# Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite

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**Abstract**

**1.** Animals switch habitats on a regular basis, and when habitats vary in suitability for parasitism, routine habitat switching alters the frequency of parasite exposure and may affect post-infection parasite proliferation. However, the effects of routine habitat switching on infection dynamics are not well understood.

**2.** We performed infection experiments, behavioural observations, and field surveillance to evaluate how routine habitat switching by adult alpine newts (*Ichthyosaura alpestris*) influences infection dynamics of the pathogenic parasite, *Batrachochytrium dendrobatidis* (*Bd*).

**3.** We show that when newts are exposed to equal total doses of *Bd* in aquatic habitats, differences in exposure frequency and post-exposure habitat alter infection trajectories: newts developed more infections that persisted longer when doses were broken into multiple, reduced-intensity exposures. Intensity and persistence of infections was reduced among newts that were switched to terrestrial habitats following exposure.

**4.** When presented with a choice of habitats, newts did not avoid exposure to *Bd*, but heavily infected newts were more prone to reduce time spent in water.

**5.** Accounting for routine switching between aquatic and terrestrial habitat in the experiments generated distributions of infection loads that were consistent with those in two populations of wild newts.

**6.** Together, these findings emphasize that differential habitat use and behaviours associated with daily movement can be important ecological determinants of infection risk and severity.

**Key words:** Host-parasite interactions, *Batrachochytrium dendrobatidis*, Habitat use, Host behaviour, Behaviour, Environmental heterogeneity, Disease risk

## Introduction

All organisms are susceptible to parasites, yet parasites do not infect all susceptible hosts equally (Wilson *et al.* 2002). While host susceptibility is always at least to some degree an intrinsic trait, extrinsic factors can also strongly influence probability and strength of infection. Environmental variation amongst habitats that hosts move between has the potential to be an important driver of infection dynamics because different environments associated with different habitats carry different risks of parasitism (Parratt, Numminen & Laine 2016). Large-scale, inter-seasonal habitat switching (i.e. migration) that exposes potential hosts to divergent environments is already known to cause spatial and temporal variation in infection (Altizer, Bartel & Han 2011). However, animals also switch habitats to complete essential, daily activities such as foraging, mate searching, and predator avoidance. The influence of this "routine" (Van Dyck & Baguette 2005) habitat switching on infection dynamics has received much less attention. Despite the shorter timescales involved, evidence is accumulating that these rapid and local habitat shifts can significantly affect rates of parasitism (Hoch, Monnet & Agoulon 2010; Byers *et al.* 2015).

Environmental heterogeneity should act on the ability of a parasite to survive, grow and reproduce, and can be broken down based on fundamental theory of transmission dynamics. Specifically, exposure frequency, parasite density and post-infection parasite proliferation should vary according to habitat suitability, and are all well accepted drivers of infection dynamics (Anderson & May 1991; Wilson *et al.* 2002). Empirical studies of the interactions amongst these factors are few and far between, though, and it is unlikely that they would be deterministic. For example, we are unaware of any study where the frequency of exposure to infectious particles was varied while the number of infectious particles was held constant, although exposure

frequency is considered to be an important driver behind infection (Leon & Hawley 2017) and disease (Rohani, Keeling & Grenfell 2002) dynamics. Nevertheless, each step of the host movement process should have specific impacts on both the probability and subsequent strength of infection. First, the time that a host spends in habitats harbouring parasites roughly corresponds to the number of exposure events over time (exposure frequency). Second, habitats with heavier parasite concentrations should pose a greater risk of infection than habitats where concentrations are light (exposure intensity). Third, even when parasites are absent, if a host is already infected, then occupying habitats that positively influence parasite growth and reproduction should also positively affect post-infection dynamics.

Animals choose whether to move between habitats, a decision-making process that can be influenced by the risk of parasitism. Such decisions can affect the frequency with which animals spend time in habitats that facilitate infection and post-infection parasite proliferation. Parasite avoidance behaviours are documented in a wide range of host taxa (Moore 2002; Hoverman & Searle 2016). Hosts may alter habitat use in response to parasites at multiple phases of the interaction, depending on the risks posed by exposure and infection, and the effect of such changes on infection dynamics likely depends on when during the interaction habitat changes are made (Wilson *et al.* 2002; Byers *et al.* 2015). Hosts may avoid parasites prior to exposure: since risk of infection often varies across habitats, avoidance may simply be a matter of preference for habitats that are less likely to carry parasites. Avoidance behaviours can also be a direct response to exposure, particularly if hosts do not easily detect parasites or habitats that inhibit parasite survival and growth are easily accessed. If the probability of exhibiting avoidance covaries to some degree with risk of infection and disease, and the effects of pathogen-inhibiting habitats are strong, hosts may switch

after infections occur when host parasite burdens have increased to potentially costly levels. As a result, avoidance can reduce infection risk and alter infection dynamics driven by extrinsic processes like dose strength and frequency. However, the efficacy of pre- and post-infection habitat switching for minimizing infection risk is uncertain, and under some conditions habitat switching may actually exacerbate infections (Hoodless *et al.* 2002; Morgan *et al.* 2007).

In this study, we assessed the role of routine habitat switching in infection dynamics of *Batrachochytrium dendrobatidis (Bd)*, a microscopic fungus that infects keratinized epidermal cells of amphibians via free-living zoospores. *Bd* is considered a major threat to global biodiversity (Fisher *et al.* 2012) but has highly variable distributions within and among susceptible host species (Bielby *et al.* 2015). Substantial advancements have been made in modeling *Bd* dynamics within aquatic habitats (Briggs, Knapp & Vredenburg 2010; Wilber *et al.* 2017). However, many adult amphibians routinely move between aquatic and terrestrial habitats. *Bd* zoospores are waterborne (Piotrowski, Annis & Longcore 2004), have limited mobility (Piotrowski *et al.* 2004), and are sensitive to environmental fluctuations like drying (Raffel *et al.* 2015), which results in heterogeneous densities of zoospores across aquatic and terrestrial habitats used by amphibians (Heard *et al.* 2015). Field surveillance (Kriger & Hero 2007), broad-scale modeling (Bielby *et al.* 2008), and experimental work (Becker *et al.* 2014) have established a general negative association between infection risk and host life histories that are biased towards terrestrial habitats. However, laboratory experiments have found that *Bd* can proliferate in hosts (Raffel *et al.* 2015) and survive outside of hosts (Kirshtein *et al.* 2007; Kolby *et al.* 2015) in sufficiently wet terrestrial habitats. There is also evidence for cryptic but persistent infection of terrestrial hosts (Minting 2012) and documented

120 cases of *Bd* infecting fully terrestrial amphibians (Kolby *et al.* 2015). Thus, whether
121 increased terrestrial use can regulate either the probability of infection or post-
122 infection parasite proliferation over short time spans associated with routine habitat
123 switching is unclear. Avoidance of *Bd*-infected habitats has been suggested
124 (McMahon *et al.* 2014) but detailed evaluations of *Bd* avoidance behaviours are
125 lacking (Raffel *et al.* 2015).

126 We used adult alpine newts (*Ichthyosaura alpestris*) as a focal host. Alpine
127 newts breed for prolonged periods in lakes and ponds during which newts mate
128 promiscuously and are largely aquatic. However, both sexes sustain varying degrees
129 of terrestrial activity during breeding periods (Weddeling *et al.* 2004), perhaps to
130 obtain nutrient-rich food (Denoel 2004), avoid predators (Winandy, Darnet & Denoël
131 2015), search for different aquatic habitats (Kopecky, Vojar & Denoël 2010), and
132 minimize parasitism (Todd 2007). Field surveillance has reported *Bd* infections in
133 wild populations of alpine newts (Wood, Griffiths & Schley 2009; Ohst *et al.* 2011;
134 Rasmussen *et al.* 2012) but with no evidence of disease or mass-mortality as in highly
135 susceptible hosts. However, recent experimental work with this species has shown
136 costs of continuous exposure to *Bd* that manifest as mortality at relatively low
137 infection levels (Miaud *et al.* 2016). Thus, while much exposure to *Bd* in the wild
138 appears to be non-lethal, newts can conceivably benefit by adopting behaviours that
139 minimize exposure to *Bd*. Our overarching aims were to establish the mechanistic
140 basis for how habitat switching alters infection dynamics and to determine if *Bd*
141 affects habitat switching behaviours. We first surveyed *Bd* infection in populations of
142 adult newts during a breeding season to characterize natural within-season variation in
143 *Bd* loads. We then conducted two experiments to test whether: a) exposure frequency
144 or exposure intensity had greater impact on the course of *Bd* infections; b) habitat type

(aquatic versus water-saturated terrestrial) influenced the persistence of infections, and; c) newts behaviourally modify use of habitats in response to changes in infection risk and post-infection loads.

## Materials and methods

*Field surveys of prevalence and infection loads*

We sampled two populations of alpine newts inhabiting networks of aquatic habitats, one in the Guadarrama Mountain National Park, Spain and one in Cornwall, U.K. The Spain network comprises permanent and ephemeral alpine ponds surrounded by moist grassland. Newts co-occur with multiple amphibian species with known histories of *Bd* infection (Bosch & Martínez-Solano 2006). The Cornish network comprises man-made ponds in residential areas. Here, alpine newts co-occur with palmate newts (*Lissotriton helveticus*) and various anuran species, and *Bd* has been detected infecting alpine newts occupying all sampled ponds (Garner, unpublished data). We dipnetted ponds during the breeding season and collected *Bd* samples by rubbing sterile swabs over the venter and appendages of newts. Swabs (MWE ltd.) were stored in 1.5mL microtubes and transported in coolers to London for quantitative molecular detection of infection (see below).

*Experiment 1*

We tested the effect of exposure frequency, exposure intensity and post-exposure habitat switching on the course of *Bd* infections in the absence of habitat choice. Male newts were collected from the Cornish sites, initially housed individually in 1.6 L plastic containers containing 750 mL of aged tap water (see Supporting Information for husbandry details). Newts had unknown infection

histories but as adults inhabited a persistently risky environment for years. For this reason we used a seven-day course of antifungals (itraconazole; Garner et al. 2009a) one week prior to the experiment to clear any preexisting *Bd* infections and confirmed clearance using qPCRs before the start of experimental exposures (Boyle *et al.* 2004). Treatments were completed under veterinary care and all newts were deemed in good health before first exposures. Newts were fed bloodworms (chironomid larvae) twice per week during antifungal treatments and throughout the experiment. We conducted antifungal treatments and the experiment in temperature-controlled rooms (18-20° Celsius) with regular airflow and a 16-hour daylight cycle.

We randomly assigned 90 newts to one of three exposure treatments: a negative control (3 x sham exposure to liquid media); a single high dose of $1.8 \times 10^6$ zoospores followed by two sham exposures (intense exposure treatment); or multiple low doses of $3 \times 6.0 \times 10^5$ zoospores (frequent exposure treatment) (Supporting Information Fig. S1). Therefore, newts exposed to *Bd* were exposed to the same number of zoospores, and the total volume of media was kept constant across all treatments. We exposed newts individually for four hours on days 1, 7 and 14 in 0.07 L containers containing 35 mL of aged tap water and their respective treatment exposure and rinsed them with aged tap water afterwards before returning to their experimental housing. We exposed newts in smaller, separate containers to decrease dose dilution and eliminate the risk of environmental contamination that could influence molecular diagnostics. We used a *Bd*GPL strain (Farrer *et al.* 2011) isolated from an alpine newt collected in Cornwall.

During exposures, we replaced water with moistened paper towels in housing for half of the newts in each exposure treatment, which served as terrestrial replicates. We kept paper towels saturated but free of standing water by misting containers with

aged tap water every other day. We changed the paper towels in terrestrial containers and changed water in aquatic containers once per week during the exposure procedures. One week after the final exposure (day 21), we placed all terrestrial newts back into aquatic containers while keeping aquatic newts in the same containers, where they were held until the end of the experiment (day 28). We simultaneously exposed ten captive bred and infection-free Mallorcan midwife toad tadpoles (*Alytes muletensis*), a host that is highly susceptible to infection (Doddington *et al.* 2013), to *Bd* according to the frequent exposure treatment, to serve as a positive control for infectivity of the *Bd* culture. To assess infection, we collected epidermal swab samples (or for midwife tadpoles, buccal swabs) on day 1, 7, and 14 (immediately prior to exposures), 21 and 28. If the skin of terrestrial newts was dry, we dipped swabs in sterile water prior to swabbing.

*Experiment 2*

Here, we tested the behavioural responses of newts when the total concentration of zoospores (i.e., risk of infection) was not held constant, as in the first experiment. We used the same collection, pre-experimental antifungal treatment, *Bd* isolate, and initial husbandry methods as in experiment 1 (see Supporting Information methods).

Newts were housed individually in 5L plastic containers divided equally into terrestrial and aquatic habitats. (Fig. S2, Video S1). For terrestrial habitat we used moistened terrarium moss (Zoo Med Laboratories, Inc., California, USA) overlaid on a pebble substrate kept saturated for the duration, and filled the aquatic habitat with 1 L of aged tap water. Pilot tests of newt activity showed that newts moved freely between habitats (data not shown).

220 We ran the experiment in three sequential batches of 30 newts, with 10 newts
221 per treatment in each batch (N = 90). We randomly assigned newts to one of 3
222 treatments (negative control, low risk, high risk). Newts were given 1 day to acclimate
223 to the tanks before experiments began. During the initial exposure, we confined newts
224 to the aquatic portion to ensure that all newts would unavoidably experience exposure
225 to *Bd* on the first day. We pipetted sterile liquid media (no risk control), 3.0 x $10^5$
226 active *Bd* zoospores (low risk), or 3.0 x $10^6$ active *Bd* zoospores (high risk) into
227 aquatic habitats, removed barriers to terrestrial habitat and began video recording
228 newt activity immediately after barriers were removed. We repeated exposures daily
229 for 7 days after removing dirt particles or excrement from aquatic habitat.
230 We digitally recorded the terrestrial and aquatic activity of exposed newts with
231 an overhead array of six webcams (Logitech C310, Newark, CA, USA), each
232 covering the aquatic portion of 5 containers (i.e. "camera blocks") and connected to a
233 computer (Dell Inspiron 350). Container locations were randomized across the array.
234 We recorded time spent in the aquatic habitat (visualizing newts against the pale
235 aquatic background was straightforward), and assumed newts spent the remaining
236 time in terrestrial habitat. Webcams captured one image per minute during simulated
237 daylight hours (6:00 – 20:00 hrs) for 8 days using iSpy webcam software
238 (www.ispyconnect.com). Newts were then transferred to clean 1.6L containers
239 containing 750 mL *Bd*-free aged tap water for 24 hours to control for environmental
240 contamination with *Bd*. Newts were then swab sampled for qPCR diagnostics.
241
242 *Parasite Detection*
243 We followed identical procedures and used the same equipment to process all
244 samples collected for this study. We quantified the amount of *Bd* DNA on each swab

in duplicate using qPCR diagnostics, appropriate negative controls (Boyle *et al.* 2004) and 4 concentration standards serving as positive controls (Garner *et al.* 2009b; Luquet *et al.* 2012; Bielby *et al.* 2015) (See Supporting Information methods for further details on qPCR assays). A sample was considered positive when both duplicates amplified, or when rerunning single amplifications generated a clear positive. *Bd* loads are reported here in genomic equivalents (GE), where one GE is equivalent to a single zoospore. Since newts consistently exhibited low-level infections (see Results), we considered GE values of at least 0.01 GE to be positive for infection.

*Data analysis*

For experiment 1 we used infection status (uninfected vs. infected) and infection intensity (log-transformed GE + 1) as response variables. We first averaged individual newt values across weeks to categorise infection status and calculate mean GE and maximum GE. Here a newt was "infected" if infection was detected on days 7, 14, and/or 21. We used generalised linear models (GLMs) to test the effect of exposure, habitat and the interaction of these two factors, using a binomial error structure when infection status was the response and a Gaussian error structure when mean and maximum *Bd* load (log-transformed) of newts were the response. For weekly analyses, we used weekly infection status and GE values, generalised linear mixed models (GLMMs) and identical error structures with newt identity as a random effect to account for repeated measures. Three aquatic newts from the control treatment, one aquatic newt from the intense exposure treatment and two aquatic newts from the frequent exposure treatment died during the experiment. None of these

269 animals exhibited symptoms of chytridiomycosis and were excluded from the
270 analysis.

271 For experiment 2, we based experiment day on 24-hour increments from the
272 start time of the experiment and omitted images captured during daily cleaning and
273 exposure times. We also omitted images during periods when webcam alignment did
274 not afford a clear view of the aquatic habitat (see Supporting Information methods for
275 times). We then calculated the time to first departure to terrestrial habitat ($t_{depart}$) and
276 the proportion of time spent on land ($t_{terrestrial}$). For $t_{depart}$ we identified the first image
277 in which individuals were absent from the aquatic habitat. We then divided the
278 position of this photograph along the sequence by the total number of images. Thus,
279 individuals that never left the aquatic habitat had a value of 1, and $t_{depart}$ decreased
280 with faster departure times. This proportion corrected for variation in total duration of
281 the experiments between batches that arose from differences in cleaning times. We
282 then estimated the proportion of total images in which individuals were present in the
283 aquatic portion of the tank ($t_{aquatic}$). We calculated $t_{terrestrial}$ as: $1 - t_{aquatic}$.

284 To ascertain if infection risk did vary on the basis of dose strength, we fitted
285 separate GLMs with exposure treatment as a fixed effect: one with a binomial error
286 structure and infection status on day 9 as the response variable, and another with a
287 Gaussian error structure and infection intensity exhibited on day 9 as the response
288 variable. We omitted newts in the control treatment from these models, as these
289 individuals were not exposed to *Bd* at any time during the experiment.

290 To assess the effects of risk and infections on $t_{terrestrial}$ and $t_{depart}$,we fitted a
291 GLM with a Gaussian error structure with cumulative $t_{terrestrial}$ (square root arcsine
292 transformed) and $t_{depart}$ as $t_{response}$ variables, respectively, with exposure treatment,

293 infection status on day 9 (0 = uninfected, 1 = infected) and GE on day 9 as fixed
294 effects.

295 We also assessed the effects of each fixed effect on daily $t_{terrestrial}$ by fitting
296 GLMMs with Gaussian error structures, $t_{terrestrial}$ (arcsine transformed) as the response
297 variable and newt identity as a random effect to account for repeated measures of
298 individuals. We included experiment day and its interaction with each factor (camera
299 block, risk level, infection status on day 9, infection intensity on day 9) in GLMMs to
300 consider temporal variation in effects of exposure and infection. Our *Bd* culture
301 completed a full growth cycle in four days (Daversa pers. obs.) so to consider phase
302 specific effects on cumulative and daily $t_{terrestrial}$ we also fitted separate GLMs (for
303 overall activity) and GLMMs (for daily activity) for two phases: days 1-3 and days 4-
304 7. We included camera block as a categorical fixed effect (there were too few levels
305 to model it as a random effect) in all GLMs and GLMMs used for the Experiment 2
306 analysis to account for potential spatial effects.

307 In all statistical analyses GEs were normalized with a $\log_{10}$ transformation,
308 and analyses for infection load as the response omitted uninfected newts. Effects of
309 body size and weight of newts were not considered, as these variables did not differ
310 among exposure or habitat treatments in either experiment (see Supporting
311 Information results). For both experiments we tested our hypotheses by comparing
312 models including factors of interest with models omitting these factors, using
313 likelihood ratio tests for GLMs ($\chi 2$ for GLMs with binomial error structures and $F$ for
314 GLMs with Gaussian error structures) and Kenward-Roger approximations for
315 GLMMs. We performed all analyses in R version 3.0.1 and used the *lme4* package to
316 run GLMMs. We used the *dropterm* function in the MASS package for model
317 comparisons and the *pbkrtest* package for Kenward-Roger approximations. The

results for all statistical analyses report the mean and standard error (SE), unless otherwise noted.

## Results

*Field Surveys*

Wild newts consistently exhibited low-level infections [Spain population (N = 49): range 0.02 – 24.46 GE, mean ± SE = 3.53 ± 0.87 GE; UK population (N = 23): range 0.04 – 56.94 GE, mean ± SE = 5.45 ± 2.57 GE; Fig. S3].

*Experiment 1*

All newts tested negative for *Bd* when experiments began. Nine out of ten of the *A. muletensis* tadpoles developed infections averaging 145.07 ± 128.67 GE, confirming the infectivity of our *Bd* culture. An aquatic newt in the frequent exposure treatment in experiment 1 exhibited an outlier *Bd* load (127.3 GE) on day 21. Removing this newt from the analysis did not qualitatively affect the results (see Supporting Information results).

*Bd* loads exhibited by newts in Experiment 1 were within the range of *Bd* loads in wild populations (Fig. S3). Newts repeatedly exposed to low doses of *Bd* were more likely to develop infections than newts exposed to a single, intense dose (dropping exposure treatment from the GLM reduced goodness of fit: $\chi^2_1 = 5.87$; p = 0.015; Fig. 1a), though mean *Bd* loads (intense GE = 0.67 ± 0.31; frequent GE = 4.03 ± 3.24; GLM, $F_{1,16} = 0.11$; p = 0.749) and maximum *Bd* loads (intense GE = 1.53 ± 0.59; frequent GE = 10.46 ± 9.00; GLM, $F_{1,16} = 0.01$; p = 0.957) did not differ among exposure treatments. Only frequently exposed newts exhibited infections by the end of the experiment (Fig. S4a,b). There was a significant interaction between week and

343 exposure treatment, as the likelihood of infection of frequently exposed newts
344 increased in later weeks (see Supporting Information results). Neither weekly mean
345 nor maximum *Bd* loads of infected newts differed between exposure treatments (Fig.
346 S4).

347 Post-exposure habitat also affected overall infection prevalence ($\chi^2_1 = 6.77$; $p$
348 = 0.009, Fig. 1a). Terrestrial newts developed weaker infections, both in terms of
349 average *Bd* loads (aquatic GE = 4.30 ± 3.22; terrestrial GE = 0.10 ± 0.03; GLM, $F_{1,16}$
350 = 11.76; $p$ = 0.003; Fig. 1b) and maximum *Bd* loads (aquatic GE = 11.83 ± 9.63;
351 terrestrial GE = 0.24 ± 0.10; $F_{1,16}$ = 15.91; $p$ = 0.001). Effects of habitat were also
352 apparent on a weekly scale (see Supporting Information results). Terrestrial newts
353 cleared infections more quickly than aquatic newts following intense exposures (Fig.
354 S4).

355 Two frequently exposed terrestrial newts that previously tested negative
356 developed detectable but weak infections on day 28, one week after being returned to
357 aquatic containers (GE = 0.14 ± 0.01; Table S1). Four aquatic newts exposed in the
358 same manner also exhibited infections on this day, though all of these individuals
359 previously tested positive. None of the terrestrial or aquatic newts that were exposed
360 to a single, intense dose of *Bd* exhibited infection on day 28 (Table S1).

361

362 *Experiment 2*

363 All newts tested negative for *Bd* when experiments began, and newts in the
364 control treatment did not develop detectable infections during the experiment. *Bd*
365 loads exhibited by newts were within the range of *Bd* loads we detected in wild
366 populations (Fig. S3). Dose strength predicted infection risk: newts in the high dose
367 tanks were more likely to develop infections (GLM; $\chi^2_1 = 18.44$; $p < 0.001$, Fig. 2a)

and developed stronger infections (low dose GE = 0.44 ± 0.15, high dose = 8.82 ± 2.72, GLM, $F_{1,51} = 24.67$, $p < 0.001$; Fig. 2b).

Risk did not affect how quickly newts first switched to terrestrial habitat (no risk $t_{depart} = 0.54 \pm 0.08$, low risk $t_{depart} = 0.55 \pm 0.09$, high risk $t_{depart} = 0.70 \pm 0.08$, GLM, $F_{1,51} = 1.66$, $p = 0.196$). Neither risk, infection status, nor infection load significantly affected cumulative $t_{terrestrial}$ (Table S2) or when breaking analysis down by *Bd* growth phases (Table S2). Terrestrial activity of newts differed between *Bd* growth phases, however (Table S3a). Both infected and uninfected newts decreased daily proportional time in terrestrial habitat throughout phase 1 (Fig. 3), with no effect of infection status or load (Table S3b, Fig. 3). In contrast, throughout phase 2 infected newts spent more time out of the water than uninfected newts (Table S3c; Fig. 3a), with newts exhibiting stronger infections spending the most time on the terrestrial habitat (Table S3c, Fig. 3b). Interactions with day for both factors reflect the predominance of these effects at the end of the second phase (Fig. 3).

## Discussion

Our first experiments demonstrated effects of exposure frequency and post-exposure habitat on the course of newt infections, and the findings indicate that discontinuous occupancy of fully aquatic habitats harbouring *Bd* reduces infection risk. While all newts were exposed to an equivalent number of zoospores, breaking the dose into multiple events produced more infections than did a single, intense exposure. Thus, infection risk for newts is not only a function of total zoospores to which newts are exposed (experiment 2; Fig. 2) but also how frequently a newt is exposed to zoospores over time (experiment 1). By extension, continuous and prolonged exposure would be most likely to manifest as increased mortality, and in

393 support of this, a recent study showed how exposing newts constantly to an infected
394 reservoir generated significant mortality (Miaud et al. 2016).

395 Removal from the aquatic environment not only reduced the likelihood that
396 newts contracted infections but also infection intensity and persistence. Despite the
397 known suitability of well-moistened terrestrial substrates to provide adequate moisture
398 for *Bd* (Garner *et al.* 2009b; Farrer *et al.* 2011; Raffel *et al.* 2015), these results
399 suggest that even saturated terrestrial habitats can be less suitable for *Bd* than aquatic
400 habitats, perhaps depending on the type of substrate (e.g. soil versus moss) or the
401 overall resistance of the host species to *Bd* infection. Emergence of infections after
402 returning terrestrial news to aquatic habitats was rare, indicating that the majority of
403 hosts completely cleared their *Bd* infections while in the terrestrial habitat.

404 While theoretical models of *Bd* dynamics have explained the occurrence of
405 low-level *Bd* infections in host populations by assuming low rates of zoospore
406 production (Briggs *et al.* 2010) and high levels of host resistance (Wilber *et al.* 2017),
407 the effects demonstrated in our first experiment suggest that escape (Altizer *et al.*
408 2011) and recovery (Shaw & Binning 2016) from infection during periods of
409 terrestrial activity could also generate these patterns in semi-terrestrial hosts.
410 Accounting for periods that newts spend outside of aquatic habitat, our experiments
411 generated infection patterns that were consistent with patterns in two populations of
412 wild newts, emphasizing the ecological relevance of our experimental infections. In
413 light of this overlap between the distributions of field and laboratory infection loads,
414 we propose that routine habitat switching by newts is a likely driver of *Bd* dynamics
415 in natural populations. Future work can test this hypothesis by considering factors not
416 tested in this study, such as prior infection history and social behaviours in aquatic
417 versus terrestrial habitats.

418 The effects of within-season habitat switching may also have implications for
419 community-scale host-parasite dynamics. Theory predicts that the persistence of
420 multi-host parasites like *Bd* is dictated by the contribution of all host species to
421 parasite reproduction (Fenton *et al.* 2015). Although newts are a dominant species at
422 our sites, our findings indicate that their fluctuating occupancy of aquatic habitats
423 lessens the actual contribution of this host to the maintenance of *Bd* in the host species
424 community. Furthermore, partial or full clearances of infection during periods of
425 terrestrial activity detract from the pool of aquatic zoospores available to infect other
426 hosts. As such, we expect that spillover transmission from alternative fully-aquatic
427 hosts, like the midwife toad tadpoles used as a positive control in our experiments, is
428 important for maintaining *Bd* in communities with adult alpine newts.
429 Although terrestrial habitats may provide a refuge for newts to escape *Bd*
430 infection, our second experiment indicated that newts do not actively avoid becoming
431 infected but may modulate time in aquatic habitats containing infective *Bd* zoospores
432 once infections proliferate. These findings support growing evidence that parasites
433 influence daily activities of hosts and sheds new light on the topic: rather than the
434 level of infection risk or even the infection status of hosts (infected vs. uninfected), in
435 certain conditions host decision-making in parasitized habitats may be best explained
436 by the intensity of infections. Such latent changes in habitat use could be indicative of
437 threshold infection levels for parasite detection by the host, or alternatively could arise
438 from costs of avoiding parasitized habitats. For example, habitats less suitable for
439 parasites may pose heighted risk of predation (Raffel *et al.* 2010). Additionally, for
440 many animals, habitats posing high infection risk also provide essential resources for
441 reproduction and foraging. In the case of newts, fully aquatic habitats are required for
442 mating and offspring development. Since *Bd*-induced mortality appears to be a

443 function of infection loads rather than infection status in various amphibian species
444 (Stockwell, Clulow & Mahony 2010; Wilber *et al.* 2017), and since newts can reduce
445 or even remove infections by switching to adjacent terrestrial habitat (as demonstrated
446 in Experiment 1), the reproductive and energetic consequences of avoiding *Bd*
447 exposure may be more costly than becoming infected. Given the conflicts that can
448 arise from avoiding parasite exposure, and since most parasite infections do not
449 deterministically lead to death, load-dependent rather than risk-dependent adjustments
450 in routine habitat use may be an expected strategy for many wildlife species.
451
452 **Conclusions**
453 Habitats comprising natural animal populations are rarely homogeneous, and
454 ecologists widely acknowledge that individuals vary in routine use of different
455 habitats (Van Dyck & Baguette 2005). Far less is known about how this potential
456 variation in abiotic and biotic factors may affect parasitism. Our results suggest that
457 hosts whose occupancy of parasitized habitats fluctuates on a routine basis face
458 reduced risks of potentially lethal infections. Disease models that neglect short-term
459 fluctuations in host occupancy may therefore overestimate the direct impact of
460 parasites in host populations. Nevertheless, our findings that habitat switching is
461 influenced by parasite loads emphasize that non-lethal effects of parasites may still
462 occur in hosts that show limited disease symptoms and in certain contexts may depend
463 more strongly on infection proliferation than infection risk.
464
465 **Ethical Statement**
466 All experimental work and treatment with itraconazole was approved by the
467 Zoological Society of London's Ethics Committee before commencement and

licensed by the Home Office (PPL 80/2466 to Garner, PIL 70/25118 to Daversa). Field surveys at our Spanish field sites were conducted with permission from the governing department for the Environment of Comunidad de Madrid and in accordance with Park regulations. Field surveys in the United Kingdom were carried out with permission of the landowners.

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**Author Contributions**

DRD formulated the hypothesis. DRD, AM, JB, JJ and TWJG designed the experiments. DRD executed the experiments. DRD, JJ and AM analyzed the data. DRD wrote the initial manuscript, which was revised according to the comments of AM, TWJG, JJ and JB.

**Conflict of interest**

The authors have no conflicts of interest

**Data accessibility**

493 Data and codes are archived in Dryad repository.

494

495 **References**


496 Altizer, S., Bartel, R. & Han, B.A. (2011) Animal migration and infectious disease
497 risk. *Science*, **331**, 296–302.

498 Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and*
499 *Control*. Oxford University Press, Oxford, UK.

500 Becker, C.G., Rodriguez, D., Toledo, L.F., Longo, A.V., Lambertini, C., Correa, D.T.,
501 Leite, D.S., Haddad, C.F.B. & Zamudio, K.R. (2014) Partitioning the net
502 effect of host diversity on an emerging amphibian pathogen. *Proceedings of*
503 *the Royal Society B: Biological Sciences*, **281**, 20141796–20141796.

504 Bielby, J., Cooper, N., Cunningham, A.A., Garner, T.W.J. & Purvis, A. (2008)
505 Predicting susceptibility to future declines in the world's frogs: Predicting
506 amphibian declines. *Conservation Letters*, **1**, 82–90.

507 Bielby, J., Fisher, M.C., Clare, F.C., Rosa, G.M. & Garner, T.W.J. (2015) Host
508 species vary in infection probability, sub-lethal effects, and costs of immune
509 response when exposed to an amphibian parasite. *Scientific Reports*, **5**, 10828.

510 Bosch, J. & Martínez-Solano, I. (2006) Chytrid fungus infection related to unusual
511 mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural
512 Park, Spain. *Oryx*, **40**, 84.

513 Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004) Rapid
514 quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*)

515 in amphibian samples using real-time Taqman PCR assay. *Diseases of aquatic*
516 *organisms*, **60**, 141–148.

517 Briggs, C.J., Knapp, R.A. & Vredenburg, V.T. (2010) Enzootic and epizootic
518 dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the*
519 *National Academy of Sciences*, **107**, 9695–9700.

520 Byers, J.E., Malek, A.J., Quevillon, L.E., Altman, I. & Keogh, C.L. (2015) Opposing
521 selective pressures decouple pattern and process of parasitic infection over
522 small spatial scale. *Oikos*, **124**, 1511–1519.

523 Denoel, M. (2004) Terrestrial versus aquatic foraging in juvenile alpine newts
524 (*Triturus alpestris*). *EcoScience*, **11**, 404–409.

525 Doddington, B.J., Bosch, J., Oliver, J.A., Grassly, N.C., Garcia, G., Schmidt, B.R.,
526 Garner, T.W. & Fisher, M.C. (2013) Context-dependent amphibian host
527 population response to an invading pathogen. *Ecology*, **94**, 1795–1804.

528 Farrer, R.A., Weinert, L.A., Bielby, J., Garner, T.W.J., Balloux, F., Clare, F., Bosch,
529 J., Cunningham, A.A., Weldon, C., du Preez, L.H., Anderson, L., Pond,
530 S.L.K., Shahar-Golan, R., Henk, D.A. & Fisher, M.C. (2011) Multiple
531 emergences of genetically diverse amphibian-infecting chytrids include a
532 globalized hypervirulent recombinant lineage. *Proceedings of the National*
533 *Academy of Sciences*, **108**, 18732–18736.

534 Fenton, A., Streicker, D.G., Petchey, O.L. & Pedersen, A.B. (2015) Are all hosts
535 created equal? Partitioning host species contributions to parasite persistence in
536 multihost communities. *The American Naturalist*, **186**, 610–622.

537 Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L.
538 & Gurr, S.J. (2012) Emerging fungal threats to animal, plant and ecosystem
539 health. *Nature*, **484**, 186–194.

540 Garner, T.W.J., Garcia, G., Carroll, B. & Fisher, M.C. (2009a) Using itraconazole to
541 clear *Batrachochytrium dendrobatidis* infection, and subsequent
542 depigmentation of *Alytes muletensis* tadpoles. *Diseases of Aquatic Organisms*,
543 **83**, 257–260.

544 Garner, T.W.J., Walker, S., Bosch, J., Leech, S., Marcus Rowcliffe, J., Cunningham,
545 A.A. & Fisher, M.C. (2009b) Life history tradeoffs influence mortality
546 associated with the amphibian pathogen *Batrachochytrium dendrobatidis*.
547 *Oikos*, **118**, 783–791.

548 Heard, G.W., Thomas, C.D., Hodgson, J.A., Scroggie, M.P., Ramsey, D.S.L. &
549 Clemann, N. (2015) Refugia and connectivity sustain amphibian
550 metapopulations afflicted by disease. *Ecology Letters*, **18**, 853–863.

551 Hoch, T., Monnet, Y. & Agoulon, A. (2010) Influence of host migration between
552 woodland and pasture on the population dynamics of the tick *Ixodes ricinus*: a
553 modelling approach. *Ecological Modelling*, **221**, 1798–1806.

554 Hoodless, A.N., Kurtenbach, K., Nuttall, P.A. & Randolph, S.E. (2002) The impact of
555 ticks on pheasant territoriality. *Oikos*, **96**, 245–250.

556 Hoverman, J.T. & Searle, C.L. (2016) Behavioural influences on disease risk:
557 implications for conservation and management. *Animal Behaviour*, **120**, 263–
558 271.

559 Kirshtein, J., Anderson, C., Wood, J., Longcore, J. & Voytek, M. (2007) Quantitative
560 PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and
561 water. *Diseases of Aquatic Organisms*, **77**, 11–15.

562 Kolby, J.E., Ramirez, S.D., Berger, L., Richards-Hrdlicka, K.L., Jocque, M. &
563 Skerratt, L.F. (2015) Terrestrial dispersal and potential environmental
564 transmission of the amphibian chytrid fungus *(Batrachochytrium*
565 *dendrobatidis)*. *PLOS ONE*, **10**, e0125386.

566 Kopecky, O., Vojar, J. & Denoël, M. (2010) Movements of alpine newts (*Mesotriton*
567 *alpestris*) between small aquatic habitats (ruts) during the breeding season.
568 *Amphibia-Reptilia*, **31**, 109–116.

569 Kriger, K.M. & Hero, J.-M. (2007) The chytrid fungus *Batrachochytrium*
570 *dendrobatidis* is non-randomly distributed across amphibian breeding habitats.
571 *Diversity and Distributions*, **13**, 781–788.

572 Leon, A.E. & Hawley, D.M. (2017) Host responses to pathogen priming in a natural
573 songbird host. *EcoHealth*. (in press)

574 Luquet, E., Garner, T.W.J., Léna, J.-P., Bruel, C., Joly, P., Lengagne, T., Grolet, O. &
575 Plénet, S. (2012) Genetic erosion in wild populations makes resistance to a
576 pathogen more costly. *Evolution*, **66**, 1942–1952.

577 McMahon, T.A., Sears, B.F., Venesky, M.D., Bessler, S.M., Brown, J.M., Deutsch,
578 K., Halstead, N.T., Lentz, G., Tenouri, N., Young, S., Civitello, D.J., Ortega,
579 N., Fites, J.S., Reinert, L.K., Rollins-Smith, L.A., Raffel, T.R. & Rohr, J.R.
580 (2014) Amphibians acquire resistance to live and dead fungus overcoming
581 fungal immunosuppression. *Nature*, **511**, 224–227.

582 Miaud, C., Dejean, T., Savard, K., Millery-Vigues, A., Valentini, A., Curt Grand
583 Gaudin, N. & Garner, T.W.J. (2016) Invasive North American bullfrogs
584 transmit lethal fungus *Batrachochytrium dendrobatidis* infections to native
585 amphibian host species. *Biological Invasions*, **18**, 2299–2308.

586 Minting, P.J. (2012) *An Investigation into the Effects of* Batrachochytrium
587 Dendrobatidis (Bd) *on Natterjack Toad (*Bufo Calamita*) Populations in the*
588 *UK*. University of Sussex, Falmer, Brighton, UK.

589 Moore, J. (2002) *Parasites and the Behavior of Animals*. Oxford University Press.

590 Morgan, E.R., Medley, G.F., Torgerson, P.R., Shaikenov, B.S. & Milner-Gulland, E.J.
591 (2007) Parasite transmission in a migratory multiple host system. *Ecological*
592 *Modelling*, **200**, 511–520.

593 Ohst, T., Gräser, Y., Mutschmann, F. & Plötner, J. (2011) Neue erkenntnisse zur
594 gefährdung Europäischer amphibien durch den hautpilz *Batrachochytrium*
595 *dendrobatidis*. *Zeitschrift für Feldherpetologie*, **18**, 1–17.

596 Parratt, S.R., Numminen, E. & Laine, A.-L. (2016) Infectious disease dynamics in
597 heterogeneous landscapes. *Annual Review of Ecology, Evolution, and*
598 *Systematics*, **47**, 283–306.

599 Piotrowski, J.S., Annis, S.L. & Longcore, J.E. (2004) Physiology of
600 *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians.
601 *Mycologia*, **96**, 9–15.

602 Raffel, T.R., Halstead, N.T., McMahon, T.A., Davis, A.K. & Rohr, J.R. (2015)
603 Temperature variability and moisture synergistically interact to exacerbate an

epizootic disease. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20142039–20142039.

Raffel, T.R., Hoverman, J.T., Halstead, N.T., Michel, P.J. & Rohr, J.R. (2010) Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology*, **91**, 1900–1907.

Rasmussen, C., Eisenberg, T., Alfermann, D. & Köhler, J. (2012) Presence of *Batrachochytrium dendrobatidis* in amphibians from central and southern Hesse, central Germany: Results from a preliminary regional screening. *Salamandra*, **48**, 166–172.

Rohani, P., Keeling, M.J. & Grenfell, B.T. (2002) The interplay between determinism and stochasticity in childhood diseases. *The American Naturalist*, **159**, 469–481.

Shaw, A.K. & Binning, S.A. (2016) Migratory recovery from infection as a selective pressure for the evolution of migration. *The American Naturalist*, **187**, 491–501.

Stockwell, M.P., Clulow, J. & Mahony, M.J. (2010) Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus: Host species determines outcome of chytrid exposure. *Animal Conservation*, **13**, 62–71.

Todd, B.D. (2007) Parasites lost? An overlooked hypothesis for the evolution of alternative reproductive strategies in amphibians. *The American Naturalist*, **170**, 793–799.

626 Van Dyck, H. & Baguette, M. (2005) Dispersal behaviour in fragmented landscapes:
627 Routine or special movements? *Basic and Applied Ecology*, **6**, 535–545.

628 Weddeling, K., Hachtel, M., Sander, U. & Tarkhnishvili, D. (2004) Bias in estimation
629 of newt population size: a field study at five ponds using drift fences, pitfalls
630 and funnel traps. *Herpetological Journal*, **14**, 1–8.

631 Wilber, M.Q., Knapp, R.A., Toothman, M. & Briggs, C.J. (2017) Resistance,
632 tolerance and environmental transmission dynamics determine host extinction
633 risk in a load-dependent amphibian disease. *Ecology Letters*.

634 Wilson, K., Bjørnstad, O.N., Dobson, A.P., Merler, S., Poglayen, G., Randolph, S.E.,
635 Read, A.F. & Skorping, A. (2002) Heterogeneities in macroparasite infections:
636 patterns and processes. *The ecology of wildlife diseases*, 6–44.

637 Winandy, L., Darnet, E. & Denoël, M. (2015) Amphibians forgo aquatic life in
638 response to alien fish introduction. *Animal Behaviour*, **109**, 209–216.

639 Wood, L.R., Griffiths, R.A. & Schley, L. (2009) Amphibian chytridiomycosis in
640 Luxembourg. *Bulletin de la Société des naturalistes luxembourgeois*, **110**,
641 109–114.

642

643 **Figures and Tables**

644 **Fig. 1.** The overall proportion of infected newts **(a)** and mean (± Standard Error) *Bd*
645 load **(b)** among aquatic (black boxes) and terrestrial (green boxes) newts after either a
646 frequent exposure or intense exposure in Experiment 1. Frequent exposure consisted
647 of three low-concentration exposure events (days 1, 7, 14), and intense exposure
648 consisted of a single exposure (day 1) that was three times the concentration
649 administered to frequently exposed newts. Total exposure dose was therefore equal
650 across exposure treatments.

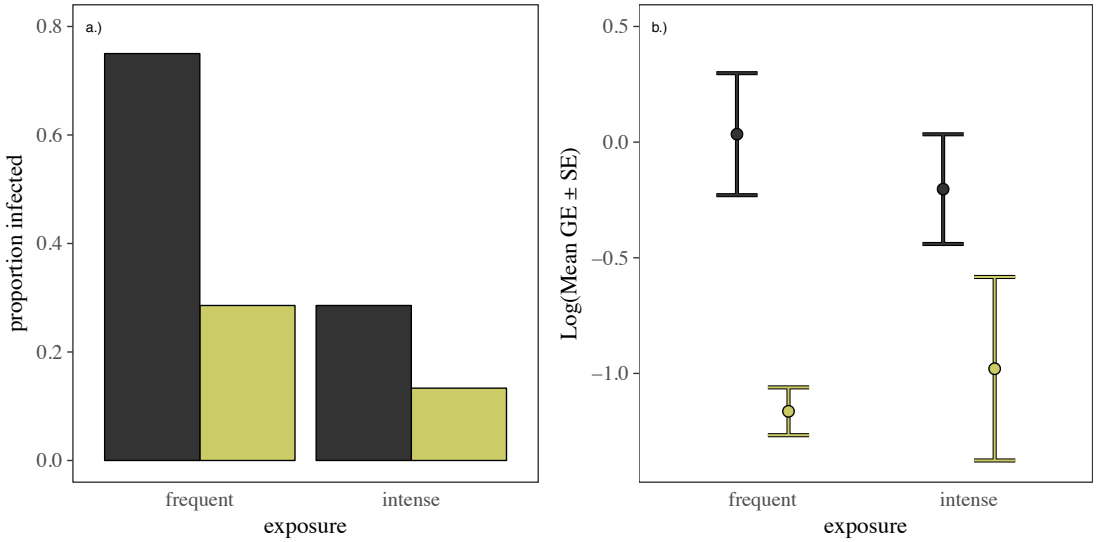
651 **Fig. 2. a.)** Overall prevalence of *Bd* infection and **b.)** infection levels of infected
652 newts exhibited on day 9 of experiment 2 exposure to a low concentration (white
653 bars) or a high concentration (grey bars) release of active *Bd* zoospores into aquatic
654 habitat on days 1-7. Error bars denote the standard error about the mean.

655 **Fig 3.** The mean proportion of recording time that newts occupied terrestrial habitat as
656 opposed to aquatic habitat throughout the seven days of our second experiment, with
657 newts distinguished by a.) infection status and b.) infection intensity exhibited on day
658 9. "Weak infections" (white bars) denote those of less than 15 GE and "strong
659 infections" (black bars) denote those of 15 GE or higher (though infection intensity
660 was a treated as a continuous explanatory variable in data analyses). Error bars
661 indicate the standard errors about the means (points).

662

663

664 Fig. 1

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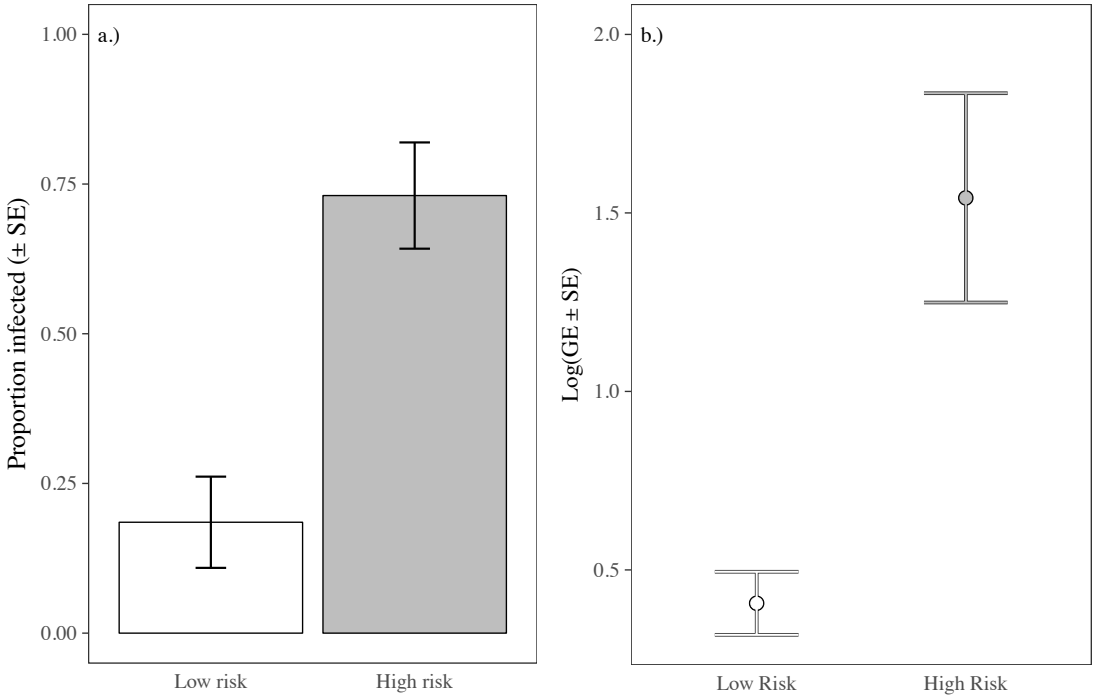
666 Fig. 2

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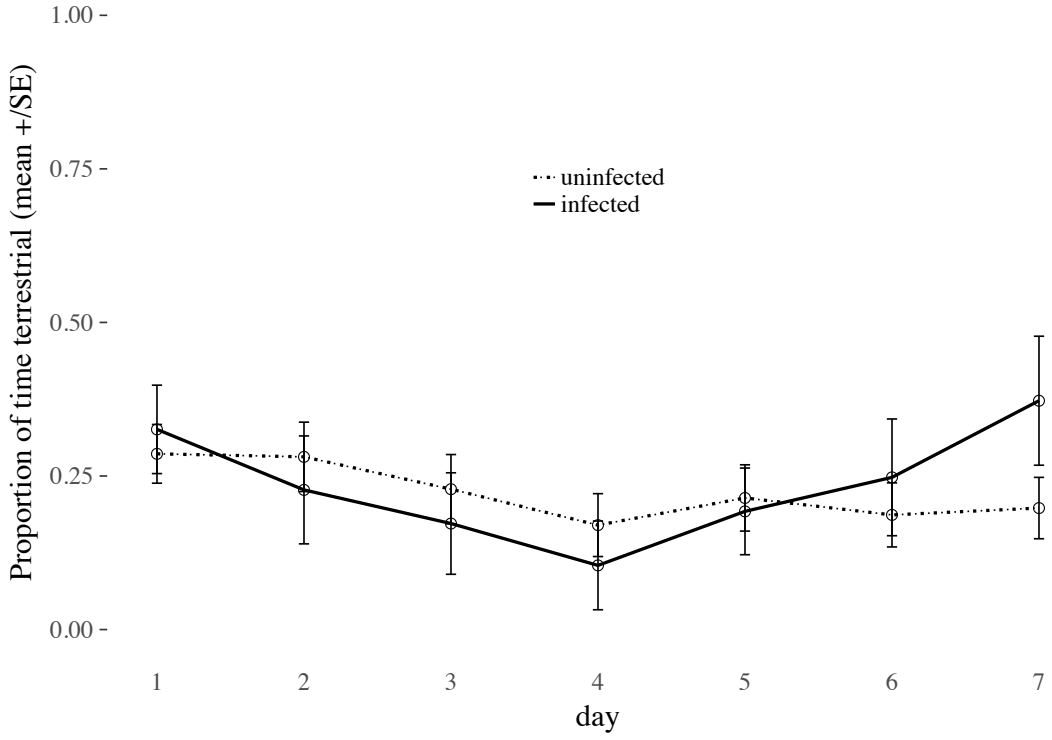
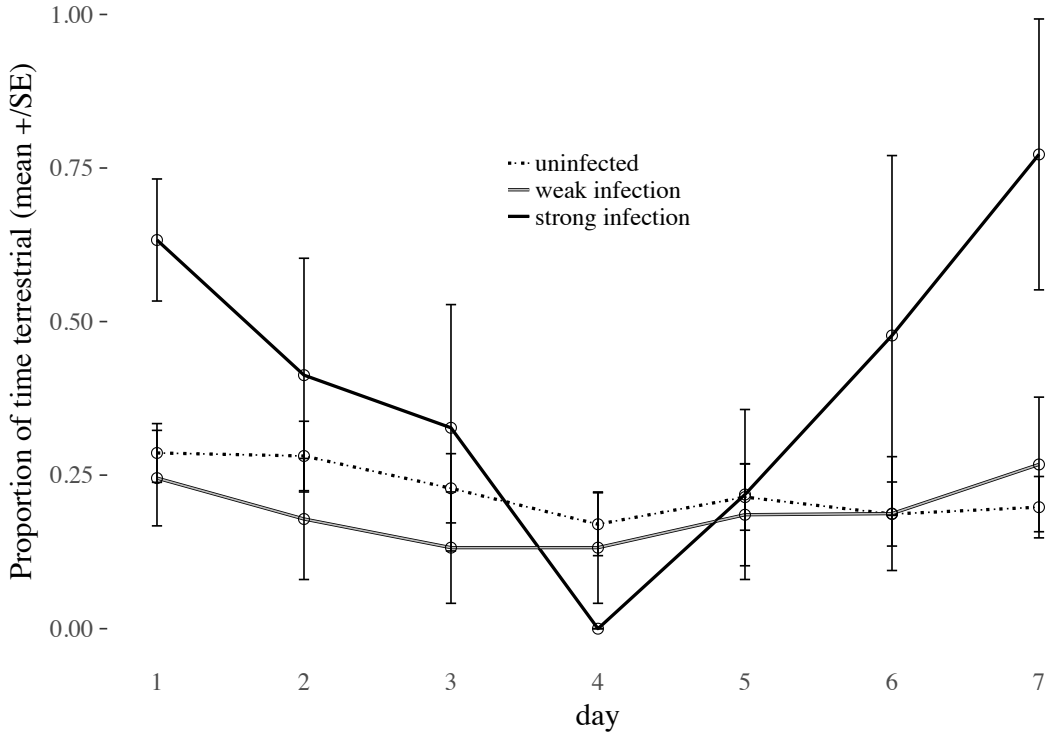
670 Fig. 3

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Supporting Information for:

*Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite*

DR Daversa, A Manica, J Bosch, JW Jolles, and TWJ Garner

## Methods

### *Experiment 1*

Husbandry

Male newts were used for the experiment to control for any sex-specific differences in behaviour and infection. Prior to experiments we cohoused newts aquatically according to collection site and fed newts an equal mixture of earthworms and frozen bloodworms twice weekly, making sure to include a feeding 1 day prior to transferring newts into experimental containers.

Parasite detection (further details)

We quantified the amount of *Bd* DNA on each swab in duplicate using qPCR diagnostics, appropriate negative controls (Boyle *et al.* 2004) and 4 concentration standards serving as positive controls. *Bd* standards used in qPCR assays were produced in-house using the same strain as in the infection experiment. We had previously run IPCs on other newt samples to assess PCR inhibition, including samples from the Cornish sites where experimental newts were collected, and saw no shift in the CT values between controls and spiked extractions (i.e. no signal of inhibition). Therefore, we did not include Internal Positive Controls (IPCs) in analyses of experiment samples.

*Experiment 2*

Husbandry

Male newts were used for the experiment to control for any sex-specific differences in behaviour and infection. Prior to experiments we cohoused newts aquatically according to collection site and fed newts an equal mixture of earthworms and frozen bloodworms twice weekly, making sure to include a feeding 1 day prior to transferring newts into experimental containers.

Image processing

When analyzing image data for experiment 2 we omitted images during the following periods when webcam alignment did not afford a clear view of the aquatic habitat: batch 1, day 1 – 0700 – 1100 hrs; batch 2, day 3 - 1130 – 13:15, day 4, - 13:16 – 2000 hrs, day 5 - 600-1130 hrs; batch 3, day 3 – 1220 – 2000 hrs, day 4- 0600 – 1115 hrs).

## Results

*Experiment 1*

Size and weight statistics

The size (snout-to-vent length) and weight of newts did not covary among the three exposure treatments (size one-way ANOVA: control mean = 4.40 ± 0.6 cm; frequent exposure mean = 4.6 ± 0.6 cm; intense exposure mean = 4.41 ± 0.10 cm; $F_{2, 84} = 2.23$; $p = 0.114$; weight one-way ANOVA: control mean ± standard error (SE) = 1.97 ± 0.07 g; frequent exposure mean = 2.19 ± 0.08 g; intense exposure mean = 2.08 ± 0.08 g; $F_{2, 84} = 1.95$, $p = 0.148$) or between terrestrial and aquatic newts (size one-way ANOVA: aquatic mean = 4.47 ± 0.05 cm; terrestrial mean = 4.47 ± 0.07 cm; $F_{1,84} = 0.00$; $p = 0.960$; weight one-way ANOVA: aquatic mean = 2.00 ± 0.06 g, terrestrial mean = 2.16 ± 0.07 g, $F_{1, 84} = 2.992$ , $p = 0.087$).

Weekly analysis

Effects of exposure treatment on infection prevalence varied over the week of the experiment (dropping exposure treatment:day interaction reduced goodness of fit: $\chi^2_1 = 12.56$; $p < 0.001$, with frequently exposed newts being increasingly likely to develop infections (Fig. S4). Infection loads did not differ between frequently exposed newts and intensely exposed newts during any week of the experiment (no effect from dropping treatment:day: $F_{1,\ 19.63} = 0.00$; p = 0.976, nor from dropping treatment: $F_{1,\ 22.54} = 0.23$; p = 0.637).

Habitat also influenced infection prevalence each week of the experiment (no effect from dropping habitat:dose: $\chi^2_1 = 1.11$; $p = 0.293$, but dropping habitat as fixed effect reduced goodness of fit: $\chi^2_1 = 6.16$; $p = 0.013$), with terrestrial newts consistently exhibiting fewer infections than aquatic newts (Fig. S4). Terrestrial newts also consistently sustained lower weekly infection loads (no effect from dropping habitat:day: $F_{1,\ 26.63} = 0.95$; p = 0.338, but dropping habitat as a fixed effect reduced goodness of fit: $F_{1,\ 18.90} = 19.92$; p < 0.001).

Statistical analyses testing effects of habitat and exposure on *Bd* loads with outlier newt omitted

One aquatic newt in the frequent exposure exhibiting *Bd* loads orders of magnitude higher than those observed in other animals on day 21 (ID = L11, Fig. S4). Since *Bd* loads tend to be overdispersed, we kept this newt in the reported analysis. However, this individual acts as a statistical outlier in our analyses. Below are results from tests of the effects on the overall and weekly infections in newts with the individual

748 removed. The results do not qualitatively differ from the analysis with the newt
749 included:
750 Newts frequently exposed to *Bd* were more likely to develop infections than
751 newts exposed to a single, intense dose (GLM with dose as fixed effect performed
752 significantly better than null models, $\chi^2_1 = 5.21$; p = 0.022; Fig. 1a). Neither the
753 overall mean *Bd* load (intense GE = 0.67 ± 0.31; frequent GE = 0.80 ± 0.31; GLM,
754 $F_{1,15} = 0.02$; p = 0.880) nor maximum *Bd* load (intense GE = 1.52 ± 0.59; frequent GE
755 = 1.46 ± 0.47; GLM, $F_{1,15} = 0.51$; p = 0.485) of infected individuals differed among
756 exposure treatments. Infected terrestrial newts developed weaker infections, both in
757 terms of average loads (aquatic GE = 1.09 ± 0.29; terrestrial GE = 0.1 ± 0.03; GLM,
758 $F_{1,15} = 15.53$; $p < 0.001$; Fig. 1b) and maximum *Bd* loads (aquatic GE = 2.21 ± 0.45;
759 terrestrial GE = 0.24 ± 0.10; $F_{1,15} = 22.46$; $p < 0.001$) when compared to fully
760 aquatic newts.
761 Effects of exposure treatment on infection prevalence varied over the week of
762 the experiment (dropping exposure treatment:day interaction reduced goodness of fit:
763 $\chi^2_1 = 12.03$; $p < 0.001$, with frequently exposed newts being increasingly likely to
764 develop infections (Fig. S4). Weekly infection loads did not differ between
765 frequently exposed newts and intensely exposed newts (no effect from dropping
766 treatment:day: $F_{1, 7.84} = 0.59$; p = 0.465, nor from dropping treatment: $F_{1, 19.67} = 0.00$; p
767 = 0.966).
768 Habitat also influenced infection prevalence each week of the experiment (no
769 effect from dropping habitat:day: $\chi^2_1 = 1.13$; $p = 0.288$, but dropping habitat as fixed
770 effect reduced goodness of fit: $\chi^2_1 = 4.88$; $p = 0.027$), with terrestrial newts
771 consistently exhibiting fewer infections than aquatic newts (Fig. S4). Terrestrial
772 newts also sustained lower infection loads each week of the experiment (no effect

773 from dropping habitat:day: $F_{1, 20.65} = 0.02$; p = 0.897, but dropping habitat as fixed
774 effect reduced goodness of fit: $F_{1, 17.01} = 23.90$; p = <0.001).
775
776 *Experiment 2*
777 Size and weight statistics
778 Neither newt size nor weight varied across treatments at the start of the experiment
779 (size One-Way ANOVA mean ± Standard Error (SE): control = 4.66 ± 0.05 cm, low
780 risk = 4.61 ± 0.06 cm, high risk = 4.63 ± 0.05 cm, $F_{2, 80} = 0.205$; p = 0.815; weight
781 one-way ANOVA mean ± SE: control = 2.7 ± 0.1 g, low risk = 2.65 ± 0.12 g, high
782 risk = 2.68 ± 0.11 g, $F_{2, 80} = 0.097$; p = 0.907) or at the end of the experiment (size
783 one-way ANOVA mean ± Standard Error (SE): control = 4.66 ± 0.05 cm, low risk =
784 4.55 ± 0.07 cm, high risk = 4.64 ± 0.05 cm, $F_{2, 80} = 1.17$; p = 0.317; weight one-way
785 ANOVA mean ± SE: control = 2.29 ± 0.09 g, low risk = 2.36 ± 0.12 g, high risk =
786 2.30 ± 0.09 g, $F_{2, 80} = 0.124$; p = 0.884).
787

## Figures and tables

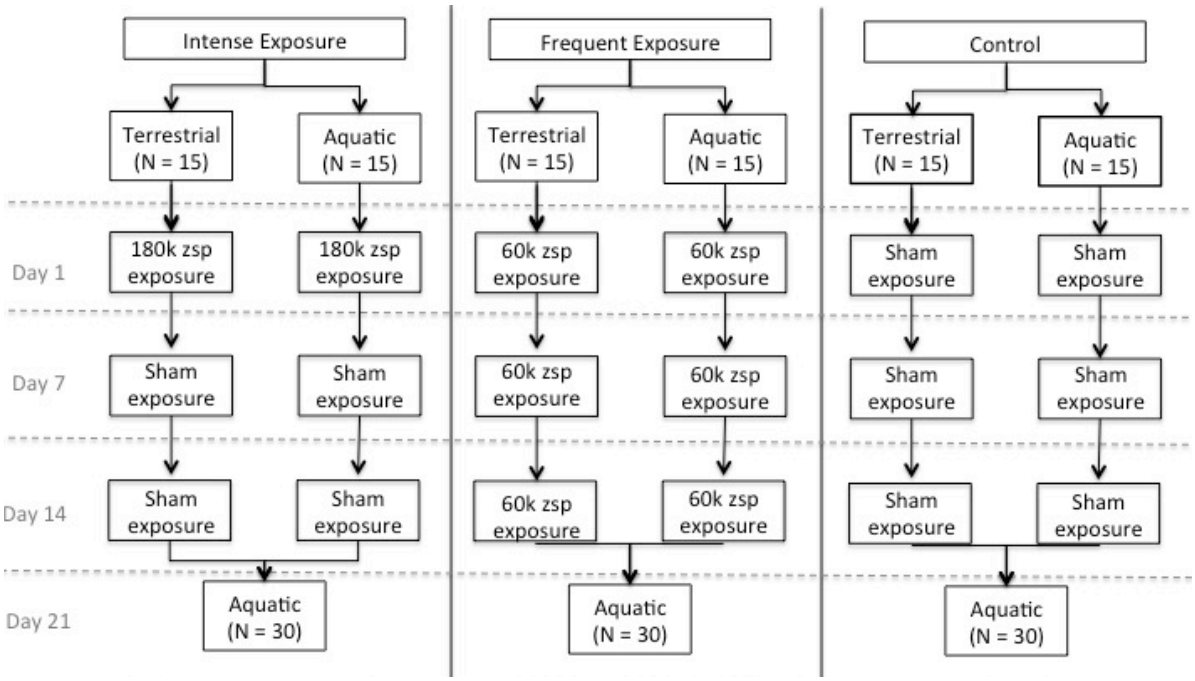


**Fig. S1.** A schematic of the design of experiment 1 is shown (zsp = *Bd* zoospores). Newts were randomly assigned to one of the following exposure treatments: intense exposure, frequent exposure, or control. Within each exposure treatment, half of the newts were housed in wet terrestrial containers when not being exposed, while the other half were housed in aquatic containers that differed from exposure containers. All terrestrial newts were returned to aquatic containers on day 21 where they were held for one week. The sham dose consisted of liquid media.

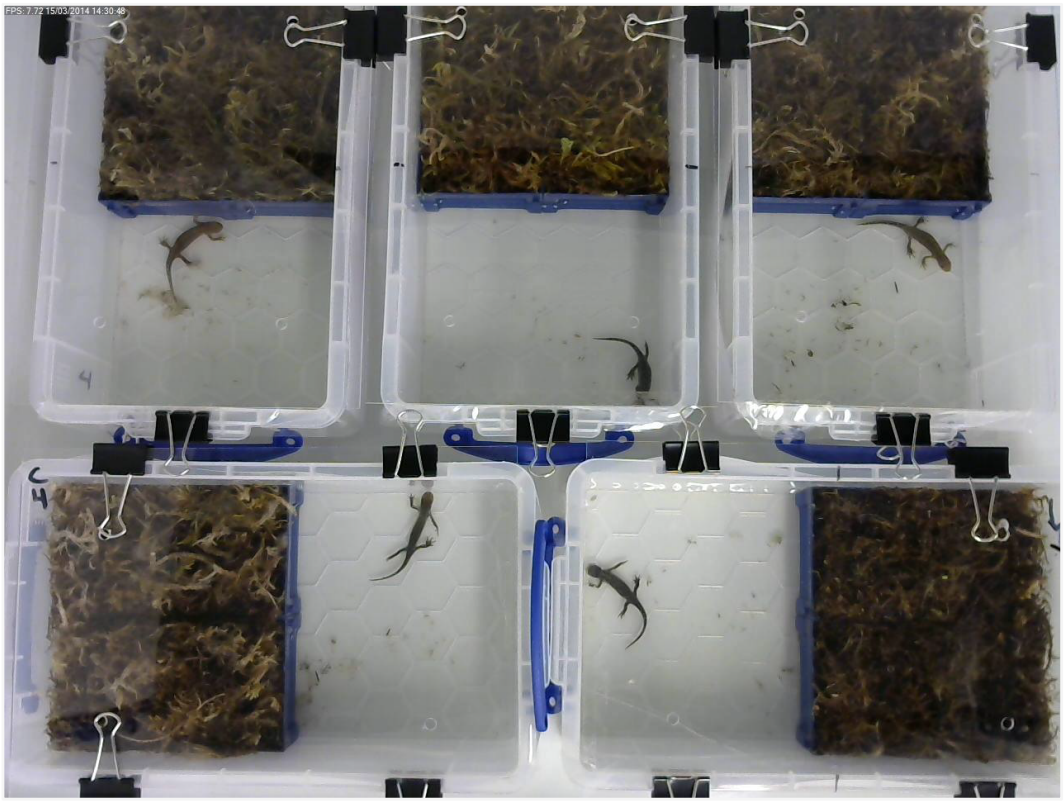

**Fig. S2:** Image captured from a webcam installed above a block of containers in experiment 2. All newts in the image are using the aquatic portion of the container (clear section) and had access to equal amounts of terrestrial habitat (brown section). Pilot observations confirmed that newts were able to freely move between the two habitats (Daversa and Garner, personal observation).

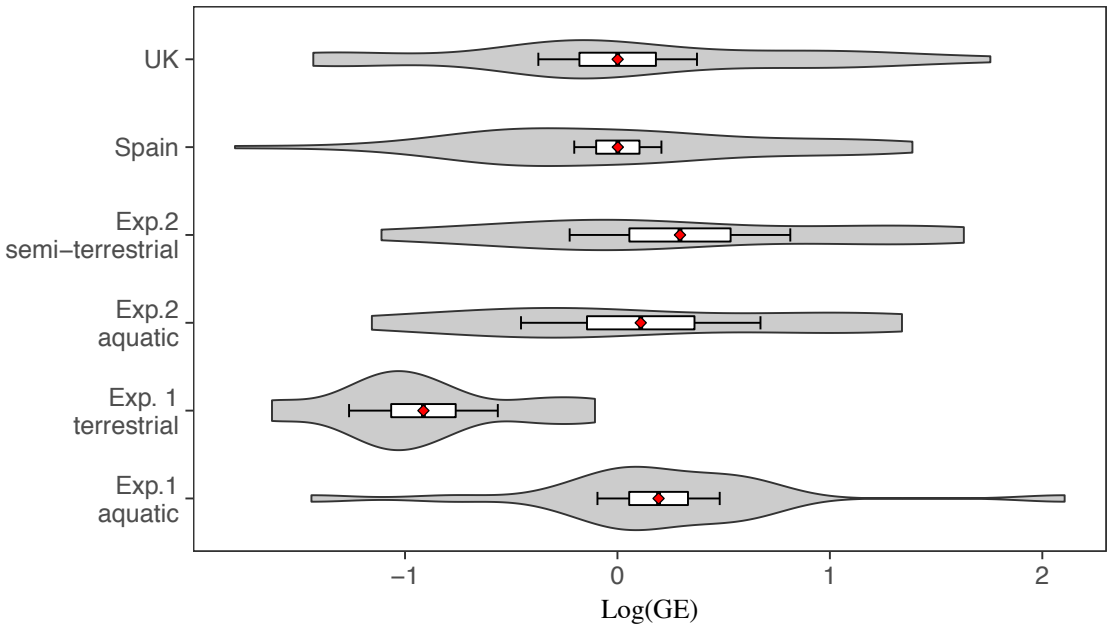


**Fig. S3.** The distribution of log-transformed infection loads for *Bd*-positive samples collected from wild newt populations in Cornwall, United Kingdom (N = 23) and Madrid, Spain (N = 49) compared to aquatic newts (N = 29) and terrestrial newts (N = 29) in experiment 1 and newts that remained fully aquatic (N = 11) or were semi-terrestrial (N = 13) in experiment 2. Red points indicate the mean load. Boxplots denote the standard error about the mean, with error bars denoting the 95% confidence intervals. The violin plots (grey) denote distributions of infection loads and their probability densities.

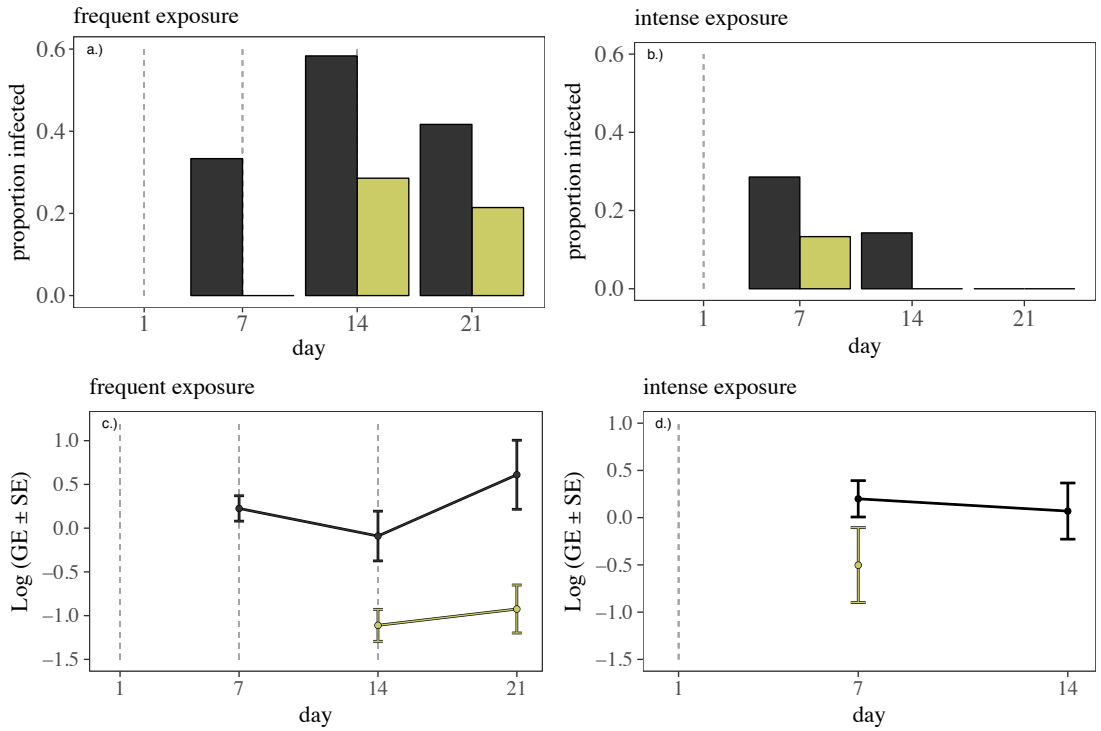


**Fig. S4.** The weekly prevalence of infection **(a, b)** and the $\log_{10}$ weekly mean *Bd* load (± Standard Error) **(c,d)** among aquatic newts (black bars and lines) and terrestrial newts (green bars and lines) throughout a frequent exposure **(a, c)** or after an intense exposure **(b, d)** in Experiment 1. Grey dashed lines denote days when newts were exposed to *Bd*.

| | | | Day of Experiment | | | |
|---|---|---|---|---|---|---|
| **Exposure** | **Habitat** | **ID** | **7** | **14** | **21** | **28** |
| Intense | *Aquatic* | H02 | 3.33 | 0.59 | | |
| | | H04 | 0.67 | | | |
| | | H08 | | | | |
| | | H09 | 0.60 | | | |
| | | H10 | 3.39 | 2.33 | | |
| | | H13 | 0.92 | | | |
| | *Terrestrial* | H16 | 0.13 | | | |
| | | H17 | 0.78 | | | |
| Frequent | *Aquatic* | L01 | | 2.02 | 4.79 | |
| | | L02 | 1.29 | | | |
| | | L03 | 4.50 | 4.57 | 1.17 | |
| | | L06 | 1.33 | 1.22 | | |
| | | L10 | | 1.08 | | |
| | | L11 | 1.03 | 0.04 | 127.38 | 31.44 |
| | | L12 | | 0.17 | | 2.30 |
| | | L13 | | 3.12 | 0.85 | 0.08 |
| | | L14 | | 0.00 | 1.86 | 2.21 |
| | *Terrestrial* | L16 | | | | |
| | | L18 | | | 0.42 | |
| | | L22 | | 0.10 | 0.07 | |
| | | L24 | | 0.17 | | |
| | | L25 | | | | 0.13 |
| | | L27 | | 0.02 | | |
| | | L29 | | | | 0.15 |
| | | L30 | | 0.09 | 0.06 | |

**Table S1.** ***Weekly Bd load of infected newts:*** *Bd* load (GE) over time of newts that tested positive for infection at least once in the experiment. Exposures were administered on day 1,7,and 14. Swabs were collected just before exposures on day 7, 14, and 21 as well as 7 d following the return of terrestrial newts to aquatic habitat (day 28). Blank boxes indicate no infection. The newt highlighted in gray carried outlying *Bd* loads. A version of the statistical analysis with this newt omitted is given in the Supplementary material methods section, above.

| **a.) Overall** | | | | | |
|---|---|---|---|---|---|
| Factor | *df* | *residual df* | *deviance* | *F* | *p* |
| risk level | 2 | 78 | 8.25 | 1.58 | 0.213 |
| bd status | 1 | 78 | 7.96 | 0.26 | 0.611 |
| bd load | 1 | 78 | 7.96 | 0.30 | 0.438 |
| **b.) Phase 1** | | | | | |
| Factor | *df* | *residual df* | *deviance* | *F* | *p* |
| risk level | 2 | 78 | 10.25 | 1.28 | 0.284 |
| bd status | 1 | 78 | 10.02 | 0.74 | 0.393 |
| bd load | 1 | 78 | 10.04 | 0.96 | 0.329 |
| **c.) Phase 2** | | | | | |
| Factor | *df* | *residual df* | *deviance* | *F* | *p* |
| risk level | 2 | 78 | 9.99 | 0.44 | 0.649 |
| bd status | 1 | 78 | 9.90 | 0.16 | 0.668 |
| bd load | 1 | 78 | 10.04 | 1.27 | 0.663 |

**Table S2.** Likelihood ratio test results (*df* = degrees of freedom) for comparing full models (GLM) of cumulative terrestrial activity of newts with nested models dropping the factors. The cumulative proportion of time that newts spent terrestrially across **a.)** all days, **b.)** in phase 1 and **c.)** in phase 2 was used as the response variable in separate GLMs with the following fixed effects: risk level (zero vs. high vs. low), infection status (infected vs. uninfected), and infection load (GE). Camera block (1-6) was included as a fixed effect (there were too few levels to include it as a random effect) in all models to account for spatial variation in tank positions in the setup.

| **a.) All Days** | | | | |
|---|---|---|---|---|
| *factor* | *numerator df* | *denomenator df* | *F* | *p* |
| **phase** | **1** | **497.00** | **5.33** | **0.021** |
| risk level:phase | 2 | 494.20 | 0.81 | 0.445 |
| risk level | 2 | 75.50 | 1.51 | 0.140 |
| infection status:phase | 1 | 495.16 | 1.31 | 0.251 |
| infection status | 1 | 76.74 | 0.01 | 0.931 |
| infection load:phase | 1 | 495.18 | 0.1147 | 0.735 |
| infection load | 1 | 76.41 | 1.28 | 0.262 |
| **b.) Phase 1** | | | | |
| *factor* | *numerator df* | *denomenator df* | *F* | *p* |
| **day** | **1** | **167.94** | **5.94** | **0.016** |
| risk level:day | 2 | 163.25 | 0.26 | 0.769 |
| risk level | 2 | 75.50 | 1.84 | 0.167 |
| infection status:day | 1 | 163.33 | 1.30 | 0.256 |
| infection status | 1 | 76.74 | 0.08 | 0.774 |
| infection load:day | 1 | 163.69 | 1.77 | 0.185 |
| infection load | 1 | 76.38 | 0.64 | 0.425 |
| **c.) Phase 2** | | | | |
| *factor* | *numerator df* | *denomenator df* | *F* | *p* |
| **day** | **1** | **251.81** | **4.10** | **0.044** |
| risk level:day | 2 | 246.54 | 0.27 | 0.762 |
| risk level | 2 | 75.37 | 1.53 | 0.223 |
| **infection status:day** | **1** | **246.42** | **8.40** | **0.004** |
| **infection load:day** | **1** | **246.85** | **16.44** | **<0.001** |

**Table S3. a)** Kenward-Rogers approximations for comparisons of nested GLMMs with the daily proportion of time that newts spent in terrestrial habitat (square root arcsin-transformed) as the response variable. Owing to the observed dependence on phase, we also performed tests of nested GLMMS of daily terrestrial activity in **b.)** phase 1 and in **c.)** phase 2. Separate GLMMs were run for each predictor variable [risk level (zero, high, low), infection status (infected vs. uninfected), infection load (GE)] to account for small sample sizes. P-values of less than 0.05 (highlighted in

858 bold) indicate a significant reduction in goodness of model fit when the factors were
859 removed. Camera block (1-6) was included as a fixed effect (there were too few
860 levels to include it as a random effect) in all models to account for spatial variation in
861 tank positions in the setup.
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