Journal of Zoology

ZSL LET'S WORK FOR WILDLIFE

Journal of Zoology. Print ISSN 0952-8369

Chytrid fungus dynamics and infections associated with movement distances in a red-listed amphibian

S. Kärvemo[1,2], G. Wikström[2], L.A. Widenfalk[1,3], J. Höglund[2] & A. Laurila[2]

1 Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
2 Department of Ecology and Genetics/Animal Ecology, Uppsala University, Uppsala, Sweden
3 Greensway AB, Uppsala, Sweden

Keywords
Batrachochytrium dendrobatidis; Chytrid fungus; movements; *Pelophylax lessonae*; pool frog; radio tracking.

Correspondence
Simon Kärvemo, Department of Ecology,
Swedish University of Agricultural Sciences,
Box 7044 75007 Uppsala, Sweden.
Email: simon.karvemo@slu.se

Editor: Mark-Oliver Rödel

Received 12 February 2019; revised 23 January 2020; accepted 24 January 2020

doi:10.1111/jzo.12773

Abstract

Amphibians are among the most threatened vertebrate taxa due to anthropogenic habitat change and emerging pathogens. The fungus *Batrachochytrium dendrobatidis* (*Bd*) may cause trade-offs between the immune responses and other important functions, such as mobility. The pool frog (*Pelophylax lessonae*) is red-listed in Sweden, and an earlier study conducted in our study area did not detect the fungus. In this study, 67 Swedish pool frogs were tested for *Bd*. Of these, 28 adults were provided with external radio transmitters and tracked during the summer and autumn to compare movement among infected and non-infected individuals. Additionally, we tested some individuals for *Bd* twice during the summer to study intra-seasonal variation in individual infections. Our results suggest that *Bd* has recently invaded these pool frog populations. During autumn, movement distances of infected pool frogs were shorter compared to uninfected individuals, and summer movements within ponds were reduced by increasing *Bd* load. We also found that the frogs can clear (or reduce) *Bd* infection during their active season. The results from this study increase understanding of the implications of *Bd* infections in an anuran fringe population, as well as in amphibian metapopulations, and can further guide amphibian conservation planning and management.

Introduction

Pathogens often reduce fitness of individuals and cause declines of animal populations. Apart from direct lethal effects, a decline in fitness may occur because resources required for elevated immune responses come at the expense of other important functions (Sheldon & Verhulst, 2008). A trade-off between resisting infection or tolerating sub-lethal infection and other important functions has previously been found in amphibians (Burrowes, Longo & Rodríguez, 2007; Chatfield *et al.*, 2013; Bielby *et al.*, 2015). Locomotion is often affected by these trade-offs, with negative consequences for the ability to escape predators, forage, disperse and reproduce (Chatfield *et al.*, 2013). However, while trade-offs between disease and movement are well studied in birds and mammals, they are poorly studied in other taxa, including amphibians (Dougherty *et al.*, 2018).

The amphibian chytrid fungus *Batrachochytrium dendobatidis* (*Bd*) has been detected in ca. 700 species and is regarded as one of the most serious threats to amphibian populations (Lips, 2016). *Bd* attacks the outer keratinized layers of skin, resulting in disruption of water, oxygen and dissolved solutes transport (Voyles *et al.*, 2010) and has caused mass mortality and population declines of amphibians in many parts of the world (e.g. Wake & Vredenburg, 2009; Scheele *et al.*, 2015; Lips, 2016).

Few studies have investigated the impact of *Bd* on amphibian movement, particularly in the case of wild animals. A laboratory study reported reduced peak speed of movement in *Bd*-infected leopard frogs *Lithobates pipiens* (Chatfield *et al.*, 2013), while Daversa *et al.* (2018) reported no effect on movement in four infected radio-tracked spiny toads, *Bufo spinosus*. *Bd* can also reduce the body condition in amphibians (e.g. Retallick & Meira 2007; Pearl *et al.*, 2009; Kärvemo, Laurila & Höglund, 2019), but several studies have found no such impact (e.g. Deguise & Richardson, 2009; Murray *et al.*, 2009; Woodhams & Alford, 2018).

Some species can, aided by antimicrobial peptides, temperature and immune responses, clear infections (Fisher *et al.*, 2009). Recovery of amphibian individuals both within and between seasons has been reported for several species of treefrogs *Litoria* spp. (Murray *et al.*, 2009; Kinney *et al.*, 2011; Sapsford *et al.*, 2017) and spiny toads *B. spinosus* (Daversa *et al.*, 2018). Reduced *Bd* prevalence between winter/spring and summer/autumn has also been shown for several amphibian species in Australia (Berger *et al.*, 2004; Kriger & Hero, 2007) and North America (Voordouw *et al.*, 2006; Duncan Pullen, Best & Ware, 2010). The level of recovery within a species is suggested to be associated with *Bd* infection loads (Briggs, Knapp & Vredenburg, 2010) and clearing is therefore likely to reflect the relative risk that *Bd* will cause to the population (Gervasi *et al.*, 2014). Thus, information about intra-seasonal dynamics of *Bd* can improve predictions of infection risks.

In this study, we radio-tracked and sampled *Bd* from pool frogs *Pelophylax lessonae*. In Sweden, this species is red-listed and occurs as a unique fringe metapopulation (Sjögren-Gulve, 1991; Orizaola, Quintela & Laurila, 2010; Orizaola & Laurila, 2016) distributed over more than 100 ponds in south-central Sweden (Lindgren, Nilsson & Söderman, 2014). A study on museum material of European amphibians did not find *Bd* in 197 Swedish amphibian samples collected between 1994 and 2004, while *Bd* was detected in other parts of Europe within the same timeperiod (Garner *et al.*, 2005). Additionally, in a previous study on Swedish pool frogs conducted between 2005 and 2008 within the same area as our study, all 137 tested individuals (a sample size large enough to ensure a correct assessment of *Bd* incidence; Adams *et al.*, 2010) were *Bd* negative (Sainsbury *et al.*, 2007). Since then, *Bd* has been found in most Swedish amphibian species (Hallengren, 2013; Kärvemo *et al.*, 2018; Meurling, 2019). This suggests that *Bd* has recently appeared in Sweden, but is now widespread. However, the current infection status of the pool frogs in Sweden is unknown.

The aims of this study were to (1) investigate evidence for recent invasion of *Bd* in pool frog populations in Sweden and if present; (2) evaluate potential costs of *Bd* infections in terms of movement distance; and (3) study intra-seasonal dynamics of *Bd* infection in individual frogs. We sampled pool frogs for *Bd* from six locations within the same region where *Bd* had not been detected more than 10 years ago (Fig. 1; Foster, 2006; Sainsbury *et al.*, 2007). Ten infected and 18 non-infected individuals were fitted with radio transmitters, allowing screening of some individuals twice for *Bd* during the study period and tracking of their movements throughout the active post-breeding period.

Methods

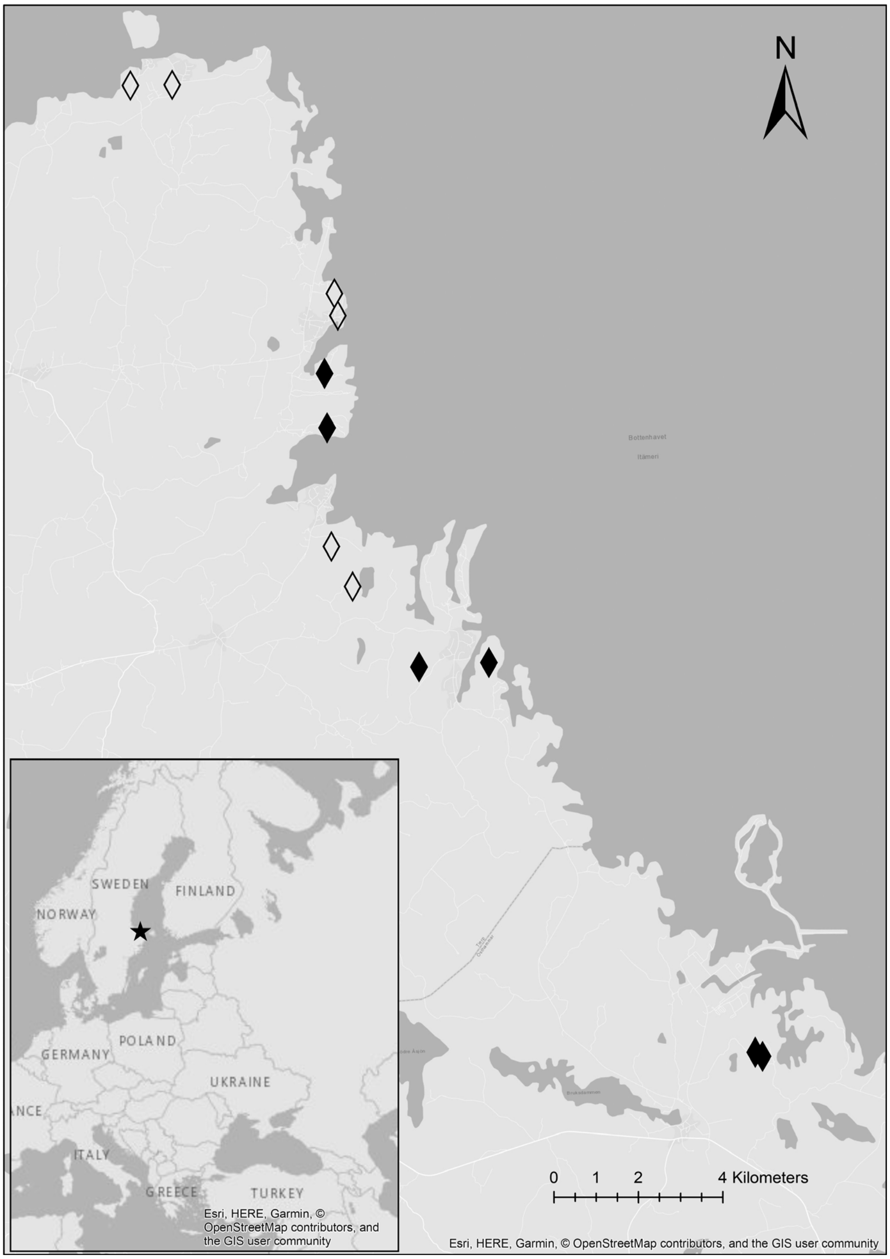
Sixty-seven pool frogs (25 adult males, 29 adult females and 13 juveniles) from six ponds were tested for *Bd* in summer and autumn 2017 (48 individuals) and in summer 2018 (19 individuals) in the Forsmark – Hållnäs area in the province of Uppland, central Sweden (Fig. 1; Table S1). The six locations were surrounded by similar forest structures of pine-dominated mixed forests (Wikström, 2008). The pool frogs were considered as juveniles when their body mass was less than 10 g. For sampling *Bd*, we used the standard swabbing protocol of Brem, Mendelson and Lips (2007) with 25 strokes on the ventral skin and feet per individual. The mean number of individuals sampled per pond was 11.2 ± 3.2 (SE). At the sites Slåtaren and Rundskär, only three and four individuals were sampled, respectively. *Bd* infection prevalence was compared with a previous disease risk analysis that sampled *Bd* from adjacent populations in 2005–2008 (Sainsbury *et al.*, 2007).

Of the 54 sampled adults, 28 were fitted with radio transmitters (PIP3 single cell tag A392; www.biotrack.co.uk) in 2017 with the intention of tracking them daily between 14 June and 25 July (summer) or 21 August and 10 October (autumn). We used external transmitters with waistbands of custom-fitted 1 mm elastic bead cord according to Groff *et al.* (2015). Terrestrial and pond positions of the pool frogs were recorded using a manual SIKA Radio Tracking Receiver combined with a flexible Yagi antenna (www.biotrack.co.uk). Each tracked position was recorded by a handheld GPS (Garmin GPSMAP 64) and 609 locations were recorded in total (summer: 423, autumn: 186). Even though the pool frog is a semi-aquatic species and spends much of its time in the breeding ponds, 1-12% of the local population can migrate annually to other ponds (Sjögren-Gulve, 1994; Holenweg Peter, 2001). However, in this study, too few of the tracked individuals performed voluntary terrestrial migrations. Thus, to be able to sample movement data, 21 individuals were translocated 500 m from their breeding ponds. Such displacement is a common method to study amphibian movement distances (e.g. Sinsch, 1996; Smith & Green, 1998). However, the translocation can change the amphibian behaviour (Pettit, Greenlees & Shine 2017) and potentially increase the movement distances of amphibians, as the animals often orient back to their breeding ponds (Sinsch, 1996; Smith & Green, 1998). Consequently, translocation was included as a factor in the land-movement models (see Statistical analyses). Fourteen individuals were translocated in the summer and seven in the autumn (Table S1). In addition, seven individuals were recorded after natural (voluntary) terrestrial movements, mainly during the autumn migration to terrestrial hibernation habitats (Table S1).

Air temperature, sex and body size are important factors influencing amphibian movement (e.g. Holenweg Peter, 2001; Mazerolle, 2001 and references therein; Todd & Winnie 2006; Wikström, 2008) and were consequently evaluated to control for these variations. Data on sex, body mass (g) and snout-vent length (mm) were recorded for all individuals and ambient air temperature for each position was recorded with a Testo 922 thermometer (Testo AG, Lenzkirch, Germany). We estimated a body condition index (BCI) from the residuals of the linear regression between body length and weight. To detect whether the frogs cleared the infection during the summer season, eleven of the radio-tracked individuals were tested for *Bd* twice, once in early June and once in late July. These individuals were designated as infected in the analyses only if they were *Bd*-positive in the first sampling occasion in June. All methods were approved by and the research was carried out under permits granted by Uppsala ethical committee for animal experiments (5.8.18-2059/2017) and the county board of Uppsala (522-420-15).

Molecular analyses

DNA was extracted from swabs using a Qiagen DNeasy Blood and Tissue kit (Qiagen, ref. 69506) following the standard protocol of Boyle *et al.,* (2004) with modifications suggested by Kosch & Summers (2013). Presence of *Bd* was assessed by



Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community

Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community

Figure 1 Study area (star) of pool frogs (*Pelophylax lessonae*) in eastern Sweden. Filled diamonds indicate detections of *Bd* in 2017 and 2018. Coordinates are presented in Table 1. Empty diamonds indicate non-detection of *Bd* in a previous survey conducted between 2005 and 2008 (Foster, 2006; Sainsbury et al., 2007).

qPCR amplification of the internal transcribed spacer (ITS)-5.8S rRNA region (Boyle *et al.*, 2004), in 25 μL reaction mixtures containing 12.5 μL of 2X Taqman Master Mix (Applied Biosystems, ref. 4318157), 2.25 μL of 10 μM solutions of the forward and reverse primers, 0.625 μL of 10 μM MGB probe and 5 μL of DNA solution (diluted x10 in water)**.** Each sample was assayed in triplicate. To detect possible false negatives caused by inhibitors, we added to one of each set of triplicates an exogenous internal positive control (IPC; Boyle *et al.*, 2004), consisting of 1 μL of 10X Exo IPC Master Mix and 0.5 μL of 50X Exo IPC DNA (VICTIM dye, Applied Biosystems ref. 4304662).

We used a Biorad CFX96 system and previously described amplification conditions (Boyle *et al.*, 2004), with standards of 0.1, 1, 10 and 100 genomic equivalents (GE), for the qPCR assays. An individual was scored as *Bd* positive if one or more of the triplicate samples exhibited a positive signal (i.e. a clear exponential amplification curve). We classified the infection level for each *Bd*-positive individual by comparing the sample cq-value to the *Bd* standards. These values were divided into three classes: <0.1, 0.1 and >0.1 < 1 GE standards, and used in additional models to evaluate the effects of relative differences in *Bd* infection loads. We used a classification of GE for a relative comparison of infection load among individuals, rather than the conversion into an absolute number of zoospores per individual. This is more suitable in our study as many methodological factors potentially affected fine-scale data (e.g. inhibitors originating from amphibian skin and the pond water, different fieldworkers carrying out the sampling). If the IPC showed signs of inhibition (no curve), negative samples were re-run once before being designated impossible to score. Scoring one of the triplicates as positive may cause some false positives due to potential contamination, which can result in overestimation of *Bd*-positive individuals. On the other hand, PCR inhibitors for *Bd* detection are very common in *Pelophylax* spp. compared to other species, which increases the risk of false negatives (Baláž *et al.*, 2014). Thus, we used the more inclusive strategy, and regarded all individuals with one (or more) positives as infected. However, to control for possible false positives we also validated our findings by also running analyses when excluding the samples when only one of the triplicates was positive (see Statistical analyses).

Statistical analyses

Movements (distance travelled) of pool frogs were analysed in linear mixed models (LMM) with *Bd* infection as a fixed factor, air temperature and body mass as covariates and individual as a random effect. To control for movement effects caused by translocations, this covariate was used in models including terrestrial movements, except for the *Bd*-load autumn-land models due to rank deficiency. Movement data were determined from the linear distances between two subsequent logger positions of each individual and were transformed to 1 + log in the analyses to attain normal distribution of the residuals. Body mass was nested within the random effect (individual), which effectively controls for pseudoreplication for all predictors (Schielzeth & Forstmeier, 2008). Explained model variance (R^2) was determined from the MuMIn package function r.squaredGLMM in program R (Barton, 2018). The LMM analyses included data with (1) pooled land and pond movements, as well as separate analyses of (2) land and (3) pond movements. These analyses were in turn divided into separate models for summer and autumn movements, resulting in six different main models of *Bd* occurrence effects on movements. This was done because we were interested in the separate movements across land and ponds, and because no time gap should occur in movement analyses (Calenge, 2006). For occasional days without field work, or if no signal was recorded, missing positions (NAs) were included in the data, resulting in regular daily trajectories (Calenge, 2006). For comparisons, data for movements from translocated and natural individuals were additionally analysed separately. We also validated the *Bd*-occurrence models where *Bd* was significant with additional models where we excluded samples with only one positive amplification (and possible risk of false positives; see Molecular analyses).

Four *Bd*-load models (including pooled land and pond movements across both seasons, pond movements in summer and land movements in autumn) were constructed to evaluate if movements of the infected individuals were associated with the amount of zoospores. Summer-land and autumn-pond movements were excluded, as the variation in *Bd* loads was too low, or there were too few pond observations of infected individuals. Potential false positives in *Bd*-load models (exclusion of single amplifications) could not be validated, as there was too little variation in *Bd* loads. Some of the models did not converged and were fitted by an optimization of the model algorithm via the Nelder–Mead function of the lmerControl parameter (Bates, 2010). This procedure minimizes the model function in a multidimensional scale (Nelder & Mead, 1965). This was done for the: pooled summer-land and pond models and summer-land models for *Bd* occurrence, and pooled autumn-land and pond models for *Bd* loads.

Chi-square tests were used to compare infection prevalence between males, females and juveniles. Differences in BCI and body mass between infected and non-infected males, females and juveniles were analysed by t-tests and by a Wilcoxon test for juvenile BCI. Daily summer and autumn movements (distances divided by days (log)) of translocated individuals were compared with t-tests. The program R (ver. 3.5.0; Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/) was used for all statistical analyses with the package lme4 (Bates, 2010) for mixed model analyses.

Results

In contrast to the previous study from 2005 to 2008, which found zero incidence of *Bd*-positive individuals (Sainsbury *et al.*, 2007), all six ponds tested positively for *Bd* in 2017-2018 (Fig. 1), that is at least one frog was tested positive on at least one sampling occasion. However, this includes Slåtaren and Rundskär with only one positive individual each, which both were based on one single amplification. The total individual prevalence was 31% (21 of 67; range 11–50% among sites/ponds; Table 1) with a mean of 29.7% (95% CI: 14.7%, 44.6%) across the populations. Infection load class in infected individuals varied between < 0.1 and 1 GE (Table S1). The percentage of infected individuals was 40% for males (10 of 25), 17% for females (five of 29) and 46% for juveniles (six of 13). We found no significant differences in mean *Bd* prevalence among males and females or juveniles (males–females: $\chi^2 = 2.42$, $P = 0.119$; males–juveniles: $\chi^2 < 0.01$, $P = 0.798$; juveniles–females: $\chi^2 = 2.53$, $P = 0.112$). We did not find any differences in mean body condition or body mass between infected and non-infected individuals, either for males, females or juveniles (Table S2).

Movements

Analyses of pooled data for land and pond movement distances of the radio-tracked frogs indicated that movements were negatively associated with occurrence of *Bd* infection, but only during the autumn (Fig. 2a–c; Table S3), and were

Table 1 Sites, sampling year and coordinates (WSG84; North and East) and results of *Bd* tested individuals from the surveyed ponds in 2017-2018

Site	Year	North	East	N	Bd+	%Bd
Vedlösa	2017	60.47104	18.04631	20	10	50
Gäddalen	2017	60.52257	18.01013	21	4	19
Slåtaren	2017	60.53415	18.00980	3	1	33
Rundskär	2017	60.47142	18.07646	4	1	25
Kungsträsket	2018	60.38564	18.18825	10	4	40
Pond 14	2018	60.38655	18.18516	9	1	11
All				67	21	31

N indicates the number of sampled individuals, Bd + is the number of *Bd*-infected individuals, and Bd% is the infection prevalence for each site.

positively associated with body mass during the summer (Fig. 2a; Table S4). When analysing land and pond data separately however, neither the association between movement distance and body mass, nor movement distance and *Bd* occurrence, were significant. The main *Bd*-occurrence models without the single-amplified *Bd* positives (to examine possible effect of false positives), also showed negative effects of *Bd* on pooled land and pond movements, and additionally on land movements in autumn (Table S5). Only including translocated individuals in analyses of autumn movements resulted in negative effects of *Bd* and positive effects of temperature on frog movement distances (Table S6). Daily mean distances (log) of translocated individuals were shorter in the summer than in the autumn (*t*-test: -3.56; $P < 0.001$), in line with their more dispersal-prone behaviour during autumn. Thus, the strongest negative effect of *Bd* occurrence on movements was observed when individuals moved during (relatively) long-distance migration to ponds. The voluntary movements that occurred, mainly towards hibernation sites, were in contrast, not affected by any of the explanatory variables (Table S7).

Bd loads were negatively associated with movement distances, but only within ponds during the summer (Fig. 3a–c; Table S8–S9). There was no association between movement distances and ambient air temperature, either in pooled or separated land and pond models. The number of radio-tracked *Bd*-infected individuals, *Bd*-load classes of tracked individuals and number of recorded positions on land (voluntary and translocated) and in ponds are presented in Table S1.

Intra-seasonal dynamics of Bd

Eleven radio-tracked individuals were sampled for *Bd* twice, once in early June and once in late July (Table 2). Over this period, individual prevalence of *Bd* was reduced from 55% to 27%. Four of eleven individuals remained *Bd* negative, two remained positive, four of the positive individuals lost or reduced their infection and one of the previous negative individuals became infected. These positives also include three single amplifications (Table 2).

Discussion

Our six study sites were located within the same area as the six sites in the previous study on *Bd* infection in Swedish pool frogs, which took place from 2005 to 2008. That study found that all 137 tested individuals (72 larvae, 29 juveniles and 36 adults) were *Bd* negative (Sainsbury *et al.*, 2007). In sharp contrast, we found *Bd*-infected pool frogs in all of our six studied ponds, with a total infection prevalence of 31%, strongly suggesting that *Bd* has either invaded the area within the last decade or become much more common. A previous study showed no difference in *Bd* detection between the two sampling methods that were used in the two compared studies, that is toe clipping and skin swabs, respectively (Livo et al., 2004). The indication of a recent invasion of *Bd* in Sweden are consistent with a study on museum material that did not find *Bd* in 197 Swedish amphibian samples collected between 1994 and 2004 (Garner *et al.*, 2005), and more recent findings (2015–2019) of *Bd* in all nine Swedish amphibian species studied, with prevalence varying from two to 61% among

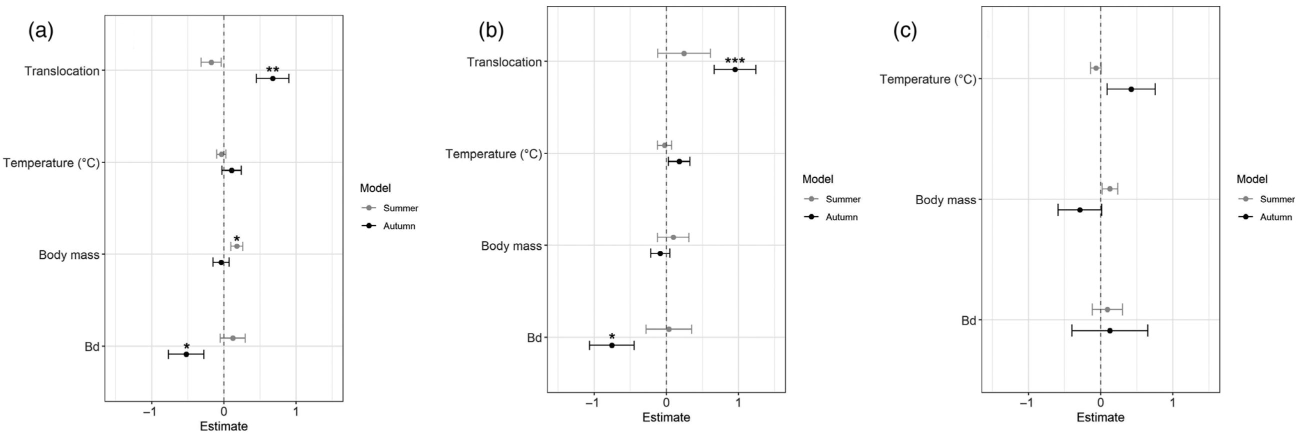


Figure 2 Estimates and standard errors of translocation (for models including land movements), ambient temperature, body mass and occurrence of *Bd*, in separate models of summer and autumn movement distances, including (a) pooled movements on land and in ponds, (b) on land and (c) in ponds. Significant effects are indicated * = 0.05> P > 0.01, ** = 0.01> P > 0.001 and *** = P < 0.001.

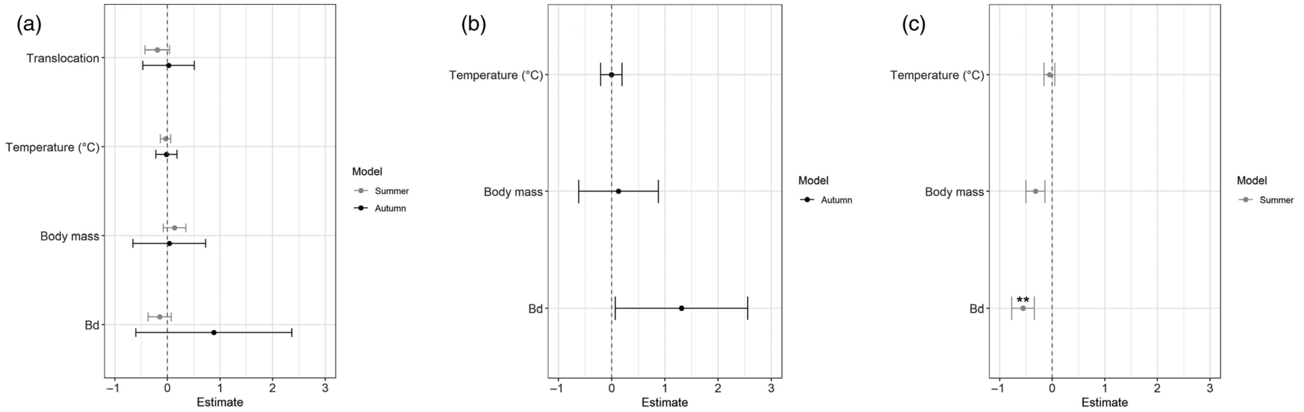


Figure 3 Estimates and standard errors of translocation, ambient temperature, body mass and *Bd* loads (three classes) in separate models of summer and autumn movement distances, including (a) pooled movements on land and in ponds, and (b) on land, and (c) in ponds. Summer-land movements and autumn-pond movements are excluded due to a too low variation in *Bd*-load classes and too few pond observations of infected individuals in the autumn. Significant effects are indicated ** = 0.01> *P* > 0.001.

Table 2 Results from repetitive *Bd* sampling of radio-tracked adult pool frogs in June and end of July 2017, from Vedlösa (V), Gäddalen (G) and Rundskär (R)

Ind	Sex	170608–170615	170725–170728
V07	Female	*Bd–*	*Bd–*
V08	Male	*Bd+*	*Bd+*
V09	Male	*Bd–*	*Bd–*
V10	Male	*Bd+*[a]	*Bd+*
V11	Male	*Bd+*[a]	*Bd–*
G03	Female	*Bd–*	*Bd–*
G07	Male	*Bd–*	*Bd–*
G08	Male	*Bd+*	*Bd–*
G12	Male	*Bd–*	*Bd+*
G13	Male	*Bd+*	*Bd–*
R03	Male	*Bd+*[a]	*Bd–*
% Bd positives		55	27

Bd– indicates no detection, and *Bd* + indicates detection of *Bd* in each sampling period.

[a] Defines samples with a single *Bd*-positive amplification.

species and nearly half of the 74 studied ponds in southern and central Sweden (Kärvemo *et al.*, 2018, 2019; Meurling, 2019). No strong signs of population decline in the present pool frog population have so far been detected (during the time-period 2001–2016; Lindgren *et al.*, 2014; Almkvist & Söderman, 2018), but the methods used (calling males) for population estimates are quite unreliable. To determine the effect of *Bd* on the viability of pool frogs in Sweden, it is critical to monitor the present population in time and space to detect potential declines.

Movements

Amphibian activity may be affected by environmental (context dependent) and behavioural (phenotype dependent) factors (Joly, 2019). For example, landscape structure and fragmentation, as well as sex, age and condition, may directly or indirectly influence migration and are consequently important features in amphibian conservation. Based on our results, we cannot exclude that pool frog movements are negatively affected by *Bd* infection, particularly during relatively long-distance movement between ponds. In addition, pond movements in the summer were negatively associated with *Bd* loads of infected individuals. Reduced movement distances of infected individuals have been reported also in other systems such as house sparrows (Bonneaud *et al.*, 2003) and wolves (Cross *et al.*, 2016). The impact of infections on amphibian movement distances is poorly investigated, but bacterial infection load was associated with reduced movement distance in cane toads *Rhinella marina* (Brown & Shine, 2014), and peak velocity and acceleration were reduced in *Bd*-infected leopard frogs *Lithobates pipiens* (Chatfield *et al.*, 2013). The reduced movement distances associated with *Bd* can be caused by a trade-off between immune system and mobility, suggesting that the energetic cost caused by the fungal infection affects movement (Chatfield *et al.*, 2013; Brown & Shine, 2014). Our results may alternatively be due to sedentary individuals that spend more time in the water where they are more exposed to infections, compared to individuals with a more mobile behaviour – that spend more time on dry land – and thus will be less exposed to *Bd* (Johnson & Speare, 2005; Terrell *et al.*, 2014).

Assuming a causal link between infection and reduced mobility, the *Bd*-mediated reduced mobility may result in higher predation risk, lower reproduction (Johnson *et al.*, 2006) and other fitness costs (Burrowes *et al.*, 2007; Bielby *et al.*, 2015). In our study population – as well as in many other amphibians (Smith & Green, 1998) – reduced movement caused by *Bd* may also have a negative impact on the metapopulation structure and survival, as proximity and migration rate to neighbouring sites is a key factor preventing local extinctions (Sjögren-Gulve, 1991). Extinctions of local populations may potentially increase the number of isolated patches in a metapopulation, which in turn can be critical for persistence of many amphibian populations (Marsh & Trenham, 2001), including the pool frog in Sweden (Sjögren, 1991). However, to draw general conclusions about movements associated with the metapopulation viability, further studies across different spatial and temporal scales and life-stages would be needed (Bailey & Muths, 2019). While we note that three of four infected individuals tracked during autumn had the lowest movement distances of all late-season individuals (Fig. 4), the limited number of radio-tracked *Bd*-infected individuals – and on some occasions a limited number of observed movements – means that broader conclusions from the present study should be made with caution. The result of reduced pond movements associated with infection loads in the summer was however, based on seven infected individuals.

We found that *Bd* occurrence and translocations had a stronger effect on autumn than on summer movements. In addition, the mean of daily distances of translocated individuals in the autumn were more than twice as long, compared to translocated individuals in the summer and the weak effect on movements during the summer may thus be caused by an unnatural migration situation for the season (Sjögren Gulve, 1998; Holenweg Peter, 2001). Translocated individuals were also moving longer distances than voluntary individuals, which may be caused by a homing behaviour that can increase movement distances considerable compared to the normal range of movements (Phillips, Adler & Borland 1995). In contrast to *Bd* occurrence, the amount of detected *Bd* zoospores in infected individuals was negatively associated with the summer-pond movements (Fig. 5). While this result may reflect a true negative effect of *Bd* load on frog movements, an alternative explanation predicts higher loads on individuals remaining in the ponds, where conditions for *Bd* growth are better than in the terrestrial habitat (e.g. Berger *et al.*, 2004; Terrell *et al.*, 2005). Summer movement distances in the *Bd*-occurrence models increased with body mass, which may be associated with higher investment in reproduction and territorial behaviour in larger individuals (Camargo *et al.*, 2005). Unlike some earlier studies on other amphibian species (Murray *et al.*, 2009; Chatfield *et al.*, 2013; Woodhams & Alford, 2005; Kärvemo *et al.*, 2019), *Bd* infection in pool frogs was not linked with body size or condition.

Models without the individuals with only one positive amplification showed similar negative effects of *Bd* on movements as the models including all individuals, indicating that the results are not driven by the single amplifications. *Bd* detection from *Pelophylax* spp. is strongly affected by PCR inhibitors (Baláž *et al.*, 2014) which increase the risk of single positive amplifications. Thus, for pool frogs, false negatives may be a larger problem than false positives. The relatively

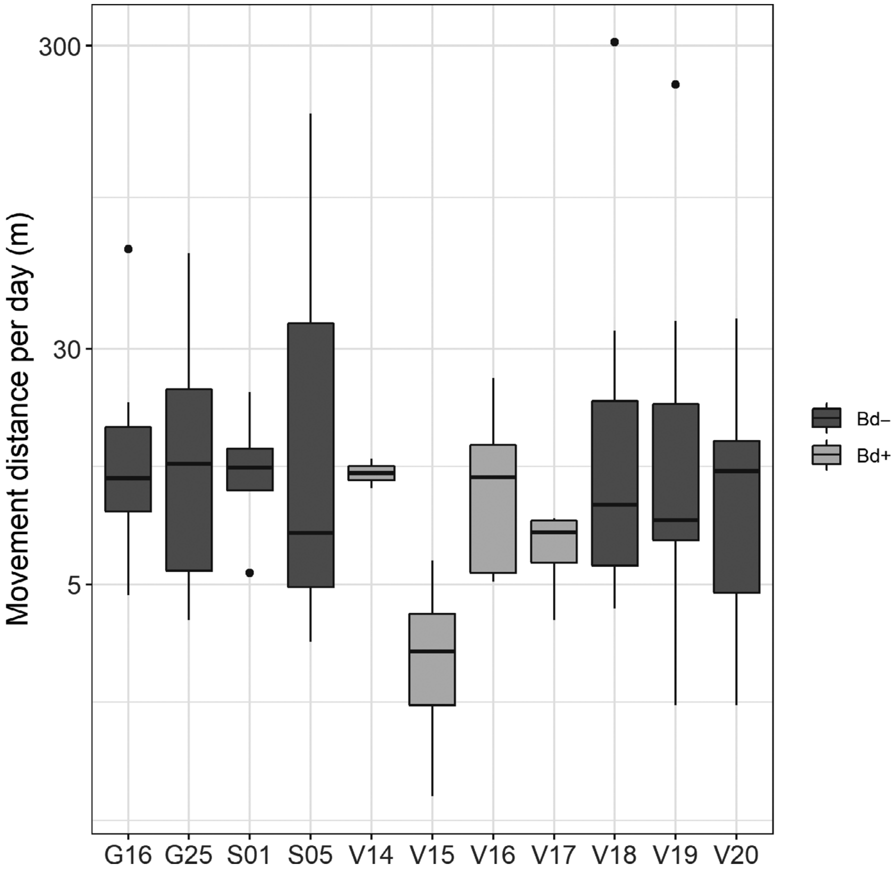


Figure 4 Boxplots of autumn movements distances per day (pooled pond and land movements) of individual *Bd*-negative and *Bd*-positive adult pool frogs (x-axis). Bar: median; box: interquartile range (IQR); whiskers: min/max. values < 1.5 × IQR below/above box; dots: outliers.

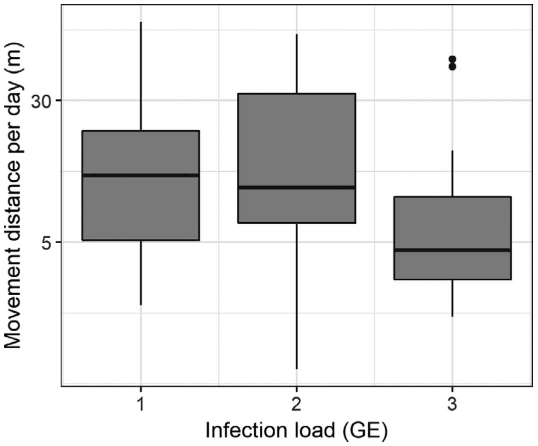


Figure 5 Boxplots of the frogs summer-pond movement distances per day, grouped by *Bd* loads based on three categorized genomic equivalents (GE standards): [1]:<0.1, [2]: 0.1 and [3]:>0.1 < 1. Bar: median; box: interquartile range (IQR); whiskers: min/max. values < 1.5 × IQR below/above box; dots: outliers.

high prevalence in Swedish pool frogs is comparable with other studies on this species (Tobler, Borgula & Schmidt, 2012; Baláž *et al.*, 2014).

Intra-seasonal dynamics of Bd

Four of the eleven individuals that were sampled for *Bd* twice lost their infection between June and July (prevalence decreasing from 55% to 27%), indicating that pool frogs can clear *Bd* infections, or that *Bd* is reduced to such low doses that it is no longer detectable (Bielby *et al.*, 2015). This is in accordance with previous studies reporting recovery of amphibian individuals within and between seasons (Murray *et al.*, 2009; Kinney *et al.*, 2011). Earlier studies have shown reduced *Bd* prevalence in temperate regions between spring and summer (Gaertner *et al.*, 2009; Duncan Pullen et al., 2010; Voordouw *et al.*, 2006, but see Kinney *et al.*, 2011). In our case, this can potentially be explained by differences in temperature between June and July, with more suitable temperatures for *Bd* occurring in early summer (Kriger & Hero, 2007). Another possible explanation for this pattern may be that three of the four individuals that cleared the infections were translocated to dry and warmer terrestrial habitats, which may help to clear infections (Johnson & Speare, 2005; Terrell *et al.*, 2005; Chatfield & Richards-Zawacki, 2011). However, two individuals remained infected despite being translocated.

Conclusions

The pool frog population in central Sweden has probably become exposed to *Bd* within the last decade. They show relatively high pond and population prevalence of *Bd* infections, possibly due to their aquatic lifestyle. This isolated metapopulation is one of the very few populations of this species in the Nordic countries (Zeisset & Hoogesteger, 2018) and is included in the Swedish Red List (VU). Our results suggest that while pool frogs can clear or reduce *Bd* infection, the infection may cause a reduction in movement. This in turn may affect their foraging performance, reproduction and dispersal, and increased susceptibility to predation. It is therefore critical to temporally monitor the present population in order to detect potential declines, and should they occur, mitigate adverse effects of *Bd* via conservation measures.

Acknowledgements

We are very grateful to Gunilla Engström and Yvonne Meyer–Lucht for laboratory work and Emelie Grabbe (Greensway AB) for field assistance. We thank Germán Orizaola, Fredrik Söderman and Frauke Ecke for valuable discussions of pool frog dispersal, ecology and methodology and Gerard Malsher for proofreading the article. The study was funded by the Carl Tryggers Foundation (15:220 and KF17:14), the Swedish Research Council Formas (215-2014-594), the Foundation for Zoological Research, Swedish Nuclear Fuel and Waste Management Company (SKB), Bergvik Skog AB and the County administrative board of Uppsala.

References

Adams, M.J., Chelgren, N.D., Reinitz, D., Cole, R.A., Rachowicz, L.J., Galvan, S., McCreary, B., Pearl, C.A., Bailey, L.L., Bettaso, J., Bull, E.L. & Leu, M. (2010). Using occupancy models to understand the distribution of an amphibian pathogen, Batrachochytrium dendrobatidis. *Ecol. Appl.* **20**, 289.

Almkvist, S. & Söderman, F. (2018). Inventering av gölgroda (Rana lessonae) i Uppsala län 2016. Uppsala: Länsstyrelsen i Uppsala län. Meddelandeserie 2018:02. (In Swedish).

Bailey, L.L. & Muths, E. (2019). Integrating amphibian movement studies across scales better informs conservation decisions. *Biol. Conserv.* **236**, 261.

Baláž, V., Vojar, J., Civiš, P., Šander, M. & Rozínek, R. (2014). Chytridiomycosis risk among Central European amphibians based on surveillance data. *Dis. Aquat. Organ.* **112**, 1.

Barton, K. (2018). MuMIn: multi-model inference. R package version 1.42.1. Retrieved from https://CRAN.R-project.org/package=MuMIn

Bates, D.M. (2010). lme4: Mixed-effects modeling with R. *Retrieved from.* https://lme4r-forger-projectorg/book

Berger, L., Speare, R., Hines, H., Marantelli, G., Hyatt, A., McDonald, K., Skerratt, L., Olsen, V., Clarke, J. & Gillespie, G. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. Vet. J.* **82**, 434.

Bielby, J., Fisher, M.C., Clare, F.C., Rosa, G.M. & Garner, T.W. (2015). Host species vary in infection probability, sublethal effects, and costs of immune response when exposed to an amphibian parasite. *Sci. Rep.* **5**, 10828.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. & Sorci, G. (2003). Assessing the cost of mounting an immune response. *Am. Nat.* **161**, 367.

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

Brem, F., Mendelson, J.R. III & Lips, K.R. (2007). Field-sampling protocol for *Batrachochytrium dendrobatidis* from living amphibians, using alcohol preserved swabs. Retrieved from https://pdfs.semanticscholar.org/36ca/5213496bae2486ddb6e9e7d37fcf3af67cbe.pdf. Cited 16 Feb 2018.

Briggs, C.J., Knapp, R.A. & Vredenburg, V.T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 9695.

Brown, G.P. & Shine, R. (2014). Immune response varies with rate of dispersal in invasive cane toads (*Rhinella marina*). *PLoS ONE* **9**, e99734.

Burrowes, P.A., Longo, A.V. & Rodríguez, C.A. (2007). Potential fitness cost of *Batrachochytrium dendrobatidis* in *Eleutherodactylus coqui*, and comments on environment-related risk of infection. *Herpetotropicos* **4**, 51.

Calenge, C. (2006). The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. *Ecol. Model.* **197**, 519.

Camargo, A., Naya, D.E., Canavero, A., da Rosa, I., Maneyro, R. & Naya, D. (2005). Seasonal activity and the body size—fecundity relationship in a population of *Physalaemus gracilis* (Boulenger, 1883) (Anura, Leptodactylidae) from Uruguay. *Ann. Zool. Fennici.* **42**, 513.

Chatfield, M.W. & Richards-Zawacki, C.L. (2011). Elevated temperature as a treatment for *Batrachochytrium dendrobatidis* infection in captive frogs. *Dis. Aquat. Organ.* **94**, 235.

Chatfield, M.W., Brannelly, L.A., Robak, M.J., Freeborn, L., Lailvaux, S.P. & Richards-Zawacki, C.L. (2013). Fitness consequences of infection by *Batrachochytrium dendrobatidis* in northern leopard frogs (*Lithobates pipiens*). *EcoHealth* **10**, 90.

Cross, P.C., Almberg, E.S., Haase, C.G., Hudson, P.J., Maloney, S.K., Metz, M.C., Munn, A.J., Nugent, P., Putzeys, O. & Stahler, D.R. (2016). Energetic costs of mange in wolves estimated from infrared thermography. *Ecology* **97**, 1938.

Daversa, D.R., Monsalve-Carcaño, C., Carrascal, L.M. & Bosch, J. (2018). Seasonal migrations, body temperature fluctuations, and infection dynamics in adult amphibians. *PeerJ* **6**, e4698.

Deguise, I. & Richardson, J.S. (2009). Prevalence of the chytrid fungus (*Batrachochytrium dendrobatidis*) in Western Toads in southwestern British Columbia, Canada. *Northwest. Nat.* **90**, 35.

Dougherty, E.R., Seidel, D.P., Carlson, C.J., Spiegel, O. & Getz, W.M. (2018). Going through the motions: incorporating movement analyses into disease research. *Ecol. Lett.* **21**, 588.

Duncan Pullen, K.D., Best, A.M. & Ware, J.L. (2010). Amphibian pathogen *Batrachochytrium dendrobatidis* prevalence is correlated with season and not urbanization in central Virginia. *Dis. Aquat. Organ* **91**, 9.

Fisher, M.C., Garner, T.W. & Walker, S.F. (2009). Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annu. Rev. Microbiol.* **63**, 291.

Foster, J. (2006). Capture of pool frogs (gölgroda) Rana lessonae in Uppsala County, Sweden, May 2006. Länsstyrelsen i Uppsala Län.

Gaertner, J.P., Gaston, M.A., Spontak, D., Forstner, M.R.J. & Hahn, D. (2009). Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). *Herp Rev.* **40**, 184.

Garner, T.W., Walker, S., Bosch, J., Hyatt, A.D., Cunningham, A.A. & Fisher, M.C. (2005). Chytrid fungus in Europe. *Emerg Infect Dis.* **11**, 1639.

Gervasi, S.S., Hunt, E.G., Lowry, M. & Blaustein, A.R. (2014). Temporal patterns in immunity, infection load and disease susceptibility: understanding the drivers of host responses in the amphibian-chytrid fungus system. *Func. Ecol.* **28**, 569.

Groff, L.A., Pitt, A.L., Baldwin, R.F., Calhoun, A.J. & Loftin, C.S. (2015). Evaluation of a waistband for attaching external radiotransmitters to anurans. *Wild. Soc. Bull.* **39**, 610.

Hallengren, A. (2013). *Chyridiomykosis: Ett hot mot svenska groddjur?* Sweden: Länsstyrelsen Skåne.

Holenweg Peter, A.K. (2001). Dispersal rates and distances in adult water frogs, *Rana lessonae*, *R. ridibunda*, and their hybridogenetic associate *R. esculenta*. *Herpetologica* **57**, 449.

Johnson, M.L. & Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Dis. Aquat. Organ.* **65**, 181.

Johnson, P.T., Stanton, D.E., Preu, E.R., Forshay, K.J. & Carpenter, S.R. (2006). Dining on disease: how interactions between infection and environment affect predation risk. *Ecology* **87**, 1973.

Joly, P. (2019). Behavior in a changing landscape: Using movement ecology to inform the conservation of pond-breeding amphibians. *Front. Ecol. Evol.* **7**, 155.

Kärvemo, S., Meurling, S., Berger, D., Höglund, J. & Laurila, A. (2018). Effects of host species and environmental factors on the prevalence of *Batrachochytrium dendrobatidis* in northern Europe. *PLoS ONE* **13**, e0199852.

Kärvemo, S., Laurila, A. & Höglund, J. (2019). Urban environment and reservoir host species are associated with *Batrachochytrium dendrobatidis* infection prevalence in the common toad. *Dis. Aquat. Organ.* **134**, 33.

Kinney, V.C., Heemeyer, J.L., Pessier, A.P. & Lannoo, M.J. (2011). Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: Affirmation of Vredenburg's "10,000 Zoospore Rule". *PLoS One* **6**, e16708.

Kosch, T. & Summers, K. (2013). Techniques for minimizing the effects of PCR inhibitors in the chytridiomycosis assay. *Mol. Ecol. Resour.* **13**, 230.

Kriger, K. & Hero, J.M. (2007). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *J. Zool.* **271**, 352.

Lindgren, B., Nilsson, J. & Söderman, F. (2014). Åtgärdsprogram för gölgroda, 2014–2019. Uppsala: Naturvårdsverket. Report 6631. (In Swedish).

Lips, K.R. (2016). Overview of chytrid emergence and impacts on amphibians. *Phil. Trans. R. Soc. B.* **371**, 20150465.

Livo, L.J., Wood, J., Annis, S., Carey, C., Epp, J., Jones, M.S. & Maine, O. (2004). Evaluation of techniques for detecting Batrachochytrium dendrobatidis from amphibians for PCR testing. In Colorado Division of Wildlife: Boreal Toad Research Report. 15–22. Rogers, K. B. (Ed.).

Marsh, D.M. & Trenham, P.C. (2001). Metapopulation dynamics and amphibian conservation. *Conserv. Biol.* **15**, 40.

Mazerolle, M.J. (2001). Amphibian activity, movement patterns, and body size in fragmented peat bogs. *J. Herpetol.* **35**, 13.

Meurling, S. (2019). The response in native wildlife to an invading pathogen: Swedish amphibians and *Batrachochytrium dendrobatidis*. PhD thesis, Uppsala University.

Murray, K.A., Skerratt, L.F., Speare, R. & McCallum, H. (2009). Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. *Conserv. Biol.* **23**, 1242.

Nelder, J.A. & Mead, R. (1965). A simplex method for function minimization. *Comput. J.* **7**, 308.

Orizaola, G. & Laurila, A. (2016). Developmental plasticity increases at the northern range margin in a warm-dependent amphibian. *Evol. Appl.* **9**, 471.

Orizaola, G., Quintela, M. & Laurila, A. (2010). Climatic adaptation in an isolated and genetically impoverished amphibian population. *Ecography* **33**, 730.

Pearl, C.A., Bowerman, J., Adams, M.J. & Chelgren, N.D. (2009). Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). *EcoHealth* **6**, 209.

Pettit, L.J., Greenlees, M.J. & Shine, R. (2017). The behavioural consequences of translocation: how do invasive cane toads (Rhinella marina) respond to transport and release to novel environments? *Behav. Ecol. Sociobiol.* **71**, 15.

Phillips, J.B., Adler, K. & Borland, S.C. (1995). True navigation by an amphibian. *Anim. Behav.* **50**, 855.

Retallick, R.W. & Miera, V. (2007). Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. *Dis. Aquat. Organ.* **75**, 201.

Sainsbury, A., Yu-Mei, R., Ågren, E., Vaughan-Higgins, R., Mcgill, I., Molenaar, F., Peniche, G. & Foster, J. (2017). Disease risk analysis and post-release health surveillance for a reintroduction programme: the pool frog *Pelophylax lessonae*. *Transbound. Emerg. Dis.* **64**, 1530.

Sapsford, S.J., Voordouw, M.J., Alford, R.A. & Schwarzkopf, L. (2015). Infection dynamics in frog populations with different histories of decline caused by a deadly disease. *Oecologia* **179**, 1099.

Scheele, B.C., Pasmans, F., Skerratt, L.F., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M.C., Flechas, S.V., Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., Gratwicke, B., Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips, K.R., Longo, A.V., Maneyro, R., McDonald, C.A., Mendelson, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.-O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R. & Canessa, S. (2008). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* **363**, 1459.

Schielzeth, H. & Forstmeier, W. (2008). Conclusions beyond support: overconfident estimates in mixed models. *Behav. Ecol.* **20**, 416.

Sheldon, B.C. & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends. Ecol. Evol.* **11**, 317.

Sinsch, U. (1991). Mini-review: the orientation behaviour of amphibians. *Herpetol. J.* **1**, 541.

Sjögren, P. (1991). Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana lessonae*). *Biol. J. Linnean Soc.* **42**, 135.

Sjögren-Gulve, P. (1994). Distribution and extinction patterns within a northern metapopulation of the pool frog. *Rana lessonae. Ecology* **75**, 1357.

Sjögren-Gulve, P. (1998). Spatial movement patterns in frogs: target-oriented dispersal in the pool frog. *Rana lessonae. Ecoscience* **5**, 31.

Smith, A. & Green, D. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**, 110.

Terrell, V.C., Engbrecht, N.J., Pessier, A.P. & Lannoo, M.J. (2014). Drought reduces chytrid fungus (*Batrachochytrium dendrobatidis*) infection intensity and mortality but not prevalence in adult crawfish frogs (*Lithobates areolatus*). *J. Wildl. Dis.* **50**, 56.

Tobler, U., Borgula, A. & Schmidt, B.R. (2012). Populations of a susceptible amphibian species can grow despite the presence of a pathogenic chytrid fungus. *PLoS One* **7**, e34667.

Todd, B.D. & Winne, C.T. (2006). Ontogenetic and interspecific variation in timing of movement and responses to climatic factors during migrations by pond-breeding amphibians. *Can. J. Zool.* **84**, 715.

Voordouw, M.J., Adama, D., Houston, B., Govindarajulu, P. & Robinson, J. (2010). Prevalence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in an endangered population of northern leopard frogs, Rana pipiens. *BMC Ecol.* **10**, 6.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerratt, L.F. & Speare, R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* **326**, 582.

Wake, D.B. & Vredenburg, V.T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 11466.

Wikström, G. (2018). Improving forest management for a red-listed anuran: movement and habitat use of pool frogs (*Pelophylax lessonae*) in Sweden. Master thesis, Uppsala University.

Woodhams, D.C. & Alford, R.A. (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conserv. Biol.* **19**, 1449.

Zeisset, I. & Hoogesteger, T. (2018). A reassessment of the biogeographic range of northern clade pool frogs (*Pelophylax lessonae*). *Herpetol. J.* **28**, 63.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pool frogs (*Pelophylax lessonae*) included in the study. Site denote names of the six studied locations, in the Sex column is M = male, F = female and J = Juvenile, Date is when the Bd sample was taken, Body mass and Body lenght is weight in gram and Snout-Vent-Length in mm. Bd denotes presence of infection, No.pos.ampl is the number of positve amplification (of the triplicate), Cq-value is the mean number (if> 1 pos) of cycles needed to detect a real pos signal, GE is genome eqvivalents class related to infection loads of positive and tracked individuals. Tracked indicate individuals that were radiotracked in each season (division of the models), landV = number of recorded voluntary land positions, landT = number of recorded translocated land positions, pond = number of recorded pond positions and all summing up recorded positions. Bd-repeated indicate positive (yes) and negative (no) individuals that were sampled twice for Bd in the summer.

Table S2. Statistical evaluation of mean body condition (BCI) and body mass between infected (Bd+: ±se) and non-infected (Bd-:±se) pool frog individuals.

Table S3. Outcomes from the linear mixed models of autumn movement distances of pool frogs with translocation (a-b; models including land data), ambient temperatures body mass and Bd occurrences as explanatory variables (a) on land and in ponds, (b) on land and (c) in ponds. Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.

Table S4. Outcomes from the linear mixed models of summer movement distances of pool frogs with translocation (a-b; models including land data), ambient temperatures body mass and Bd occurrences as explanatory variables (a) on land and in ponds, (b) on land and (c) in ponds. Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.

Table S5. Outcomes from the linear mixed models of pooled data for autumn-land and pond movements and autumn-land movement distances of pool frogs, with translocation, ambient temperaturesexclusively with more than one positive amplification, body mass and Bd occurrences as explanatory variables (a) on land and in ponds (autumn), (b) on land (autumn) and (c) in ponds (summer). Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.

Table S6. Outcomes from the linear mixed models of autumn-land-voluntary movement distances of pool frogs with ambient temperatures, body mass and Bd occurrences as explanatory variables. Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.

Table S7. Outcomes from the linear mixed models of autumn-land movement distances of translocated pool frogs with ambient temperatures, body mass and Bd occurrences as explanatory variables. Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.

Table S8. Outcomes from the linear mixed models of summer movement distances of pool frogs with ambient temperatures, body mass and Bd loads (Genomic equivalents) as explanatory variables (a) on land and in ponds, and (b) in ponds. Land movements did not converge. Individual (random effect) were nested with body mass in the models.

Table S9. Outcomes from the linear mixed models of autumn movement distances of pool frogs with ambient temperatures, body mass and Bd loads (Genomic equivalents) as explanatory variables (a) on land and in ponds, and (b) on land (no variation in Translocation and thus excluded and pond observation of infected individuals were too few). Individual (random effect) were nested with body mass in the models. Deviations are based on the ML of the fixed effect variables.