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No evidence for effects of infection with the amphibian chytrid fungus on populations of yellow-bellied toads

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ABSTRACT: The parasitic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) can cause the lethal disease chytridiomycosis in amphibians and therefore may play a role in population declines. The yellow-bellied toad *Bombina variegata* suffered strong declines throughout western and northwestern parts of its range and is therefore listed as highly endangered for Germany and the federal state of Hesse. Whether chytridiomycosis may play a role in the observed local declines of this strictly protected anuran species has never been tested. We investigated 19 Hessian yellow-bellied toad populations for *Bd* infection rates, conducted capture-mark-recapture studies in 4 of them over 2 to 3 yr, examined survival histories of recaptured infected individuals, and tested whether multi-locus heterozygosity of individuals as well as expected heterozygosity and different environmental variables of populations affect probabilities of *Bd* infection. Our results show high prevalence of *Bd* infection in Hessian yellow-bellied toad populations, but although significant decreases in 2 populations could be observed, no causative link to *Bd* as the reason for this can be established. Mass mortalities or obvious signs of disease in individuals were not observed. Conversely, we show that growth of *Bd*-infected populations is possible under favorable habitat conditions and that most infected individuals could be recaptured with improved body indices. Neither genetic diversity nor environmental variables appeared to affect *Bd* infection probabilities. Hence, genetically diverse amphibian specimens and populations may not automatically be less susceptible for *Bd* infection.

KEY WORDS: *Bombina variegata* · *Batrachochytrium dendrobatidis* · Amphibian decline · Population decline · Microsatellites · Chytridiomycosis

INTRODUCTION

The emerging infectious disease chytridiomycosis, caused by the aquatic chytridiomycete fungus *Batrachochytrium dendrobatidis* (*Bd*), is thought to play a main role in the worldwide amphibian decline (Fisher et al. 2009a). The fungus can disrupt the normal regulatory functions of the amphibian skin and can cause electrolyte depletion and osmotic imbalance, which ultimately can result in the death of the infected individuals (Voyles et al. 2009, 2012, Rosenblum et al. 2010). Amphibians can be ordered after their susceptibility (for the disease) as susceptible (*Bd* causes chytridiomycosis, either followed by recovery or death), tolerant (*Bd* infection without outbreak of the disease) or resistant species, but the response of a

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species, population or individual to *Bd* furthermore depends on many abiotic and biotic factors (Van Rooij et al. 2015). The following variables should be seen as examples. Together with altitude, climate is an important factor for presence of *Bd* and outbreak of chytridiomycosis (Rödder et al. 2009). Furthermore, different *Bd* strains cause different mortality rates in amphibians (Fisher et al. 2009b). Susceptibility to *Bd* infection is related to life history traits, at least in anuran species (Bielby et al. 2008). Some species are protected by the type and composition of their skin peptides or their hosted microbial communities (e.g. Pasmans et al. 2013, Van Rooij et al. 2015), and the genetic diversity of amphibian populations can affect the probability of infection and outbreak of chytridiomycosis. In general, loss of genetic diversity in animal populations can decrease their disease resistance (Spielman et al. 2004). With regard to amphibians and *Bd* infections, Luquet et al. (2012) found that mortality after metamorphosis only increased in *Bd*-infected larvae of the European treefrog *Hyla arborea* from genetically impoverished populations.

Although the fungus has already spread over many parts of the world (Fisher et al. 2009a) from different proposed origins (Africa, North America, Asia, Brazil: see Van Rooij et al. 2015), outbreaks of the disease have not been reported from all affected areas. Mass mortalities and even species extinctions are known from mountainous areas of the Americas, Australia, and southern Europe (Berger et al. 1998, Bosch et al. 2001, La Marca et al. 2005, Bosch & Martínez-Solano 2006). In a European-wide study on *Bd* infections, members of the families Alytidae and Bombinatoridae were significantly more likely to be infected than other European amphibian groups, and the highest infection rates were found in juvenile yellow-bellied toads *Bombina variegata* from Hungary (Baláž et al. 2014). Hence, *B. variegata* is at least a tolerant species, and the question is if the species or populations/individuals are also susceptible to chytridiomycosis (see Van Rooij et al. 2015)

In Germany, *Bd*-infected individuals have been found in populations of almost all autochthonous amphibian species (Lötters et al. 2012, Ohst et al. 2013). In the first nationwide *Bd* screening by Ohst et al. (2013), 15 out of 108 tested yellow-bellied toads and 5 out of 13 tested populations were infected with *Bd*. For the German federal state of Hesse, Rasmussen et al. (2013) conducted a regional *Bd* screening. The authors found infected individuals in 6 out of 9 tested species but did not sample yellow-bellied toads.

Populations of the yellow-bellied toad are declining in western and north-western parts of its range, but over other parts of its range such as the Carpathian Mountains, Poland, and Slovenia, it is still common in suitable habitat, so that the species is listed as Least Concern by the IUCN (Kuzmin et al. 2009). In Germany, this anuran species has suffered and still suffers strong declines and local extinctions. Therefore, it is listed as highly endangered for Germany and also for the federal state of Hesse (Kühnel et al. 2009, Alfermann et al. 2010). Although chytridiomycosis has not been suggested to play a main role in yellow-bellied toad declines (Kuzmin et al. 2009) and outbreaks of chytridiomycosis have not yet been observed in any amphibian species in Germany, impacts on populations cannot be ruled out (Ohst et al. 2013) without long-term monitoring of infected populations.

For these reasons, we tested 19 Hessian yellow-bellied toad populations for *Bd* in 2011 and furthermore conducted capture-mark-recapture (CMR) studies in 4 of them over the following 2 to 3 yr. In conjunction with population size estimates and with the help of individually marked individuals, our goals were (1) to investigate survival histories of infected individuals, (2) to test whether individual multi-locus heterozygosity affects infection rate, (3) to identify parameters which influence the prevalence of *Bd* in yellow-bellied toad populations, and (4) to check whether the presence of *Bd* in yellow-bellied toad populations negatively affected population sizes in the following years.

MATERIALS AND METHODS

Study sites and field work

We screened 374 individuals from 19 populations of the yellow-bellied toad for *Bd* in the northern part of the German federal state of Hesse (Fig. 1). We followed the protocol of Hyatt et al. (2007) and swabbed ventral surfaces of the body as well as fore and hind feet of toads using fine-tip swabs (Medical Wire & Equipment, MW 100–100). Ordinary cotton cosmetic swabs were only used in 1 population (Bebra gravel pit). Swabs were kept at ambient temperature during fieldwork and frozen at −80°C upon return from the field (Hyatt et al. 2007). In 4 of the tested populations, CMR studies were conducted over 2 to 3 yr (see Table 1). Adults and juveniles (excluding metamorphs) were captured during the main reproductive activity period from April to August, with 3 capture occasions conducted each year for the Baumbach

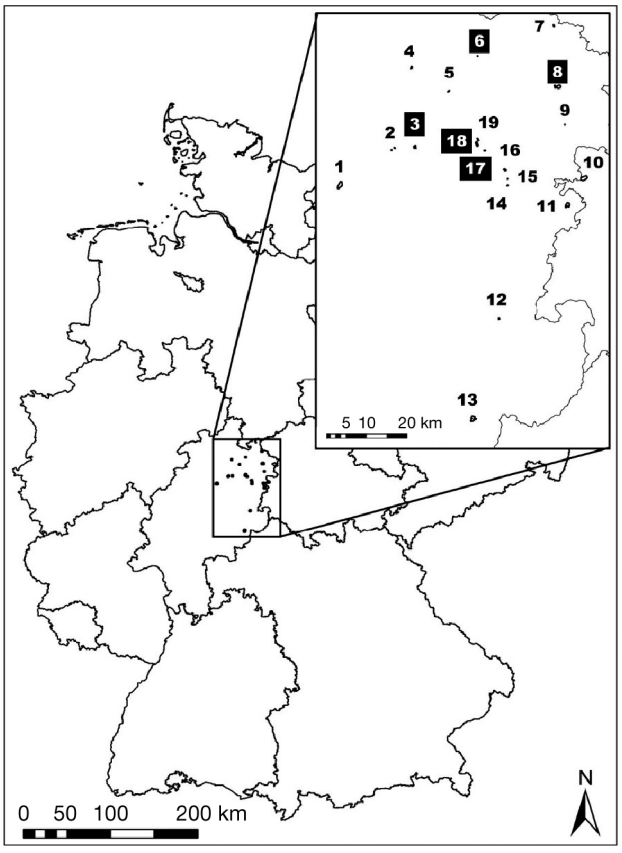

Fig. 1. Location of the northern Hessian study sites in Germany. Location numbers refer to the numbers of studied populations of the yellow-bellied toad *Bombina variegata* in Table 1. The 5 locations with white numbers on black backgrounds refer to the only 5 populations that tested negative for *Batrachochytrium dendrobatidis* (*Bd*) (but note that in these populations, only 1 to 4 individuals were tested, cf. Bayesian 95% credible intervals in Table 1)

gravel pit, 4 capture occasions each year for Blankenheim and the Bebra gravel pit, and 5 capture occasions each year for the Baumbach marl pit/Hergershausen stone pit. At breeding sites, toads were captured with dip nets or by hand, the ventral patterns were photographed for individual identification (after metamorphosis, the ventral pattern of a yellow-bellied toad does not change over its whole life span), and mass and snout-to-vent-lengths were recorded. Animals were released immediately afterwards.

Laboratory work

For *Bd* screening, we used quantitative real-time PCR of the ITS-1/5.8S ribosomal DNA region of *Bd* following the protocols of Boyle et al. (2004) and Kriger et al. (2006). Samples were diluted 1:10 and each sample was run in duplicate. A *Bd* standard from ecogenics was used, with concentrations of 0.1, 1.0, 10.0, and 100.0 zoospores according to Hyatt et al. (2007) to obtain a calibration curve. One modification was that 100 µl of PrepMan Ultra (Applied Biosystems) were used for the DNA-extraction of samples from the Bebra gravel pit population (sampled with ordinary cotton cosmetic swabs). As a second modification, we included for each run an additional non-infectious positive control consisting of a sample with known *Bd* DNA concentration instead of a swab dipped in a broth of *Bd* culture as suggested by Kriger et al. (2006). Reactions yielding 0.05 genomic equivalents (GE) or above were considered *Bd* positive (Göçmen et al. 2013).

Statistical analysis

Prevalence

To obtain a Bayesian 95% credible interval for prevalence of *Bd* infection in each sampled population, the software R (R Development Core Team), the package 'R2WinBUGS' (http://cran.r-project.org/web/packages/R2WinBUGS/), and the software WinBUGS (Lunn et al. 2000) were used to estimate the posterior distribution of prevalence (Kéry 2010, Böll et al. 2012, Lötters et al. 2012). We used a uniform prior for prevalence (e.g. prevalence ~ $U(0,1)$). We used a Bernoulli distribution model for *Bd* prevalence. Three parallel Markov chains with 20 000 iterations each were run, discarding the first 5000 iterations as burn-in. Chains were not thinned.

Population sizes and survival probabilities

Estimating population sizes using count data is based on the assumption that detection probability is equal to 100%, but this assumption is violated in nearly all wild animal populations (Schmidt 2004). Count data often underestimate true population sizes, especially of so-called 'prolonged breeding' amphibian populations (Wagner et al. 2011). 'Prolonged breeding' sensu Wells (2007) means that the reproductive part of the population is asynchronously present at the breeding sites (where sampling takes place). The yellow-bellied toad shows 'prolonged breeding' behavior (Gollmann & Gollmann 2002). For such 'open populations,' the POPAN model, which is implemented in the software MARK, can be used (White & Burnham 1999). The POPAN model is a

modification of the Jolly-Seber model (Schwarz et al. 1993, Schwarz & Arnason 1996), which primarily aims at estimating abundances (Pollock et al. 1990). One disadvantage is that population sizes are only estimated for the capture occasions (i.e. animals that were present at a certain point in time) (Bailey et al. 2004). The POPAN model furthermore estimates the number of individuals present during a capture occasion and then estimates the number of individuals that enter the population between this and the following occasion. Thus, the total or cumulative population size is additionally estimated (super population approach [N], for details see Schwarz & Arnason 2007, Wagner et al. 2011). For each data set, we tested whether models with constant or time-varying detection probabilities (p) provided a better fit to the data. Survival (φ) and the probability of entry (b_i) were always kept time-varying to account for possible effects of *Bd* infection on population sizes. We used the sinus or logit function for φ and p. For b_i, we always used the Mlogit link function and for N, the identity or log-link function (as recommended by Schwarz & Arnason 2007). Best-fitting models were chosen by their small-sample corrected Akaike information criterion (AICc) values and used for parameter estimation. Models with ΔAIC values ≤ 2 were considered plausible (Burnham & Anderson 2002). Significant differences ($\alpha = 0.05$) in estimated population sizes of each location were checked by overlap test of their 95% confidence intervals.

To account for the asymmetric sampling design, we furthermore calculated the overall and daily survival probabilities for each population (see Table 3). For this reason, we first calculated the overall survival rate for the entire yearly sampling period by multiplying all survival probabilities of time intervals. From these we calculated average daily survival rates by extracting the n^{th} root, with n being the number of sampling days, as the weighted average of the daily interval survival rates.

Survival histories and probabilities of uninfected vs. infected individuals

We calculated an index of weight condition (IWC) according to Hemmer & Kadel (1972) for each individual from the equations a = body mass / SVL^b and IWC = $a \times 10^{-3}$, where SVL is the snout-to-vent length and b is calculated as the linear regression coefficient of the function log[body mass] = f(log[SVL]) for all measured specimens.

To investigate potential differences in survival probabilities of uninfected and infected animals, we calculated survival probabilities between the different capture occasions using the POPAN model in MARK (see above). Survival probabilities for uninfected and infected subgroups were compared using a paired *t*-test in R.

Potential factors influencing infection

Individual level. Other studies have found effects of the genetic diversity of amphibians on infection with *Bd* (e.g. Addis et al. 2015). Therefore, we used the multi-locus heterozygosity from individuals of the considered *Bombina variegata* populations as factor potentially influencing *Bd* infection rates (in GE). In a parallel study, the genetic structures of the populations were assessed by genotyping a total of 281 individuals from 14 tested populations (laboratory methods and detailed results can be found in the Supplement at www.int-res.com/articles/suppl/d123p055_supp.pdf and in Guicking et al. in press). In short, 6 microsatellite markers were used, and in 88.61% (n = 249) of the tested individuals, all 6 loci were amplified (5 loci in 8.19% [n = 23], and 4 loci in the remaining 3.20% [n = 9]). Of the 281 genotyped individuals, 274 were also checked for *Bd* infection. For these 274, we calculated the multi-locus heterozygosity in a given individual by dividing the number of heterozygous loci by the number of successfully amplified loci (data from Guicking et al. in press). We hypothesized a negative relationship between the extent of polymorphism (as expressed by the multi-locus heterozygosity in a given individual) and infection rate with *Bd*. To test for this assumption, we calculated a generalized linear model (GLM) with log link for Poisson distributed data between the *Bd* infection rates (in GE) and the multi-locus heterozygosity of individuals.

Population level. From the parallel study of Guicking et al. (in press), we took the expected heterozygosity of the considered *B. variegata* populations as one potential explanatory variable influencing *Bd* infection rates. Following Scheele et al. (2015), we also considered several environmental variables to potentially explain the probability of infection in populations. First, the proportion of forest cover in the terrestrial habitats was chosen because higher forest cover and hence cooler and wetter habitat conditions that are more favorable for *Bd* may positively affect infection rates (Scheele et al. 2015). Proportions of forest cover were calculated in a 300 m buffer around breeding sites using ArcMap 10 (ESRI) and the latest version of the CORINE land cover data

(www.eea.europa.eu). We merged all occurring forest types (CORINE land cover classes 311 [broad-leaved forest], 312 [coniferous forest], and 313 [mixed forest]). A 300 m buffer is expected to cover the average terrestrial habitat used by *B. variegata* (Hartel 2008). Second, elevation is well known to affect *Bd* infection rates (Scheele et al. 2015). We used a digital terrain model (www.geodatenzentrum.de) with a grid width of 200 m and calculated in ArcMap the average elevation (in m above sea level) of the polygons representing the habitats of the different *B. variegata* populations (Fig. 1). Third, permanent waters are known to be reservoirs of *Bd* (Scheele et al. 2015). We measured the distance from the populations to fish ponds, lakes, and perennial streams using aerial photographs and ArcMap. Detailed information on the study sites can be found in the Supplement.

As response variable, we used the prevalence (%) of infection in the populations. The data set for prevalence was checked for normal distribution and homogeneity of variances prior to analysis. We calculated a GLM with identity link (normal distribution of prevalence data) with the software R.

RESULTS

Bd screening

Infected animals were found in 14 out of 19 populations (73.68%) with estimated Bayesian credible intervals for prevalence ranging from 0.00 (0%) to 0.93 (93%) (Table 1; note that for populations with only 1 sampled individual, a calculation of the credible interval is not possible). Furthermore, in the 5 populations which were tested negative for *Bd*, only 1 to 4 individuals were analyzed (cf. Bayesian 95% credible intervals in Table 1).

Table 1. *Batrachochytrium dendrobatidis* (*Bd*) prevalence in northern Hessian populations of the yellow-bellied toad *Bombina variegata*. *Bd* was detected in 14 out of 19 populations (73.68%). GE: genomic equivalents; CMR: capture-mark-recapture. Bayesian 95% credible intervals (CI) were computed as described by Lötters et al. (2012). Sites are numbered as in Fig. 1. Dates are given as mm/dd/yy

Location	Site no.	Sampling date(s)	N ind.	N positive	Mean GE ± SD	Range of GE loads	Prevalence (%)	Bayesian 95% CI	CMR data available
Former military training area Treysa	1	07/3/2011 07/22/2011	13	5	2.44 ± 4.04	0.1–9.6	38.46	0.17; 0.64	–
Military training area Homberg	2	05/14/2011 07/3/2011 07/8/2011	35	10	2.68 ± 5.05	0.1–14.7	28.57	0.16; 0.45	–
Homberg Remsfeld	3	07/03/2011	3	0	–	–	0.00	0.01; 0.61	–
Ellenberg	4	05/9/2011 07/19/2011	33	10	1.00 ± 1.51	0.05–4.9	30.30	0.17; 0.47	–
Melsungen	5	05/9/2011	35	2	0.15 ± 0.07	0.1–0.2	5.71	0.02; 0.19	–
Fürstenhagen	6	06/12/2011 06/28/2011	3	0	–	–	0.00	0.01; 0.61	–
Bad Soden Allendorf Nord	7	06/12/2011 06/28/2011	33	9	0.38 ± 0.24	0.1–0.8	27.27	0.15; 0.44	–
Trimberg	8	07/10/2011	4	0	–	–	0.00	0.00; 0.51	–
Breitau	9	06/12/2011 06/18/2011	20	8	0.35 ± 0.33	0.06–0.7	40.00	0.22; 0.61	–
Gravel pit Obersuhl	10	06/4/2011 06/1/2011	32	3	2.40 ± 2.10	0.1–4.2	9.38	0.03; 0.25	–
Obere Aue Heringen	11	06/4/2011 07/1/2011	21	1	0.10	–	4.76	0.01, 0.24	–
Hünfeld Rückers	12	06/19/2011 07/5/2011	16	1	0.20	–	6.25	0.01; 0.29	–
Disposal site Schrimpf	13	06/19/2011 07/5/2011	37	4	0.25 ± 0.17	0.1–0.5	10.81	0.05; 0.25	–
Wet meadow near Meckbach	14	07/15/2011	3	1	0.7	–	33.33	0.08; 0.82	–
Im Sand	17	08/3/2012	1	0	–	–	0.00	–	–
Blankenheim	15	06/14/2011 08/23/2011	3	2	0.33 ± 0.39	0.05–0.6	66.67	0.20; 0.93	2012, 2013
Bebra gravel pit	16	09/25/2012	45	13	0.28 ± 0.29	0.05–0.96	28.89	0.18; 0.44	2012, 2013
Baumbach gravel pit	18	06/14/2011	3	0	–	–	0.00	0.01; 0.61	2012, 2013
Baumbach marl pit/ Hergershausen stone pit	19	06/14/2011 07/15/2011 07/29/2012	34	14	0.87 ± 1.79	0.07–6.6	41.18	0.26; 0.57	2011, 2012, 2013
Total ind.			**374**	**83**			**22.19**	**0.18; 0.27**	

Table 2. Survival histories of *Batrachochytrium dendrobatidis* (*Bd*)-infected yellow-bellied toad *Bombina variegata* individuals from the population Baumbach marl pit/Hergershausen stone pit. Values in the table show the index of weight condition of the infected individuals based on mass and snout-to-vent length. Higher values indicate better individual body condition. M: adult male; J: juvenile; F: adult female. X: specimen captured, but no metrics taken. Dates are given as mm/dd/yy

Ind. ID	Stage, sex	06/07/ 2011	06/14/ 2011	06/27/ 2011	07/15/ 2011	08/11/ 2011	Stage, sex	05/19/ 2012	06/19/ 2012	07/19/ 2012	08/09/ 2012	Stage, sex	06/12/ 2013	07/03/ 2013	08/14/ 2013
277	M	X	2.4				M			0.9		M	2.7		
278	M	X	3.1	X	2.1	X									
280	M	X	1.1												
282	M		1.3	X			M	1.9							
283	J	X	0.2				F	1.6							
284	J	X	0.2	X											
292	J	X	0.1									F	1.1	3.2	
296	M				0.7		M			2.1		M	3.8		2.6
299	J	X			0.3		F	0.8							
300	J				0.3		M	0.5							
301	J	X			0.1										
302	J				0.1		F				1.6				
303	J				0.3		F		1.1						

Survival histories and probabilities of uninfected vs. infected individuals

Only in 1 out of 3 *Bd*-infected populations with CMR data (Baumbach marl pit/Hergershausen stone pit), 13 out of 14 individuals (92.86%) that were tested positive for *Bd* in 2011 could be recaptured at least 1 time (Table 2). Hence, 13 out of a total of 29 *Bd*-positive individuals (=44.83%) from all 3 *Bd*-infected populations with CMR data could be recaptured over 3 yr. None of the recaptured individuals showed any obvious signs of chytridiomycosis outbreak. In most cases, body indices increased over time (Table 2).

Survival probabilities between capture occasions were compared for uninfected and infected animals of this population for the year 2011 when *Bd* swabbing took place. The best-fitting POPAN model had a logit link for time-varying survival and detection probabilities, respectively. Averaged daily survival probabilities per interval ranged from 0.87 ± 0.03 to 0.99 ± 0.00 for uninfected toads and from 0.90 ± 0.03 to 1.0 ± 0.00 for infected toads and did not significantly differ (t = 0.0085, df = 3, p = 0.9937).

Population sizes

The estimated population sizes do not only refer to the reproductive part but the total population, as many juveniles were captured and recaptured (Table 3). Recapture rates (i.e. that an individual could be recaptured at least once within a year) in the different populations and years ranged from about 30 to >50% (Table 3). Survival and detection probabilities as well as the best-fitting models and their population size estimates are summarized in Tables 3 & 4. Based on overlap tests of 95% confidence intervals, the population size estimates for 2 *Bd*-infected populations (Blankenheim and Bebra gravel pit) were significantly (α = 0.05) lower in 2013 compared to 2012 (see Fig. 2A,B). Conversely, estimated sizes of the uninfected population (Baumbach gravel pit) did not differ between the 2 years (Fig. 2C). However, the fourth population (Baumbach marl pit/Hergershausen stone pit), where sufficient CMR data were available for 3 years, showed a significant increase in population size between 2011 and 2012 (Fig. 2D), although this population is highly infected with *Bd* (see Table 1).

Effects of explanatory variables on infection

There was no correlation between the *Bd* infection rates and the multi-locus heterozygosity of individuals averaged across loci (Z = 1.332, p = 0.183). Likewise, at the population level, the second GLM did not find significant relationships between any of the potentially explanatory variables and the prevalence of infection (Table 5).

DISCUSSION

First of all, we stress that in the absence of long-term data, any statements on population trends re-

Table 3. Survival probabilities (φ) ± SE and detection probabilities (p) ± SE for yellow-bellied toad *Bombina variegata* populations. N: total number of individuals; J: juvenile; M: adult male; F: adult female; OSP: overall survival probability from first to last capture occasion in a sampling year (calculated as Σ from first to last occasion); DSP: daily survival probability during that period (calculated as the n^{th} root, with n being the number of sampling days, as the weighted average of the daily interval survival rates). Dates are given as mm/dd/yy

	$N_{captured}$	$N_{recaptured}$ (recapture rate, % yr^{-1})	Capture occasions	Survival probability	Detection probability
Blankenheim	41 (36 J, 1 M, 4 F)	13 (31.71)	05/19/2012	–	0.81 ± 0.14
			06/07/2012	0.99 ± 0.01	0.81 ± 0.14
			06/21/2012	0.98 ± 0.01	0.81 ± 0.14
			07/18/2012	1.00 ± 0.01	0.81 ± 0.14
			OSP	0.97	
			DSP	0.9995	
	38 (27 J, 4 M, 7 F)	25 (66.79)	05/07/2013	–	1.00 ± 0.00
			06/11/2013	0.98 ± 0.01	0.73 ± 0.09
			07/04/2013	1.00 ± 0.00	0.73 ± 0.09
			07/23/2013	0.97 ± 0.01	1.00 ± 0.01
			OSP	0.95	
			DSP	0.9993	
Bebra gravel pit	64 (58 J, 2 M, 4 F)	30 (46.88)	06/07/2012	–	0.72 ± 0.06
			06/21/2012	1.00 ± 0.00	0.72 ± 0.06
			07/18/2012	0.99 ± 0.01	0.72 ± 0.06
			08/10/2012	1.00 ± 0.00	0.72 ± 0.06
			OSP	0.99	
			DSP	0.9998	
	18 (4 J, 9 M, 5 F)	7 (38.89)	05/07/2013	–	1.00 ± 0.00
			06/11/2013	0.99 ± 0.02	0.12 ± 0.10
			07/04/2013	0.99 ± 0.02	1.00 ± 0.00
			07/23/2013	0.91 ± 0.03	1.00 ± 0.00
			OSP	0.89	
			DSP	0.9985	
Baumbach gravel pit	23 (22 J, 1 M)	13 (56.52)	05/19/2012	–	1.00 ± 0.00
			06/07/2012	1.00 ± 0.01	0.88 ± 0.11
			07/19/2012	0.99 ± 0.01	1.00 ± 0.00
			OSP	0.99	
			DSP	0.9998	
	19 (6 J, 7 M, 6 F)	8 (42.11)	05/06/2013	–	0.40 ± 0.10
			06/12/2013	0.95 ± 0.04	0.40 ± 0.10
			07/03/2013	1.00 ± 0.00	0.40 ± 0.10
			OSP	0.95	
			DSP	0.9991	
Baumbach marl pit/ Hergershausen stone pit	55 (35 J, 10 M, 10 F)	28 (50.91)	06/07/2011	–	1.00 ± 0.00
			06/14/2011	0.97 ± 0.02	0.68 ± 0.11
			06/27/2011	1.00 ± 0.00	0.48 ± 0.10
			07/15/2011	0.96 ± 0.03	0.60 ± 0.29
			08/11/2011	0.92 ± 0.02	1.00 ± 0.00
			OSP	0.86	
			DSP	0.9976	
	88 (50 J, 15 M, 23 F)	30 (34.09)	04/26/2012	–	0.90 ± 0.08
			05/19/2012	1.00 ± 0.00	0.90 ± 0.08
			06/19/2012	0.98 ± 0.01	0.90 ± 0.08
			07/19/2012	0.97 ± 0.01	0.90 ± 0.08
			08/09/2012	0.95 ± 0.01	0.90 ± 0.08
			OSP	0.90	
			DSP	0.9990	
	105 (51 J, 19 M, 35 F)	71 (67.62)	05/06/2013	–	0.62 ± 0.05
			06/12/2013	0.99 ± 0.00	0.62 ± 0.05
			07/03/2013	1.00 ± 0.00	0.62 ± 0.05
			07/24/2013	0.98 ± 0.01	0.62 ± 0.05
			08/14/2013	0.96 ± 0.01	0.62 ± 0.05
			OSP	0.93	
			DSP	0.9993	

Table 4. Best-fitting POPAN models chosen by their values of Akaike's information criterion adjusted for small sample sizes (AICc); *K* is the number of parameters. Link functions are given in parentheses for survival (φ) and detection probabilities (p), and superpopulation size (N). An Mlogit link was used for entry probabilities (pent = bi). CI: confidence interval

Population	Year	Model	AICc	*K*	Est. population size (95% CI)
Blankenheim	2012	φ(t)p(.)pent(t)N(id) (logit link for φ and p)	108.79	8	68.77 (55.66; 81.89)
Blankenheim	2013	φ(t)p(t)pent(t)N(log) (logit link for φ and p)	135.17	7	45.53 (39.82; 51.24)
Bebra gravel pit	2012	φ(t)p(.)pent(t)N(id) (logit link for φ and p)	116.52	5	78.47 (67.41;89.53)
Bebra gravel pit	2013	φ(t)p(t)pent(t)N(log) (logit link for φ and p)	57.69	7	23.96 (14.45; 33.47)
Baumbach gravel pit	2012	φ(t)p(t)pent(t)N(log) (logit link for φ and p)	49.18	5	25.01 (22.90; 27.13)
Baumbach gravel pit	2013	φ(t)p(.)pent(t)N(id) (logit link for φ and p)	50.52	3	27.30 (15.32; 39.27)
Baumbach marl pit/Hergershausen stone pit	2011	φ(t)p(t)pent(t)N(log) (logit link for φ and p)	191.70	11	71.13 (58.09; 87.10)
Baumbach marl pit/Hergershausen stone pit	2012	φ(t)p(.)pent(t)N(id) (logit link for φ and p)	166.83	9	124.60 (107.03; 145.07)
Baumbach marl pit/Hergershausen stone pit	2013	φ(t)p(.)pent(t)N(id) (logit link for φ and p)	413.67	9	128.44 (115.42; 142.94)

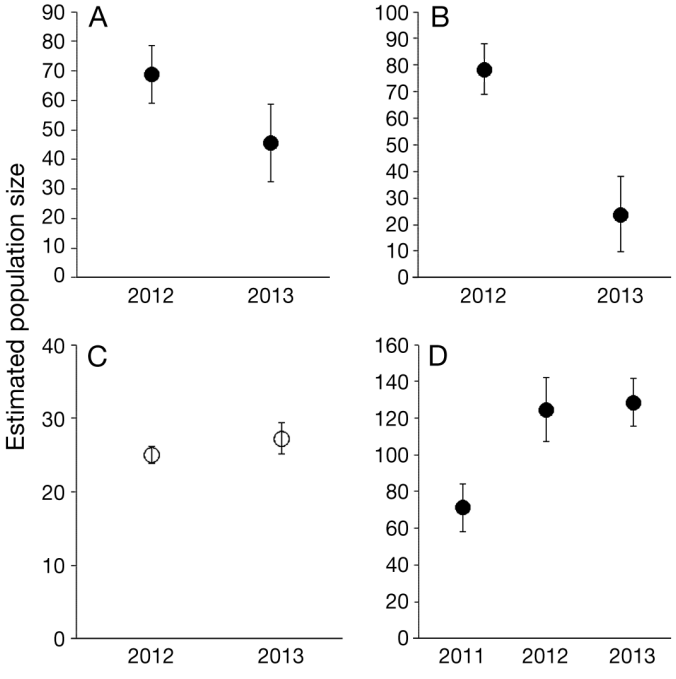

Fig. 2. *Batrachochytrium dendrobatidis* (*Bd*)-infected (filled circles) and -uninfected (open circles) yellow-bellied toad *Bombina variegata* populations with capture-mark-recapture (CMR) data. For the infected populations at the (A) Blankenheim and (B) Bebra gravel pit, significantly smaller population sizes were estimated in 2013 compared to 2012, whereas (C) the uninfected population at the Baumbach gravel pit showed no trend. (D) The population at the Baumbach marl pit/Hergershausen stone pit (where CMR data were also available for 2011) increased in size despite *Bd* infection

main equivocal. Amphibian populations show natural fluctuations in their population sizes, and, strictly speaking, monitoring must be conducted over decades to draw causative links (e.g. Meyer et al. 1998). Our goal was to investigate survival rates of infected individuals and to check whether strong declines occur in *Bd*-infected populations of yellow-bellied toads.

Our results suggest that *Bd* is widely present in Hessian yellow-bellied toad populations. In the 2 *Bd*-infected populations with CMR data where no *Bd*-positive toad could be recaptured (Blankenheim and Bebra gravel pit), the detection probabilities on the sampling occasions ranged from 12 to 100% (Table 3). Hence, the fact that infected individuals could not be recaptured does not automatically mean that they died (or emigrated from the study sites). Apart from the fact that no *Bd*-infected individuals were found dead during the whole study period, the high estimated survival probabilities of the toads (nearly 100% over all occasions; Table 3; all φ values were always time-varying and not constant in the best models; Table 4) argue for simple non-detection rather than death or emigration. Together with the observation that all recaptured *Bd*-infected toads improved their body indices (Table 2) and the survival probabilities of uninfected and infected toads did not significantly differ, the individual data suggest that *Bd* infection had no measurable impact on survival at the individual level.

At 2 *Bd*-infected localities (Blankenheim and Bebra gravel pit), population sizes significantly decreased

Table 5. No effects of co-variables on prevalence of *Batrachochytrium dendrobatidis* infection in yellow-bellied toad *Bombina variegata* populations from northern Hesse, Germany

	Estimate	SE	*t*	p
Intercept	3.26	63.68	0.05	0.96
Coefficients				
Expected heterozygosity	44.21	94.22	0.47	0.65
Forest cover (%) in terrestrial habitats	0.36	0.31	1.15	0.28
Elevation (m above sea level)	−0.07	0.10	−0.69	0.51
Distance (m) to permanent waters	0.03	0.03	1.28	0.23

by about 30 and even 70%, respectively, whereas the monitored uninfected population apparently remained stable. The third infected population (Baumbach marl pit/Hergershausen stone pit) even significantly increased by about 40% (Fig. 2). This increase was only visible due to monitoring data over 3 instead of 2 yr, again pointing to the importance of long-term monitoring (see Meyer et al. 1998). Tobler et al. (2012) analyzed count data series of 26 *Bd*-infected and uninfected midwife toad *Alytes obstetricans* populations in Switzerland over 4 to 8 yr. The authors did not find any negative effects of the presence of *Bd* in a population on growth rate. By contrast, infected populations were even able to increase despite the presence of the fungus. Thus, our observation in the yellow-bellied toad population Baumbach marl pit/Hergerhausen stone pit with high prevalence of *Bd* but population growth confirms that of Tobler et al. (2012), who also concluded that observed negative effects of *Bd* on survival of individuals do not necessarily translate into negative effects at the population level.

In yet another study, Canestrelli et al. (2013) showed that *Bd* has been present in Apennine yellow-bellied toad populations *Bombina pachypus* at least since the late 1970s, but strong declines only started after the mid-1990s. From this, the authors concluded that *Bd* cannot be the only reason for declines but possibly interacts with other stressors. However, deadly chytridiomycosis in captive toads has been previously observed and was thought to be responsible for declines (Stagni et al. 2004). Hence, a role for *Bd* in the widespread decline of the Apennine yellow-bellied toad still appears plausible (Canestrelli et al. 2013), and it could be hypothesized that the closely related *B. variegata* is also suceptible under certain conditions.

As already mentioned, Luquet et al. (2012) observed *Bd*-induced mortality in European treefrogs *Hyla arborea* only from genetically impoverished populations. Conversely, Addis et al. (2015) found that more heterozygous boreal toads *Bufo boreas* were more likely to be infected with *Bd* in Glacier National Park (Montana, USA) and suggested a relationship of migrating toads, genetic diversity, and *Bd* infection probability. In the present study, neither individual multi-locus heterozygosity nor expected heterozygosity in populations had any obvious effect on infection rates and prevalence, respectively. Hence, genetically diverse *B. variegata* populations may not necessarily be less susceptible (Luquet et al. 2012) or more susceptible (Addis et al. 2015) than genetically impoverished populations to *Bd* infection, but further studies are necessary.

Conversely to Scheele et al. (2015), distance to perennial water bodies (and also the remaining environmental variables checked in the present study) had no measurable effects on *Bd* infection probability. One simple reason for this could be that most *B. variegata* populations in this study region are located next to perennial water bodies (i.e. larger rivers, especially in restored floodplains).

Taken together, although significant decreases in population size could be observed in 2 *Bd*-infected populations, no causative link to *Bd* as the (single) reason for these decreases could be established. Mass mortalities or obvious signs of disease in individuals were not observed. Conversely, we could show that *Bd*-infected populations can even increase in size under favorable habitat conditions, supporting the findings of Tobler et al. (2012). Apart from long-term monitoring of yellow-bellied toad populations, we therefore call for maintenance measures that improve aquatic and terrestrial habitat conditions to keep or bring populations into a favorable conservation status. This could help yellow-bellied toad populations to be in overall better condition and hence individuals to be better protected against potentially negative effects of *Bd*.

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