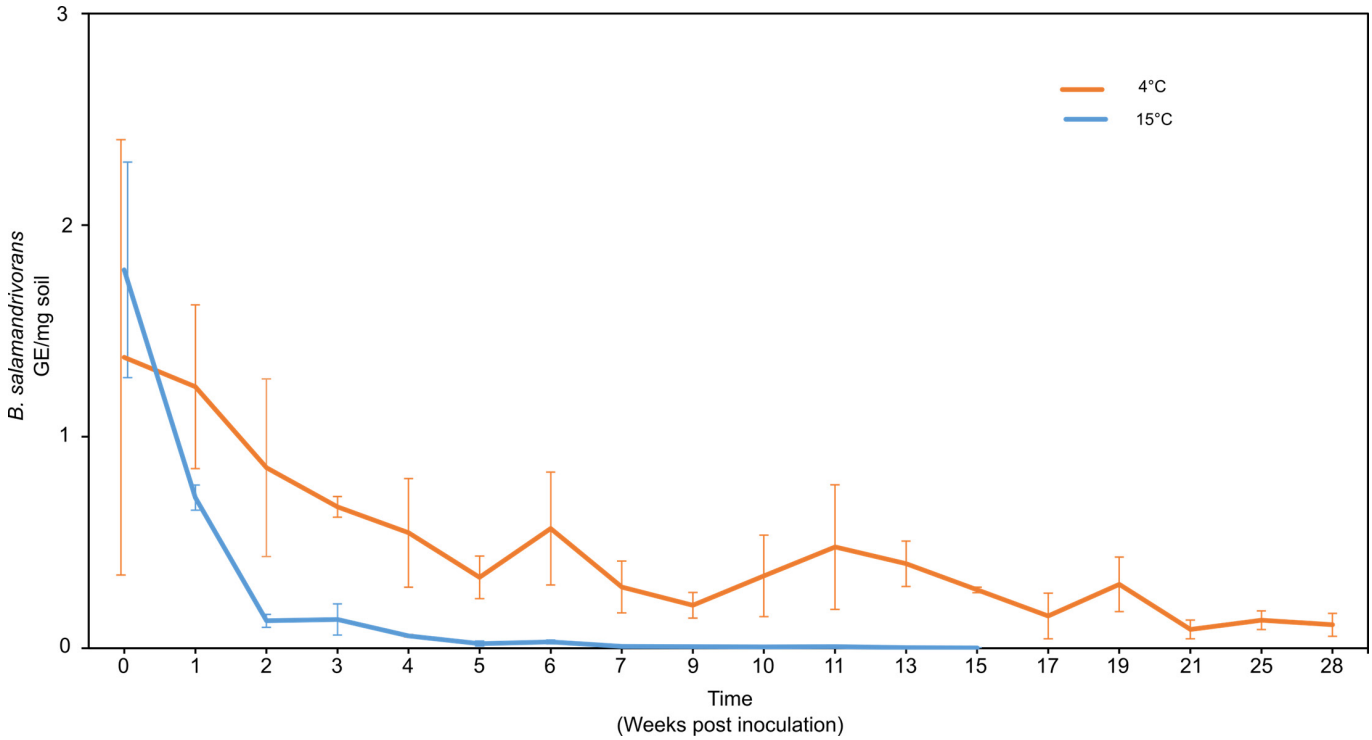
Data analysis. To assess the response of animals to *B. salamandrivorans* infection under different conditions, we carried out a two-step analysis. First, we modelled the presence or absence of infection across all individuals using logistic regression; second, we modelled the average load of positive individuals only using linear regression. For estimation of the infection load, we always used the natural logarithm of the GE as the response variable. For all analyses where repeated measures of the same individual or sample were taken, we used a random effect to account for pseudoreplication. We modelled probability of infection and average load as a linear function of treatment and as an asymptotic (*S. salamandra*), quadratic (*I. alpestris*) or exponential (*A. obstetricans*) function of time, and included the appropriate treatment–time interactions. For the zoospore predation experiment, we used an open-population N-mixture model with a robust design[28], accounting for sampling variability in the repeated measures for each sample and mortality between sampling occasions. We fitted all models in JAGS[25] through package jagsUI in R.[24], using uninformative priors for all coefficients including intercepts, sampling 10,000 posterior values from three Markov chains after a burn-in of 10,000 iterations. Convergence was inferred by $\hat{R}$ values <1.1.

Data availability. The authors declare that the data supporting the findings of this study are available within the paper and the Supplementary Information files (available in the online version of the paper).

19. Blooi, M. *et al.* Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in Amphibian samples. *J. Clin. Microbiol.* **51,** 4173–4177 (2013).
20. Blooi, M. *et al.* Correction for Blooi *et al.*, Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in Amphibian samples. *J. Clin. Microbiol.* **54,** 246 (2016).
21. Mao, J., Hedrick, R. P. & Chinchar, V. G. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. *Virology* **229,** 212–220 (1997).
22. Williams, B. K., Nichols, J. D. & Conroy, M. J. *Analysis and management of animal populations.* (Academic Press, 2002).
23. Lebreton, J. D., Nichols, J. D., Barker, R. J., Pradel, R. & Spendelow, J. A. Modeling individual animal histories with multistate capture–recapture models. *Adv. Ecol. Res.* **41,** 87–173 (2009).
24. Kéry, M. & Schaub, M. *Bayesian population analyzing using WinBUGS – a hierarchical perspective* (Academic Press, 2012).
25. Plummer, M. JAGS: a program for analyzing of Bayesian graphical models using Gibbs sampling. In *Proc. 3rd International Workshop on Distributed Statistical Computing* (Hornik, K., Leisch, F. & Zeileis, A., 2003).
26. Blooi, M. *et al.* Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. *Sci. Rep.* **5,** 8037 (2015).
27. Schmeller, D. S. *et al.* Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Curr. Biol.* **24,** 176–180 (2014).
28. Dail, D. & Madsen, L. Models for estimating abundance from repeated counts of an open metapopulation. *Biometrics* **67,** 577–587 (2011).



Extended Data Figure 1 | *B. salamandrivorans* GE loads in soil. To investigate whether *B. salamandrivorans* can be detected in terrestrial environments, soil samples were taken in the close vicinity of experimentally infected animals (experimental samples) and naturally infected salamanders in the Robertville outbreak area (outbreak samples). Error bars depict s.d.



Extended Data Figure 2 | *B. salamandrivorans* GE loads detection in experimentally infected soil, incubated at 4 °C and 15 °C. Error bars depict s.d.

Extended Data Table 1 | Infection loads (expressed in GE/PCR reaction) for midwife toads (green) and fire salamanders (orange)

Strain	Weeks after inoculation of Alytes muletensis																							
	week 1	week 2			week 3		week 4		week 5		week 6		week 7		week 8		week 9		week 10	
	AL	*AL*	*		*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*	*AL*	*SAL*
AMFP13/1																				
1	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	6	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	6	-			-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-
4	10	-			-	-	-	-	-	-	-	-	-	-	22	-	-	-	-	-
5	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	34,6	-			-		-		-		-		-		-		-		-	
7	-	-			-		26,4		14		-		-		-		-		-	
8	-	-			-		-		-		-		-		-		-		-	
AMFP14/1																				
1	6	46			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	10	250			-	-	-	-	-	-	-	-	36	600	156	8609	-		42	
3	-	134			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	26	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	24	84			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	56,2			-		-		-		-		24,6		-		-		-	
7	-	24,4			-		65,4		-		-		-		-		-		-	
8	-	15,2			12		-		-		-		-		-		-		-	
AMFP14/2																				
1	46	-			20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	30	-			1210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	32	-			-	-	-	-	-	-	-	-	22	-	-	-	-	-	-	-
4	4	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	66	-			52		35		-		28		4		-		-		-	
7	-	-			-		-		-		-		-		-		-		-	
8	-	-			-		-		-		-		-		-		-		-	
AMFP15/1																				
1	4	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	1140			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	24	1940			52	-	152	-	14	-	3,2	36	1660	9564	340		-		-	
6	88,6	-			3,16		-		-		-		-		-		-		-	
7	252	422			7,32		1,6		-		-		6,3		-		-		-	
8	39,4	23,4			150		100		-		-		-		-		-		-	

Four *B. salamandrivorans* isolates (AMFP13/1, AMFP14/1, AMFP14/2, and AMFP15/1) were used to expose 32 juvenile midwife toads to 10^5 spores. Eight animals were inoculated per isolate. To assess whether infected midwife toads are capable of transmitting *B. salamandrivorans* to susceptible fire salamanders, from 14 days after inoculation, five randomly selected midwife toads per isolate were selected and each toad was housed together with a juvenile fire salamander in a new terrarium (* indicates the time point of co-housing of *midwife toads* with *fire salamanders in* a new terrarium). The course of disease was followed up by daily clinical inspection and weekly sampling of the animals. Increased infection load and the development of ulcerations was used as an endpoint to stop the experiment for the fire salamanders: two animals were taken out of the experiment (blue).

Extended Data Table 2 | Infectivity of experimentally infected *B. salamandrivorans* soil at 4 °C and 15 °C

Time points	Incubation temperature	*B. salamandrivorans* positive animals (% of individuals)
0 h	/	83%
8 h	4°C	67%
	15°C	0%
24 h	4°C	67%
	15°C	33%
48 h	4°C	67%
	15°C	33%
96 h	4°C	0%
168 h	4°C	0%

Extended Data Table 3 | Infectivity of *B. salamandrivorans* in soil

	Infection load (GE) of the source animal	*B. salamandrivorans* transmitted to sentinel animal
Immediate replacement	168	NO
	8560	YES
	10	NO
	204	NO
	12240	YES
	3,33	NO
Replacement after 24h	3714	YES
	9421	YES
	335	NO
	3329	YES
	625	NO
	245	NO

Infected animals (source animals) were housed in a terrarium at 15 °C with a forest soil substrate and replaced with a healthy individual after 24 h (immediate replacement) or removed after 24 h and replaced by another 24 h later (replacement after 24 h). GE, genomic equivalent.

Web summary	The authors investigated the disease ecology of the fast-spreading fungal pathogen *Batrachochytrium salamandrivorans* in fire salamanders; on the basis of their research, they call for Europe-wide monitoring systems and conservation strategies for threatened species.