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EPIDEMIOLOGY OF AN AMPHIBIAN PATHOGEN: DISTRIBUTION OF AN ENDEMIC LINEAGE AND DISTRIBUTION IN AN ENDEMIC ISLAND COMMUNITY

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Abstract

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) causes the amphibian disease chytridiomycosis. This pathogen is a major threat to amphibians throughout the world. The severity of the threat of *Bd* to the host can be related to many factors. We study two factors: pathogen and host genotype (i.e., species). Pathogen genotype may affect the epidemiology of the disease as some recently discovered *Bd* genotypes were found to be hypovirulent. In the first part, we investigate the distribution of one recently discovered lineage of *Bd* in Switzerland by creating lineage-specific primers and sampling sites where *Bd*CH is likely to occur. We were unable to find *Bd*CH anywhere in Switzerland, which leads us to believe that this lineage may be being outcompeted by the hypervirulent global panzootic lineage. Further studies with a larger sample size are needed to confirm this. Host genotype (i.e., species) also determines the effect of *Bd* on the host. Endemic species are known to be highly susceptible to *Bd*. Here we investigate the distribution of *Bd* on the largely endemic amphibian population of Corsica by analyzing *Bd* infection data. We found *Bd* on Corsica to be widely distributed and our risk assessment revealed many vulnerable species. Further monitoring of the amphibian population on Corsica is needed.

27 Résumé

28 Le pathogène fongique *Batrachochytrium dendrobatidis* (*Bd*) est responsable de la
29 chytridiomychose chez les amphibiens. Ce pathogène est une menace majeure pour les
30 amphibiens tout autour de la planète. La sévérité de la menace de *Bd* face à son hôte peut être
31 reliée à plusieurs facteurs. Nous avons étudié deux facteurs: les génotypes du pathogène et de
32 l'hôte (i.e., espèce). Le génotype du pathogène peut influencer l'épidémiologie de la maladie.
33 Certains génotypes de *Bd* hypo virulents ont été découverts récemment. Dans la première
34 partie, nous étudions la distribution d'une lignée de *Bd* récemment découverte en Suisse en
35 créant des amorces spécifiques pourcette lignée et des échantillons ont été prélevés sur des
36 sites où *Bd*CH est probablement présent. Nous n'avons pas pu trouver *Bd*CH en Suisse, ce
37 qui mène à croire que cette lignée doit être supplantée par la lignée panzootique hyper
38 virulente. Des futures études sur un plus grand nombre d'échantillons sont nécessaires afin de
39 le confirmer. Le génotype de l'hôte (i.e., espèce) détermine aussi l'effet de *Bd* sur ce dernier.
40 Les espèces endémiques sont connues comme étant très sensibles à *Bd*. Nous étudions la
41 distribution de *Bd* dans la grande population endémique d'amphibiens de la Corse en
42 analysant les données d'infection de *Bd*. Nous avons trouvé que *Bd* était largement répondu
43 en Corse et notre évaluation des risques a montré, que beaucoup d'espèces étaient
44 vulnérables. Une future surveillance des populations d'amphibiens de la Corse est nécessaire.

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48 Keywords: *Alytes obstetricans*, amphibians, *Batrachochytrium dendrobatidis*, *Bd*CH, *Bufo*
49 *viridis*, chytridiomycosis, Corsica, *Discoglossus montalentii, Discoglossus sardus,* endemic
50 species, *Euproctus montanus*, *Hyla sarda*, lineage, pathogen, *Pelophylax bergeri,*
51 *Salamandra corsica*, Switzerland.

52 Introduction

53 In recent years, pathogens have been found to play an increasing role in host population
54 declines and species extinctions (Smith et al. 2009). The majority of these species extinctions
55 have been cause by fungal pathogens, which are fast becoming a real threat to biodiversity
56 (Fisher et al. 2012). One fungal pathogen in particular, *Batrachochytrium dendrobatidis*
57 (Longcore et al. 1999) (hereafter *Bd*), is known to cause the emerging infectious disease
58 chytridiomycosis. This pathogen is a waterborne obligate parasite of amphibians which can
59 have lethal effects on individual amphibians and can cause mass mortalities within amphibian
60 communities (Berger et al. 1998, 2002, Longcore et al. 1999, Tobler & Schmidt 2010, Fisher
61 & Garner 2007, Kriger & Hero 2007). Berger et al. (1998) first identified the disease in 1998
62 after amphibian mass mortalities in Australia and Central America. Now, it is found on every
63 continent where the hosts are present (www.bd-maps.net) and has been named by some the
64 worst vertebrate pathogen in history (Gascon et al. 2007). This globally-spread disease has
65 been linked to rapid population declines and is responsible for local extinctions in up to 40%
66 of anuran species (Crawford et al. 2010, Skerratt et al. 2007, Lips 1999, Bosch et al. 2001,
67 Walker et al. 2010). As amphibians are already the most threatened vertebrates on the planet
68 (Stuart et al. 2004) the consequences of this emerging infectious disease have been
69 devastating.

70 In Europe, the disease is widespread, although it appears to be irregularly distributed (Bosch
71 et al. 2007). Despite the fact that *Bd* has spread through much of Europe there have been far
72 fewer mass die-offs seen than in Australia, Central and South America (Bosch et al. 2007,
73 Cheng et al. 2011). Prevalence is found to be exceptionally high in Spain (Bosch 2007) and
74 Switzerland (Garner et al. 2005) but mass mortalities have only been seen in a few high
75 altitude locations in Spain, France and Sardinia in Italy (Bosch et al. 2007, Bielby et al.

76 2009). In contrast, in some countries, such as the Netherlands *Bd* does not seem to have
77 caused many negative effects at all (Spitzen-van Der Sluijs et al. 2014).

78 Chytridiomycosis has been established as a major threat to amphibians (Skerratt et al 2007).
79 The severity of this threat is influenced by many different factors related to the parasite: *Bd,*
80 the host amphibian species and environmental conditions (Bielby et al. 2013). Recent
81 research has demonstrated that the genotype of *Bd* may have an important role in the
82 parasite's ability to infect the host (Ferrer et al. 2011, Schoegel et al. 2012). The threat of *Bd*
83 to the host may be determined by the genotype of the *Bd* lineage. *Batrachochytrium*
84 *dendrobatidis* was originally thought to consist of one invariant clone, based on the evidence
85 collected from sites of mass amphibian die-offs (Farrer et al. 2011). In 2011, it was
86 discovered that one lineage of *Bd* was responsible for the mass mortalities around the world,
87 the global panzootic lineage (hereafter *Bd*GPL) (Farrer et al. 2011). At the same time, *Bd*
88 isolates from non-die off sites were found to have deeper phylogenetic structure than was
89 previously thought (Farrer et al. 2011, Schloegel et al. 2012, Goka et al. 2009, Rosenblum et
90 al. 2013). These older, rare lineages appear to be endemic. Many of these endemic lineages
91 have been discovered from sites all over the world; *Bd*CAPE from South Africa and Mallorca
92 (Farrer et al. 2011), *Bd*CH from Switzerland (Farrer et al. 2011), *Bd*-Brazil from Brazil
93 (Schloegel et al. 2012) and the Asian endemic lineages found in China (Bai et al. 2012), India
94 (Dahanukar et al. 2013) and Japan (Goka et al. 2009). Generally, these lineages are not
95 associated with mortalities and have been found to be much older than *BdGPL* (Rosenblum
96 et al 2013, Schloegel et al. 2012, Farrer et al .2011). Furthermore, one study, while doing a
97 direct comparison of *Bd*GPL and an endemic lineage found that *Bd*GPL is much more
98 virulent (Farrer et al. 2011). Many amphibian communities coexist with *Bd* and although this
99 could be due to the species-specific susceptibility of the host or possibly environmental

100 factors, it is now possible that *Bd* genotype may play an important role (Ferrer et al. 2011,
101 Ohst et al. 2013).

102 The threat of *Bd* to its host is also determined by host genotype (i.e., species).
103 Chytridiomycosis affects the host by disrupting the skin's integrity, which in turn affects the
104 uptake of electrolytes and leads to hyperkeratosis and cardiac arrest (Berger et al. 1998,
105 Voyles et al. 2009). Host species are not all threatened equally: the effect of *Bd* on its host is
106 very species-specific. It is not fully understood why some amphibians are resistant, others are
107 tolerant and some die when infected (Fisher et al. 2009). In Europe, *Bd* is spreading but levels
108 of *Bd* seem to differ substantially among its species (Balaz et al. 2014). A recent study has
109 found that Alytidae and Bombinatoridae families consistently showed high prevalence,
110 whereas Ranidae and Salamandridae species did not (Balaz et al. 2014). There is a need to
111 understand the effect *Bd* will have on host communities by identifying particularly
112 susceptible species (Balaz et al. 2014). Studies need to focus on vulnerable species, such as
113 endemic species, which are known to be particularly at risk of *Bd* (Bielby et al. 2009).
114 Endemic species are especially vulnerable due to the fact they often have limited ranges and
115 relatively small population sizes. *Bd* has the ability to spread quickly (Lips et al. 2008) and
116 the limited ranges of many endemic species means the disease could cover an entire range
117 quickly. There are a number of reported cases of extinctions caused by chytridiomycosis
118 among these vulnerable populations (La Marca et al. 2005, Schloegel et al. 2006). Within
119 Europe, one of the first places to have confirmed cases of chytridiomycosis outside Spain was
120 the island of Sardinia, Italy in 2008 (Bovero et al. 2008). Sardinia is home to many endemic
121 and endangered species and the Tyrrhenian painted frog has already suffered extensive
122 mortalities possibly due to *Bd* (Bielby et al. 2009). Interestingly, the neighbouring island of
123 Corsica, which shares similar amphibian communities and habitats with Sardinia, has been
124 the focus of very little research regarding the presence or distribution of *Bd*.

125 This study focuses on two factors that can influence the effects of *Bd* on its host. Firstly, as
126 *Bd* genotype has proven to play an important role in the severity of *Bd*, but studies on
127 endemic lineages are lacking, I will investigate the distribution of one such lineage of *Bd*,
128 isolated from a site in Switzerland (Farrer et al. 2011). In Switzerland, in addition to the
129 global *Bd*GPL lineage, an older lineage, called *Bd*CH has been identified. Switzerland is
130 known to have a high prevalence of *Bd*, but unlike Spain there has been no mass mortalities
131 observed (Tobler et al. 2012). Furthermore, *Bd* was recently identified in a museum sample in
132 Ticino from 1901, which is well before the emergence of *Bd*GPL is suspected (Peyer 2010,
133 Schloegel et al. 2012, Farrer et al. 2011), therefore it would be plausible that older lineages of
134 *Bd* are present. To date the distribution of *Bd*CH is completely unknown and to my
135 knowledge there have been no studies of endemic lineages of *Bd* in Europe. I will investigate
136 the occurrence and distribution of *Bd*CH lineage by surveying *Alytes obstetricans* in Northern
137 Switzerland with the aim of identifying and describing the distribution of *Bd*CH.

138 Secondly, I will investigate the role of host genotype on the threat of *Bd*. I will focus on the
139 amphibian population on the island of Corsica, France, home to a largely endemic population
140 of amphibians. The Corsican fire salamander (*Salamandra corsica*), Corsican brook newt
141 (*Euproctus montanus*), Corsican painted frog (*Discoglossus montalentii*) are all endemic to
142 Corsica; the Corsican painted frog being listed as near-threatened by the IUCN Red List.
143 Tyrrhenian painted frog (*Discoglossus sardus*) and Tyrrhenian tree frog (*Hyla sarda*) are
144 found only in Corsica, Sardinia and Tuscany. The restricted range of these species puts them
145 at particular risk of *Bd*. There has been no assessment of *Bd* in these populations of
146 amphibians on Corsica, but the neighbouring island of Sardinia, which shares closely related
147 and endemic species (*Discoglossus* and *Euproctus*), has a known history of *Bd* and has seen
148 population declines (Bovero et al. 2008, Bielby et al. 2009, Bielby et al. 2013) which leads us
149 to believe the amphibians of Corsica are at risk. Here I will present the first risk assessment

150 of chytridiomycosis on Corsica. To do so, I will analyse *Bd* infection data and determine the
151 geographical and taxonomic distribution of *Bd* on Corsica. I will also compare presence and
152 distribution of *Bd* among the host species with neighbouring Sardinia to generate a risk of
153 chytridiomycosis on Corsica.

154

155

156 Materials & Methods

157 **Review of *Bd* Biology**

158 *Bd* is a member of the chytrid family, which is found primarily in soil and water, usually
159 living as saprobes (Berger et al. 1998). Some chytrids are parastic, but *Bd* is one of only two
160 obligate parasites of vertebrates (Dix & Webster 1995, Fisher et al. 2009). *Bd* is an aquatic
161 fungus with two life stages. The infectious stage is characterized by free-living, swimming
162 flagellated zoospores that move through water to their anuran host and entire the keratinized
163 skin cells and encyst (Longcore et al. 1999, Berger et al. 2005). It then forms a thallus into
164 the host and produces more zoospores. This life cycle takes four to five days but zoospores
165 can survive in water for up to seven weeks (Johnson et al. 2003). *Bd* infection leads to the
166 development of the disease chytridiomycosis.

167 **Switzerland**

168 *Study Sites and Species*

169 To determine the distribution of *Bd*CH in Switzerland we collected field samples from April
170 to July 2014. We focused our survey on sites in and around the canton of Zurich, as *Bd*CH
171 was first discovered and described from a pond near the village of Gamlikon, Zurich in 2007.
172 Sites were also selected based on the presences of *Alytes obstetricans,* the species from which
173 *Bd*CH was isolated. We used KARCH data (www.karch.ch) and unpublished data from the
174 PhD thesis of Ursina Tobler (Tobler 2011) to determine sites that contain *A. obstetricans* and
175 that are known to be *Bd* positive. The range of *A. obstetricans* is restricted to northern
176 Switzerland, therefore the majority of our sites were in the cantons of Zurich, Baselland and
177 Lucerne. We also sampled a site in Ticino since we suspected that there might be older

178 lineages present here as *Bd* was identified from a museum sample from 1901 in Ticino (Peyer
179 2010).

180 In total, samples were collected from ten sites across four cantons (Table 1, Figure 1). Habitat
181 types included: ponds in open meadows, garden ponds, firewater reservoirs, pools, and
182 streams. We collected samples of *A. obstetricans* tadpoles. At site Gamlikon 2, in previous
183 years *A. obstetricans* have been found at this site and tested for *Bd*, furthermore this is the site
184 where *Bd*CH was isolated from, but in 2014 no *A. obstetricans* were found so adult
185 *Ichthyosaura alpestris* (Alpine Newts) were swabbed instead. In Ticino, adults *Pelophylax*
186 *esculentus* were sampled from a large permanent pond.

187 *Sample collection and analysis*

188 Sampling for *Bd*CH was done opportunistically; the first amphibians captured were swabbed.
189 *Alytes obstetricans* were collected with nets and swabbed with sterile swabs (155C Copan,
190 Italy) around the mouth for 40 seconds, because in tadpoles *Bd* is found in only keratinized
191 mouthparts (Vredenburg & Summers 2001, Garner et al. 2005, Hyatt et al. 2007). Swabs of
192 adult *P. esculentus* and *I. alpestris* were taken from the ventral abdomen, pelvis and feet, as
193 this is where zoospores and sporangia are found on the skin (Longcore et al. 1999, Pessier et
194 al. 1999, Green & Sherman 2001, Berger et al. 1999). Standard swabbing protocols were
195 followed (Hyatt et al. 2007). Once samples were taken, the swabs were stored on ice and then
196 frozen at -20°C until extraction. In addition to the samples I collected from Gamlikon 1 site,
197 previously extracted DNA samples of *A. obstetricans* tadpoles, collected from this site in
198 2011 were used to test for *Bd*CH. These samples were collected and DNA was extracted by
199 Dr. Leyla Davis from Zurich University.

200 In total, 112 samples were collected and used for *Bd*CH analysis. In order to test for *Bd*CH
201 we had to first create primers and probe that are specific to this lineage of *Bd*. We extracted
202 the DNA and ran diagnostic qPCR tests for both *Bd* and *Bd*CH. For extractions we used
203 standard *Bd* extraction protocols as per Boyle et al. 2004 with the following modifications:
204 2ml Safe-Lock Eppendorf tubes were used instead of 1.5ml screw top centrifuge tubes for
205 steps 1 to 10. Samples were homogenized in TissueLyser II (Quigen) for 45 seconds, not a
206 Bead beater (Mini-beadbeater-8) for step 4. Once *Bd* DNA was extracted samples were then
207 frozen at -20° C. 1:10 dilutions of DNA samples were then made and ready for the qPCR
208 analysis. Standard *Bd* qPCR protocol was followed (Boyle et al. 2004) with some changes.
209 Namely, we used the Rotorgene qPCR machine for analysis instead of ABI Real Time PCR
210 machine. Preliminary test were done to ensure that the Rotorgene machine worked with the
211 standard ABI machine protocol. The *Bd* qPCR assay uses the species-specific primers ITS1-3
212 Chytr and 5.8S Chytr, in addition to the fluorescently labeled probe Chytr MGB2 to amplify
213 *Bd* ITS-1 and 5.8S regions. DNA standards were diluted to 100, 10, 1 and 0.1 zoospore
214 genome equivalents for use in the Taqman assay. Each reaction well contained 25ul, made up
215 of the master mix with 12.5ul Taqman Universal PCR Mix (Applied Biosystems), 1.25ul of
216 both the forward and reverse primers, 0.0625ul of MGB probe, 0.2ul of Bovine Serum
217 Albumin and 4.9375ul of de-ionized water. 20ul of master mix was added to each well with
218 5ul of 1:10 diluted sample DNA. Amplification conditions were as follows: 50°C for two
219 minutes, 95° C for ten minutes, followed by 50 cycles including 95° C for 15 seconds and 60°
220 C for one minute. For each assay, samples were run in duplicate with standards and negative
221 control with no DNA. A sample was considered positive if both replicates had a clear
222 sigmoid amplification curve. All samples positive for *Bd* were then tested for *Bd*CH.

223 To test for the presence of *Bd*CH, we designed a primer/probe that would be able to
224 distinguish between the different lineages of *Bd*. For this we used mitochondrial DNA, as this

225 is found in high copy number and therefore yields a highly sensitive PCR in comparison to
226 single-copy targets. To design a lineage specific Taqman primer and probe, identification of
227 polymorphic sites that contain *Bd*CH/GPL/CAPE polymorphism were needed. Lineage
228 specific primers were designed against mitochondrial sequence polymorphisms. From all the
229 variants (excluding indels) that are found in all isolates of a given lineage (*Bd*GPL, *Bd*CAPE,
230 *Bd*CH), consensus sequence was created, and then tallied using a non-overlapping window.
231 The windows were sorted in Excel by CH SNPs (max to min), CAPE SNPs (max to min) and
232 GPL SNPs (min to max). Six promising sequences were then extracted from the consensus
233 sequences for each of the lineages by identifying homologous sequences that contained
234 lineage-specific single-nucleotide polymorphisms (SNPs). Candidate sequences were
235 BLASTed to the nuclear genome to confirm they did not match. These candidate sequences
236 were assumed to be able to distinguish between *Bd*GPL and *Bd*CH by the use of TaqMan
237 probes. Bioinformatic work was completed by Rhys Farrer.

238 mtDNA Sequence 1 was selected to design primers and MBG FAM-labelled probe.

239 Primer sequences:

240 MTDNACH_F **GCGCAGCGAAATCATATAAGATACTT**

241 MTDNACH_R **CTCATCGCGGTTGGGTTT**

242 TaqMan® probe sequence:

243 **ACTTAAGTATCGAGAACGGTG**

244 To verify the specificity and sensitivity of the new primers and probe, test qPCRs were
245 carried out. The *Bd*CH primers and probe were highly sensitive and were completely
246 discriminatory between *Bd*CH and *Bd*GPL. As the *Bd*CH primers and probe targeted a SNP

247 that was also found in *Bd*CAPE, it was therefore determined that for all samples that tested
248 positive for *Bd*CH, a subsequent test would be run for *Bd*CAPE. As *Bd*CH would not amplify
249 with the *Bd*CAPE primer and probe, in this way we determined the lineage.

250 All samples that tested positive for *Bd* were then tested for *Bd*CH using protocol as per Boyle
251 et al. 2004. Amplification conditions modified as follows: 50° C for two minutes, 95° C for
252 ten minutes, followed by 50 cycles including 95° C for 15 seconds and 62° C for one minute.
253 Detector layer FAM was specified to detect the probe. The cycle threshold was adjusted
254 manually to 0.200 for FAM detector. Standard curves were plotted to observe the linear
255 relationship between Ct and log concentration of GEs. Any samples in question were sent to
256 Imperial College London to run qPCR with alternate *Bd*CH mtDNA primers and probe.

257 **<u>Corsica Data</u>**

258 To determine the geographical and taxonomic distribution of *Bd,* a multi-year survey took
259 place on Corsica, a French Mediterranean island off the west coast of Italy. The island is
260 8680km^2 with and elevation of 2706m and is characterized by a mountainous landscape, with
261 both Mediterranean and alpine influences.

262 Field data was collected from 2009 to 2013, from sites throughout Corsica. Species sampled
263 were mainly endemic (or had very limited ranges) such as *Salamandra corsica, Euproctus*
264 *montanus, Discoglossus montalentii, Discoglossus sardus, Hyla sarda,* with the exception of
265 *Pelophylax bergeri* and *Bufo viridis,* which have a broad distribution (Table 2).
266 Geographically, data was collected from 34 sites located over the entire island, with the
267 exception of the far north. The northernmost site was at 42.48394 N latitude and the
268 southernmost at 41.45735 N. The altitude of the sites ranged from 7m to 1162m. The
269 majority of the sites are located in the centre, and north-east side of the island.

270 Four scientists collected the data used in this study: Dr. Benedikt Schmidt (University of
271 Zurich and KARCH) collected data from 2013, Dr.Dirk Schmeller (CNRS Moulis
272 Experimental Ecology Station) collected data from 2009, Dr. Claude Miaud (Université
273 Montpellier 1) collected data from 2009 and 2013 and Dr. Frank Pasmans (University of
274 Ghent) collected data from 2011. The field samples were collected opportunistically and
275 standard sampling *Bd* protocols were followed (see *Bd*CH Methods). Site data was
276 documented at the time of sampling, such as co-ordinates and altitude. *Bd* infection status
277 was determined by qPCR at each scientist's home university laboratory.

278 **Data Analysis**

279 The observed prevalence of *Bd* in both Switzerland and Corsica was calculated by dividing
280 the number of infected individuals by the total sampled individuals for each site and/or
281 species. Confidence intervals were calculated from prevalence with R (v 3.1.2; R Core Team
282 2014) using the PropCIs package (Scherer 2014). The mean infection loads of Corsica
283 samples were calculated based on the raw GE zoospore equivalency for each species. Maps
284 for species prevalence were drawn with the R packages: maps (Becker et al 2014), mapdata
285 (Becker et al. 2014) and plotrix (Lemon 2006).

286 We used logistic regression to assess the effects of selected variables on both infection
287 intensity and prevalence for the Corsica data. Firstly, to assess the impact of variables on
288 prevalence we created two models, each with a different definition of what would be
289 considered an infected individual. Each individual was tested for *Bd* in duplicate. Normally
290 both of these tests need to be positive for an amphibian to be considered infected but we also
291 wanted to use a model that included those with only one positive test, as these amphibians
292 might just have a low infection load. Prevalence model one determined an individual as
293 infected if one of the two qPCR tests resulted in a GE output > 0. Prevalence model two

294 determined an individual as infected if both of the qPCR tests results in GE outputs > 0. For
295 both of these models we had site as a random effect and fixed effects for this model were
296 stage (juveniles = 0 vs adult = 1) and altitude (standardized). These models measure the
297 effect of stage and altitude on the prevalence.

298 Secondly, we assessed the impact of variables on infection intensity. For this we used GE
299 zoospore equivalences from a single individual as a measure of infection intensity for all
300 individuals. The model measured the effects of stage (juveniles = 0 vs adults = 1), lab (from
301 which the sample was analysed) and altitude as a factor of infection load. We removed all
302 non-infected individuals; log transformed the GE values and standardized altitude. For this
303 model we had two random effects: one for individual and another for site as well as fixed
304 factors for lab, stage and altitude. All models were run on all species that had enough data,
305 namely *P. bergeri*, *D. sardus, E. montanus* and *S. corsica*. We ran each model individually
306 for each species. We used R (v 3.1.2; R Core Team 2014) to run these models with the Linear
307 mixed-effects model (lme4; Bates et al. 2014) package.

308 Recently, it has become apparent for wildlife disease studies that implementing an analytical
309 method that can account for imperfect detection and sampling errors is necessary
310 (McClintock et al. 2010). In this study, we used a two-step hierarchical Bayesian model
311 created by Miller et al. 2012 to determine if corrected estimates from this model were
312 substantially different from the uncorrected estimates from the observed data we used. This
313 Bayesian model accounts for imperfect detection and sampling errors by using MCMC
314 sampling and maximum likelihood mark-recapture-like estimators. We modified the code
315 provided by Miller et al. (2012) and used the software program-JAGS (Plummer 2003) to run
316 the Bayesian MCMC sampling and R version (v 3.1.2; R Core Team 2014) with the jagsUI

317 (Kellner 2014) package. The analysis produces means and credible intervals for posterior
318 distributions of parameters such as the presence or absence of the pathogen.
319 We modelled each species individually and adapted the model based on the data we had.
320 Sample sizes were too small to analyse data for *D. montalentii, B. viridis and H. sarda,* while
321 models for *D. sardus*, *P. bergeri* and *S. corsica* failed to converge and we were therefore
322 unable to include them in this study. We analysed the data from the one species with a
323 sufficient sample size, *Euproctus montanus*. For *E. montanus*, we were able to include a
324 covariate with prevalence as a function of altitude and prevalence as a function of stage
325 (juvenile versus adult). Uniform priors were used for all parameters N(0, 0.001). We ran
326 three chains for 15000 iterations, with a burnin of 2000 and a thinning rate of 5. Traceplots
327 and Gelman-Rubin statistic Rhat <1.1 were checked to confirm convergence.

328

329 <u>Results</u>

330 **Switzerland**

331 We tested 112 samples from ten sites throughout Switzerland for *Bd*. Overall prevalence of
332 *Bd* observed in Switzerland was 43% (n = 48, 95% CI 0.335 – 0.525) (Table 3). Of the ten
333 sites tested, only two were found negative for *Bd*. All *Bd* positive samples were subsequently
334 tested for *Bd*CH. We did not detect *Bd*CH in any samples.

335 In order to confirm there wasn't a problem with the primers being used for *Bd*CH, a selection
336 of *Bd* positive samples from different sites were sent to Imperial College London to be tested
337 for *Bd*CH with different mtDNA primers; we confirmed that no *Bd*CH was present (Ghosh
338 2014).

339 **Corsica**

340
341 In total, 718 individuals from seven species were sampled on Corsica. Taxonomically, our
342 samples were largely *Discoglossus sardus*, which made up 54% of our samples, *Euproctus*
343 *montanus,* which accounted for another 22% and *Pelophylax bergeri,* with 10% of our
344 samples. The remaining 14% was made up of *Bufo viridis, Discoglossus montalentii* and
345 *Hyla sarda*. Of these samples, 12.6 % (n= 86, 95% CI 0.096 – 0.146) were infected with *Bd*.
346 Among sites and across all species containing *Bd*, observed prevalence ranged from 1.7% to
347 100%, but it should be noted that sample sizes varied greatly among the sites (Table 4). In *Bd*
348 positive samples, infection intensity ranged from 0.009 GE zoospores to 699 GE zoospores
349 with a mean of 2.229 (SE ± 0.843).
350 Geographically, *Bd* is found from sea level to the highest mountains (Figure 2). Throughout
351 Corsica, half of the sites (n=17) were infected with *Bd*. The southernmost site, Site 21 (Table
352 4), located near Bonifacio has the highest observed prevalence of 88% (n=26, 95% CI 0.698-
353 0.976) (with the exception of one site which has only one positive sample). This site was at a

354 low altitude of 55m, and the majority of the samples at this site were adult Italian pool frogs
355 (*P. bergeri)*. Sites 24 and 30 have the highest infection intensity but had relatively low
356 prevalence (Figure 3).

357 The observed prevalence for species ranged from 0 to 26% (n=73, 95% CI 0.17 – 0.38)
358 (Figure 4, Table 5). No *Bufo viridis* samples tested positive for *Bd,* but the sample size was
359 very small (n= 5) and only collected from two sites (Fig 5g). *Salamandra corsica* and *D.*
360 *sardus* both had relatively low prevalence levels (Fig 5e,a). *Pelophylax bergeri,* had the
361 highest prevalence (Fig 5b) but *D. montalentii, E. montanus* and *H. sarda* also had relatively
362 high infection rates (Table 5, Figure 5d,f,c). Infection intensity ranged among the species. *D.*
363 *sardus* had a high infection load with a mean of 3.574 (SE ± 2.326) log(GE) zoospore
364 equivalents, while *D. montalentii, H. sarda* and *S. corsica* all had low infection levels (Table
365 5).
366 For *E. montanus,* our first prevalence model found that altitude had a significant negative
367 impact with an estimate of -0.610 (SE ± 0.003, p-value = 0.001) (Figure 6) and adult stage
368 had positive impact with an estimate of 1.550 (SE ± 0.003, p-value = 0.001) (Table 6).
369 Similar to the first model, we found that adult stage had a positive impact with an estimate of
370 2.080 (SE ± 0.738) but altitude was not significant for this model (Table 7). Our infection
371 intensity model found that samples analysed in laboratory two had significant impact on
372 estimated infection of *E. montanus*, with an estimate of log(GE) zoospores at 3.99 (SE ±
373 1.581) when compared to GE results from laboratory one (Table 8). However neither altitude
374 nor stage was found to be significant. The estimated infection intensity for *E. montanus* was
375 -4.019 (SE ± 1.630).
376 We also saw a significant increase in prevalence with adults for *D. sardus* for both of our
377 prevalence models with estimates of 4.119 (SE ± 0.737, p-value = 0.001) and 3.622 (SE ±
378 0.730) respectively (Table 6 and 7); altitude was not significant for either model. None of the

379 factors had a significant impact on the infection intensity of this species. *Discoglossus sardus*
380 had a relatively high estimated infection intensity of 3.052 (SE ± 1.992) log(GE) zoospores,
381 which is fitting with our observed infection intensity.
382 Our second prevalence model found altitude had a significant positive effect on *P. bergeri*
383 with an estimated coefficient of 0.221 (SE = 0.003, p-value = 0.001) (Figure 7), but altitude
384 was not significant for the first model and stage was not seen to have an effect in either
385 model (Table 6 and 7). *Pelophylax bergeri* had a low estimated infection intensity of -3.410
386 (SE ± 1.324) and none of the variables tested were found to have a significant impact on this
387 species.
388 *Salamandra corsica* had an estimated infection intensity of -1.186 (SE ± 5.685). While we
389 found none of the explanatory variables affected prevalence or infection intensity for this
390 species, this may be due to its sample size, as we were unable to run our second prevalence
391 model for this species due to insufficient data.
392 From the Miller et al. (2012) model, prevalence at the mean altitude was estimated at 24.4%
393 (95% CI 0.017-0.873)(Table 9), which is close to our observed prevalence of 19%. We
394 therefore determine that for the purposes of this study we are justified in using the
395 uncorrected observed values of prevalence and infection intensity for our GLM analysis. We
396 also note that altitude has a negative effect on prevalence with an estimate of -1.563 (95% CI
397 -4.384 to 1.146), although here the effect is not determined to be significant (the 95%
398 credible interval includes zero), this estimate is in line with our GLM analysis for this
399 species.
400

401 **Discussion**

402 Despite the dramatic impacts of chytridiomycosis worldwide there still remain many gaps in
403 our knowledge of the disease. The distribution of older lineages has not been explored and
404 the impacts of *Bd* on many vulnerable and endemic species remain unknown. Here we will
405 first attempt to describe the distribution of an endemic lineage of *Bd* and secondly assess the
406 risk of chytridiomycosis on Corsica.

407 Firstly, we studied the unknown distribution of a lineage of *Bd, Bd*CH in Switzerland. This
408 lineage was isolated in 2007 from *A. obstetricans* tadpoles from single pond near Gamlikon
409 village near Zurich (Farrer et al. 2011). Our survey found the prevalence of *Bd* among the
410 common midwife toads (*A. obstetricans*) to be high throughout its range in Switzerland. We
411 revisited the site where *Bd*CH was originally isolated from twice during this study;
412 furthermore in addition to our samples we analyzed samples from this site from 2011.
413 Despite all this, *Bd*CH could not be found neither there or anywhere else.

414 We can hypothesize several possible reasons why we are unable to detect *Bd*CH in
415 Switzerland. It is possible that the number of samples collected was insufficient to detect
416 *Bd*CH. As we do not know the prevalence of this lineage, it could be very low. In which case,
417 the sample size would need to be very large to detect the disease (DiGiacomo & Koepsell
418 1986). Furthermore, all samples analyzed for *Bd*CH were taken from swabs and a recent
419 study has shown that *Bd* samples taken from swabs can be found to yield inconsistent results
420 (Shin et al. 2014). Additionally, low infection loads may be more difficult to detect. If
421 *Bd*CH's infection loads are low, as is found with other endemic lineages (Shin et al. 2014),
422 the swab results could be unreliable (Miller et al. 2012).

423 The lack of *Bd*CH seen in this study could be due to competition. As hypothesized by
424 Schloegel et al. 2012, due to *Bd*GPL's high infectivity (Farrer et al. 2011), it is possible that

425 *Bd*GPL has simply been outcompeting other lineages. It is possible that *Bd*CH has been
426 outcompeted to the extent that it has possibly become extinct or is now so rare that we were
427 unable to detect it. Further studies are needed with larger sample sizes to confirm if the
428 lineage has indeed become extinct.

429 Other unknown lineages could be present in Switzerland. The accepted emergence of *Bd*GPL
430 is relatively recent (Rosenblum et al. 2013, Schloegel et al. 2012, Farrer et al. 2011). A study
431 of museum specimens in Switzerland confirmed that *Bd* has been present in Switzerland
432 since 1901 (Peyer 2010). Since this is well before the emergence of *Bd*GPL it is possible that
433 other older lineages may be present here. During this study, a selected subsample of extracted
434 DNA was sent to Imperial College in London to be tested against all available lineage
435 specific primers (*Bd*GPL, *Bd*CH and *Bd*CAPE). One sample collected from Zunzgen
436 Hefleten that was confirmed *Bd* positive tested negative for all of the lineage specific primers
437 (Ghosh 2014). Therefore, it is possible that another novel lineage could be present.

438 A deeper understanding of the distribution of pathogen lineages may explain why no negative
439 effects of *Bd* on host populations are observed in Switzerland. Although Switzerland has one
440 of the highest prevalence's in Europe (Garner et al. 2005), it appears to have less of an impact
441 on host species than in Spain, where there have been mass die-offs (Bosch et al. 2007, Tobler
442 et al. 2012). The reasons behind this are unknown. One possible explanation lies in host
443 immunity. All anuran hosts are found to have differing levels of innate immunity to *Bd*
444 (Woodhams et al. 2012). A recent study has suggested that frogs may also be able to acquire
445 immunity (McMahon et al. 2014). This study showed frogs have the ability to develop
446 behavioral or immunological resistance to *Bd*. Therefore, if hypovirulent lineages of *Bd,*
447 endemic to Switzerland, such as *Bd*CH, have been present before the invasion of the *Bd*GPL
448 lineage, then it is possible that *A. obstetricans* may carry prior immunity that buffered the

449 population against the invading lineage, resulting in the observed high prevalence but low
450 mortalities (Louca et al. 2014).

451 Describing the distribution of *Bd* is as an important first step in assessing the risk of the
452 pathogen to the amphibian hosts (Balaz et al. 2014). In Europe, *Bd* is expanding (Garner et al.
453 2005, Walker et al. 2008, 2010) and sampling efforts need to be expanded to include areas of
454 Europe where the presence and prevalence of the disease is unknown (Balaz et al. 2014). Our
455 analysis of Corsica revealed that *Bd* is distributed throughout the island. Geographically, we
456 found *Bd* scattered over the entire island, from sea level to high elevations. All species tested
457 where found to have *Bd* positive individuals, with the exception of the European Green Toad
458 (*B. viridis),* which only has a small sample size (n=5). In total, the observed prevalence of
459 infection was 12.6% (n=86, 95% CI 0.096 – 0.146) and half of all the sites sampled
460 contained *Bd* (n=17). This is slightly higher than the overall infection level of Europe, which
461 is approximately 11% (Balaz et al. 2014).

462 The general *Bd* levels on Corsica should cause concern. The overall incidence is slightly
463 higher than the average and much higher than many other European countries (Ohst et al.
464 2013, Balaz et al. 2014). The prevalence observed among the sites is also relatively high.
465 These levels of *Bd* are concerning as the amphibian populations of Corsica live in a limited
466 range with the majority of species being endemic to the area and therefore particularly
467 vulnerable. Reports from the neighboring island of Sardinia have lead us to believe several
468 species on Corsica are at particular risk, such as *Discoglossus* and *Euproctus*.

469 In Sardinia, there have been observed mortalities of *Discoglossus* species (Bielby et al.
470 2009). On Corsica we sampled two species of *Discoglossus*, the Tyrrhenian painted frog (*D.*
471 *sardus*) and the Corsica painted frog *(D. montalentii*). Tyrrhenian painted frog, which is
472 endemic to Corsica and Sardinia showed a relatively low observed prevalence of 7% (95% CI

473 0.05 - 0.11) on Corsica. Unfortunately, although the prevalence we found is low, the infection
474 loads of *D. sardus* were the highest of any species we tested at 3.571 (SE ± 2.326) log (GE).
475 On the neighbouring island of Sardinia, *D. sardus* has also been found to have a high
476 prevalence (41%) and lethal *Bd* infections (Bielby et al. 2013) with high infection loads that
477 ranged from low (0.1 ± 0.03) to very high (13203.1 ± 32.8) genomic equivalencies (Bielby et
478 al. 2009). What should also cause concern is that *D. sardus* populations of northern Sardinia
479 have undergone mass die-offs in 2004 and 2006 for reasons unknown (Bielby et a.l 2013,
480 Tessa et al. 2013). Therefore this species appears to be vulnerable and possibly at risk for
481 chytridiomycosis on Corsica.

482 The Corsican painted frog *(D. montalentii*) was recently differentiated from *D. sardus*. This
483 species is listed as Near Threatened by the IUCN, and is found only in a very limited range of
484 5000km^2 on Corsica. There is limited information on this species and no previous studies of
485 *Bd* prevalence are known. We found an observed prevalence of 15% (n=13, 95% CI 0.19 –
486 0.45) for this species. Although the sample size is small and therefore the prevalence might
487 be unreliable the fact that *Bd* was detected in such a vulnerable population is very
488 concerning. Further studies of *Bd* prevalence for this species are urgently needed.

489 In this study, two species from the Salamandridae family were sampled, Corsican brook
490 salamander (*E. montanus*) and Corsican fire salamander (*S. corsica*). We found that
491 *Euproctus montanus* has a relative high prevalence of 19% (n=156, 95% CI 0.13 - 0.26). On
492 Sardinia, closely related *Euproctus platycephalus* was also found to have a high prevalence
493 of 36% (95% CI 0.27 - 0.45) (Bielby et al. 2013) and has suffered declines possibly due to
494 chytridiomycosis (Bielby et al. 2009). *Salamandra corsica* has relatively low observed
495 prevalence, the lowest of all species we tested here (prevalence = 2%, 95% CI 0.00 - 0.097).
496 The infection intensity was also very low (log(GE) = -0.072, SE ± -0.528). Both these

497 species are endemic to Corsica and have a limited range but are found to be locally abundant
498 and not at risk for extinction (IUCN redlist). Regardless, the presence of *Bd* in these endemic
499 populations is of concern as we have seen other *Salamandra* species suffer die-offs because
500 of *Bd* (Bosch et al. 2006)

501 We found the observed prevalence of *Hyla sarda,* the Tyrrhenian tree frog was relatively
502 high at 19% (n=27, 95% CI 0.06 - 0.38), especially when compared to neighbouring Sardinia
503 where they found no infected *H. sarda* (Bielby et al. 2013). This species might be tolerant to
504 *Bd* infections, as is the case with close relative *Hyla arborea* (Luquet et al. 2012). Our data
505 showed this species has very low infection loads and therefore could be undergoing some
506 kind of tolerance to *Bd*. Tyrrhenian tree frogs spend relatively little time in the water as
507 adults, spending more time in the sun at higher temperature leads to lowered prevalence and
508 load levels. Even so, the high prevalence seen on Corsica is concerning, further studies are
509 needed to determine the risk of *H. sarda* to *Bd*.

510 *Bufo viridis*, the European green toad, had a very small sample size (n =5), too small to make
511 any reliable inference with regards to the presence of *Bd*. But we found no *Bd* in our sample;
512 additionally we were unable to find any evidence in other studies of infected green toads in
513 Europe. This along with the fact that this species is widely distributed leads me to believe that
514 this species isn't particularly vulnerable to *Bd*.

515 We found the Italian pool frog (*P. bergeri*) to have a particularly high observed prevalence
516 (26%, 95% CI 0.17 – 0.38). Throughout Europe, *Pelophylax* species are found to have high
517 prevalence when compared to the background rate (Balaz et al. 2014). But prevalence rates of
518 this species are inconsistent among sites throughout Europe, indicating that environmental
519 factors play a role (Balaz et al. 2014). In Italy, *Peloyphylax* have long been found to be
520 infected with *Bd* (Tessa et al. 2013) but the number of infected individuals is low (Simoncelli

521 et al. 2005). Furthermore, studies have shown *Pelophylax* species to be tolerant to *Bd*, even in
522 populations with high prevalence no mortalities are seen (Woodhams et al. 2012). Therefore
523 we don't believe this species is at risk.

524 It should be noted that there are some inherit problems with this kind of ad hoc analysis of
525 opportunistic sampling (Muths et al. 2009). One major issue with this kind of data is the
526 variation in sample sizes. The wide confidence interval seen for our prevalence estimates
527 shows that these estimates are uncertain. Site and species are often confounded, with only
528 one species tested at a site. Recurrently, with the opportunist sampling done with *Bd* surveys
529 there is insufficient sample sizes to get accurate estimates of the presence and prevalence of
530 the disease. With low sample sizes, sites could be mistakenly considered negative for *Bd,*
531 when in fact the sample size is too small to detect it and in sites where *Bd* is detected the
532 prevalence calculated will not be accurate (DiGiacomo & Koepsell 1986). Studies of these
533 kinds often result in false negatives and the failure to detect pathogen when it's present
534 (Adams et al. 2010). This non-detection leads to underestimation of prevalence and
535 overestimation of infection intensity (Miller et al. 2012).

536 In addition to problems related to low sample size, there are inherent difficulties in these kind
537 of studies when dealing with comparing a population being monitored by different
538 researchers, in different years, when samples are analyzed by different laboratories (Miller et
539 al. 2012). Accuracy in estimates of prevalence and infection intensity is reduced when
540 detection is not the same across all the data (Miller et al. 2012). During this study we
541 attempted to use a Bayesian two-step hierarchical model (Miller et al. 2012), which takes into
542 account errors and detection abilities. We successfully fitted the model to the *E. montanus*
543 data, the other species either had insufficient data or we were unable to get the model to

544 converge. We did run the data through several GLM models to gain insight into parameters
545 affecting prevalence and infection intensity for these species.

546 Despite the limitations of opportunistic sampling there are still legitimate reasons to sample
547 this way, particularly in areas where there is no data on *Bd* presence (Muths et al. 2009). The
548 results of this study are robust even if the small sample sizes of some populations caused us
549 to fail to detect *Bd* when it was present. Furthermore, we know that some endemic species are
550 highly infected with *Bd*, such as *Euproctus* (Bovero et al. 2008) and as there is an effect of
551 phylogeny on species susceptibility to *Bd*, we can safety conclude that for example, *E.*
552 *montanus* is at risk because *E. platycephalus* is. But future studies would be greatly aided by
553 coordinated efforts in the sampling of *Bd* (Gascon et al. 2007, Skerratt et al. 2008).

554 In conclusion, our results increase the understanding of the role that both *Bd* genotype and
555 host genotype (i.e. species) play in the threat of chytridiomycosis. Our survey of *Bd*CH in
556 Switzerland has shown the difficulties in studying these endemic lineages. Although it often
557 wasn't possible to capture large enough numbers of tadpoles to determine if *Bd*CH has
558 become extinct, our results lead us to believe that it might be outcompeted by the *Bd*GPL
559 (Schloegel et al. 2012). Further studies could find that endemic lineages of *Bd* may play an
560 important role in the effects of chytridiomycosis by serving as a buffer for the host amphibian
561 population against the hypervirulent lineages; future studies should aim to better understand
562 the roles of these endemic lineages. Host susceptibility to *Bd* infection varies greatly among
563 species. It is unclear why this occurs but it is critical to conservation efforts to determine
564 which species are particularly susceptible (Balaz et al. 2014). Our survey of Corsica shows
565 that *Bd* is widely distributed throughout the island and many of the most vulnerable species
566 are at risk for chytridiomycosis. Furthermore, as this study was done opportunistically, with
567 limited sample sizes our results could underestimate the prevalence of *Bd* on Corsica. This

568 study illustrates the need for further sampling of both endemic *Bd* lineages and for focused
569 field monitoring of the amphibian population of Corsica. Moreover, there is an urgent need
570 for improved large-scale coordinated field monitoring practices throughout Europe (Gascon
571 et al. 2007) to better understand the risks of chytridiomycosis.
572

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884 **Table 1.** Sites, species and sample sizes of samples collected in Switzerland.

Region	Location	Coordinates Ch- 1903		Species	Sample size
		Cx	Cy		
ZH	Rutiboden	684177	236636	*A. obstetricans*	10
ZH	Tufiweiher	682073	242014	*A. obstetricans*	4
ZH	Gamlikon 1	680050	240750	*A. obstetricans*	10 (2014) 12 (2011)[a]
ZH	Gamlikon 2	680080	240115	*I. alpestris*	19
LU	Ehrendingen	662260	209930	*A. obstetricans*	10
LU	Hergiswald	660550	208350	*A. obstetricans*	9
BLS	Chalchofen	624710	258587	*A. obstetricans*	9
BLS	Zunzgen Hefleten	627350	254050	*A. obstetricans*	9
BLS	Geissgrube	624200	259980	*A. obstetricans*	10
TIC	Arcegno	701100	114300	*P. esculentus*	10

885 [a]Gamlikon 1 samples from 2011 where collected and DNA extractions were done by Dr. Leyla Davis of Zurich University.
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894 **Table 2**. Summary of data collected from Corsica.

Species	Number of sites	Number of individuals[a]	Adults	Tadpoles	Altitude range (m)	Number of labs[b]
Bufo viridis	2	3	5	0	24 - 44	2
Discoglossus montalentii	4	2	13	0	124 - 1113	1
Discoglossus sardus	20	18	42	346	24 - 1037	3
Euproctus montanus	10	15	102	54	41 - 1041	2
Hyla sarda	2	14	27	0	24 - 44	2
Pelophylax bergeri	8	5	68	8	7 - 417	4
Salamandra corsica	10	5	13	42	41 - 1162	1

895 [a] Average number of individuals per site [b]Number of labs that contributed data on the species.
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905 **Table 3.** Observed prevalence of *Bd* in Switzerland.

Region	Location	Species	Bd+ Samples[a]	Prevalence (95%CI)
ZH	Rutiboden	*A. obstetricans*	3/10	0.30 (0.066-0.652)
ZH	Tufiweiher	*A. obstetricans*	1/4	0.25 (0.006-0.805)
ZH	Gamlikon 1 2014	*A. obstetricans*	8/10	0.80 (0.444-0.974)
	Gamlikon 1 2011		8/12	0.67 (0.349-0.900)
ZH	Gamlikon 2	*I. alpestris*	0/19	0.00 (0.000-0.176)
LU	Ehrendingen	*A. obstetricans*	10/10	1.00 (0.025-1)
LU	Hergiswald	*A. obstetricans*	9/9	1.00 (0.025-1)
BL	Chalchofen	*A. obstetricans*	7/9	0.78 (0.400-0.972)
BL	Zunzgen Hefleten	*A. obstetricans*	1/9	0.11 (0.003-0.482)
BL	Geissgrube	*A. obstetricans*	0/10	0.00 (0.000-0.308)
TC	Arcegno	*P. esculentus*	1/10	0.10 (0.002-0.445)

906 [a]number of Bd+ individuals/number of samples tested
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938 **Table 4.** Observed Prevalence and Infection Intensity of *Bd* on Corsica by Site

Sites	Co-ordinates N/E	Altitude (m)	Bd+ Samples[a]	Infection Intensity log (GE)	Prevalence (95% CI)
Site 1 -	42.45944/ 9.12309	417	2/10	-0.199 SE ± 1.187	0.200 (0.025 -0.556)
Site 2 -	42.439651 / 9.032922	610	3/11	0.433 SE ± 0.390	0.270 (0.060 – 0.609)
Site 3 -	42.466632 / 8.680475	44	3/16	0.150 SE ± 0.553	0.190 (0.041 – 0.456)
Site 4 -	42.46664 / 8.68048	192	0/4	0.000	0.000 (0.000 - 0.602)
Site 5 -	42.47253 / 9.2104	113	4/19	-0.395 SE ± 1.180	0.210 (0.061 - 0.456)
Site 6 -	42.48394/ 9.20098	7	0/2	0.000	0.000 (0.000 – 0.841)
Site 7 -	42.379314/8.748538	119	0/1	0.000	0.000 (0.000 – 0.975)
Site 8 -	42.54199/9.20552	124	0/3	0.000	0.000 (0.000 – 0.708)
Site 9 -	42.53323/9.24373	194	0/1	0.000	0.000 (0.000 – 0.975)
Site 10 -	42.524/9.25141	275	0/1	0.000	0.000 (0.000 – 0.975)
Site 11 -	42.52411/9.25145	1162	0/6	0.000	0.000 (0.000 – 0.459)
Site 12 -	42.06584/9.06414	1113	1/4	2.879 SE ± 0.378	0.250 (0.006 – 0.806)
Site 13 -	42.0842/9.08425	833	0/1	0.000	0.000 (0.000 – 0.975)
Site 14 -	42.21065/9.08308	612	0/1	0.000	0.000 (0.000 – 0.975)
Site 15 -	42.27113/9.13357	344	0/2	0.000	0.000 (0.000 – 0.841)
Site 16 -	42.24355/9.21197	712	1/2	3.190 SE ± 0.141	0.500 (0.013 – 0.987)
Site 17 -	42.21032/9.24303	798	0/1	0.000	0.000 (0.000 – 0.975)
Site 18 -	42.15348/9.2759	330	1/1	2.223 SE ± 0.869	1.000 (0.025 – 1.000)
Site 19 -	42.13283/9.26475	98	1/4	-1.201 SE ± 2.231	0.250 (0.006 – 0.806)
Site 20 -	42.12477/9.18492	150	1/2	0.128 SE ± 0.19	0.500 (0.013 – 0.987)
Site 21 -	41.45735/9.20401	51	23/26	10.008 SE ±3.536	0.880 (0.698 – 0.976)
Site 22 -	42.526/9.3661	379	7/42	1.118 SE ± 0.506	0.170 (0.070 – 0.314)
Site 23 -	41.5875/9.21155	50	11/108	18.709 SE ±6.156	0.100 (0.052 – 0.175)
Site 24 -	41.69763/9.00108	394	9/56	75.908 SE ± 52.898	0.160 (0.076 – 0.283)
Site 25 -	41.65608/8.97923	24	0/52	0.000	0 .000 (0.000 – 0.068)
Site 26 -	42.02836/9.03844	1041	0/25	0.000	0.000 (0.000 – 0.137)
Site 27-	42.33011/9.30561	998	8/75	1.442 SE ± 0.777	0.110 (0.047 – 0.199)
Site 28 -	42.22708/9.23473	207	0/28	0.000	0.000 (0.000 – 0.123)
Site 29 -	42.30972/9.28419	570	0/32	0.000	0.000 (0.000 – 0.109)
Site 30 -	42.30380/9.31861	1037	1/30	87.300 SE ± 0.600	0.030 (0.000 – 0.172)
Site 31 -	42.31522/9.32244	1020	0/20	0.000	0.000 (0.000 – 0.168)
Site 32 -	42.17558/9.41541	41	1/57	1.980 SE ± 0.840	0.020 (0.000 – 0.094)
Site 33 -	42.41630/9.44033	425	6/37	8.023 SE ± 5.739	0.160 (0.062 – 0.320)
Site 34 -	41.64625/9.21683	428	0/15	0.000	0.000 (0.000 – 0.218)

939 [a]Number of Bd+ individuals/number of samples tested. Samples were only considered positive if two qPCRs results were positive
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Table 5. Observed prevalence and infection intensity of *Bd* on Corsica by species. Based on 718 samples. Infection intensity is mean log(GE) zoospores.

Species	Bd+ Samples[a]	Infection Intensity	Prevalence (95% CI)
Bufo viridis	0/5	0.00	0.00 (0.00–0.52)
Discoglossus montalentii	2/13	-0.53 (SE ± -1.06)	0.15 (0.19-0.45)
Discoglossus sardus	29/389	3.57 (SE ± 2.33)	0.07 (0.05-0.11)
Euproctus montanus	30/156	1.86 (SE ± 0.22)	0.19 (0.13-0.26)
Hyla sarda	5/27	-0.80 (SE ± -3.24)	0.19 (0.06-0.38)
Pelophylax bergeri	19/73	1.93 (SE ± 0.73)	0.26 (0.17-0.38)
Salamandra corsica	1/55	-0.07 (SE ± -0.53)	0.02 (0.00-0.10)

[a]Number of Bd+ individuals/number of samples tested

983 **Table 6.** Summary of estimates from GLM Prevalence Model 1. All data are on the logit scale except
984 intercept estimates, which are on the decimal scale. All significant results are in bold.

Prevalence Model 1. Formula: prev1 ~ lmer(1 l site) + as.factor(lab) + as.factor(stage) + altitude			
P. bergeri[a]			
Random Effects	Variance	Std. Dev	
Intercept (site)	15.3	3.911	
Fixed Effects	Estimate	Std. Error	z value
Intercept	0.025	3.166	-1.159
Altitude	0.659	1.488	0.444
D. sardus			
Random Effects	Variance	Std. Dev	
Intercept (site)	0.987	0.994	
Fixed Effects	Estimate	Std. Error	Z value
Intercept	0.008	0.730	-6.654
Stage 1 (adult)	**4.119**	**0.737**	**5.553**
Altitude	-0.439	0.482	-0.908
E. montanus			
Random Effects	Variance	Std. Dev	
Intercept (site)	2.963	1.721	
Fixed Effects	Estimate	Std. Error	Z value
Intercept	0.108	0.003	-769.9
Stage 1(adult)	**1.550**	**0.003**	**565.9**
Altitude	**-0.610**	**0.003**	**-223.1**
S. corsica			
Random Effects	Variance	Std. Dev	
Intercept (site)	0	0	
Fixed Effects	Estimate	Std. Error	Z value
Intercept	0.050	0.726	-4.06
Stage 1 (adult)	0.310	1.349	0.230
Altitude	0.241	0.671	0.357

985 [a]Model for *P. bergeri* was modified to not include the stage effect, as all individuals infected were adults.
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1011 **Table 7.** Summary of estimates for GLM Prevalence Model 2. All values are on the logit
1012 scale except intercept estimates, which are on the decimal scale. Significant results are in
1013 bold. No model was run for *S. corsica* due to insufficient data.

Prevalence Model 2. Formula: prev2 ~ lmer(1 | site) + as.factor(lab) + as.factor(stage) + altitude			
P. bergeri[a]			
Random Effects	Variance	Std. Dev	
Intercept(site)	7.918	2.814	
Fixed Effects	Estimate	Std. Error	z value
Intercept	0.048	0.003	-1137.1
Altitude	**0.221**	**0.003**	**83.6**
D. sardus			
Random Effects	Variance	Std. Dev	
Intercept (site)	0.764	0.874	
Fixed Effects	Estimate	Std. Error	Z value
Intercept	0.007	0.750	-6.559
Stage 1 (adult)	**3.622**	**0.730**	**4.988**
Altitude	-0.498	0.491	-1.011
E. montanus			
Random Effects	Variance	Std. Dev	
Intercept (site)	1.855	1.362	
Fixed Effects	Estimate	Std. Error	Z value
Intercept	0.090	0.956	-3.364
Stage 1(adult)	**2.080**	**0.738**	**2.820**
Altitude	0.036	0.641	0.055

1014 [a]Model for *P. bergeri* was modified to not include the stage effect, as all individuals infected were adults.
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1045 **Table 8.** Estimates from GLM Infection Intensity Model. All results of log(GE) zoospores.
1046 Significant results are in bold.

Infection Intensity Model. Formula: GE ~ lmer(1 ǀ ind) + (1 ǀ site) + as.factor(lab) + as.factor(stage) + altitude			
P. bergeri[a]			
Random Effects	Variance	Std. Dev	
Intercept(ind)	2.376	1.541	
Intercept(site)	0	0	
Residual	2.792	1.671	
Fixed Effects	Estimate	Std. Error	t value
Intercept	-3.410	1.324	-2.576
Lab 1	4.6834	1.691	2.770
Lab 2	2.334	2.407	0.969
Altitude	0.912	0.726	1.255
E. montanus			
Random Effects	Variance	Std. Dev	
Intercept (ind)	1.824	1.350	
Intercept (site)	1.298	1.139	
Residual	1.808	1.345	
Fixed Effects	Estimate	Std. Error	t value
Intercept	-4.019	1.630	-2.465
Lab 2	**3.993**	**1.582**	**2.524**
Stage 1 (adult)	0.505	0.756	0.669
Altitude	-0.664	0.572	-1.161
D. sardus			
Random Effects	Variance	Std. Dev	
Intercept (ind)	4.333	2.082	
Intercept(site)	0	0	
Residual	3.472	1.863	
Fixed Effects	Estimate	Std. Error	t value
Intercept	3.052	1.992	1.532
Lab 2	-1.373	1.418	-0.968
Lab 3	0.390	1.488	0.262
Stage 1(adult)	-2.048	1.590	-1.288
Altitude	0.472	0.521	0.906
S. corsica[b]			
Random Effects	Variance	Std. Dev	
Intercept (ind)	17.130	4.138	
Residual	11.890	3.449	
Fixed Effects	Estimate	Std. Error	t value
Intercept	-1.186	5.685	-0.209
Lab 2	-1.585	7.444	-0.213
Altitude	-0.631	3.844	-0.164

1047 [a]Removed the stage effect for this model because all infected individuals were adults. [b]Model used contained no site fixed
1048 effect.
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1057 **Table 9**. Posterior distribution of prevalence parameters from Bayesian Model for *E.*
1058 *montanus*. Mean and credible intervals for prevalence as a function of altitude and of stage.
1059 All values are in the logit scale except the intercept, which is in decimal.

Prevalence	Mean	2.5%	97.5%
Intercept	0.244	0.017	0.873
Altitude	-1.563	-4.384	1.146
Stage	0.190	-1.316	1.906

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1104 Figure Legends
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1106 **Figure 1**. **Map of Swiss Sites.** Red dots represent the sites. Green dot and arrow is the
1107 location of Gamlikon 2, where *Bd*CH was first found.
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1109 **Figure 2. Map of sites on Corsica.**
1110 Red sites are *Bd* positive and white sites are *Bd* negative
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1112 **Figure 3. Prevalence and Mean Infection Intensity by Site.** Plotted points are the
1113 prevalence and mean log(GE) zoospore equivalents for all site containing *Bd*. Error bars
1114 represent standard error of the log(GE) and confidence intervals of the prevalence.
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1116 **Figure 4. Prevalence and Mean Infection Intensity by Species.** Plotted points are the
1117 prevalence and mean log(GE) zoospore equivalents for all species. Error bars represent
1118 standard error of the log(GE) and confidence intervals of the prevalence.

1119 **Figure 5. Observed Prevalence of each species on Corsica**. Red dots show *Bd* infection
1120 and white dots show no infection. For sites with both infected and non-infected individuals a
1121 pie chart shows the prevalence.

1122 **Figure 6. Predicted Estimates from GLM Prevalence Model 1 for species *E. montanus*.**
1123 The effect of altitude on prevalence for a) tadpoles and b) adults.
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1125 **Figure 7. Predicted Estimate from GLM Prevalence Model 2 for species *P. bergeri*.** The
1126 effect of altitude on prevalence.
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1153 Figure 1

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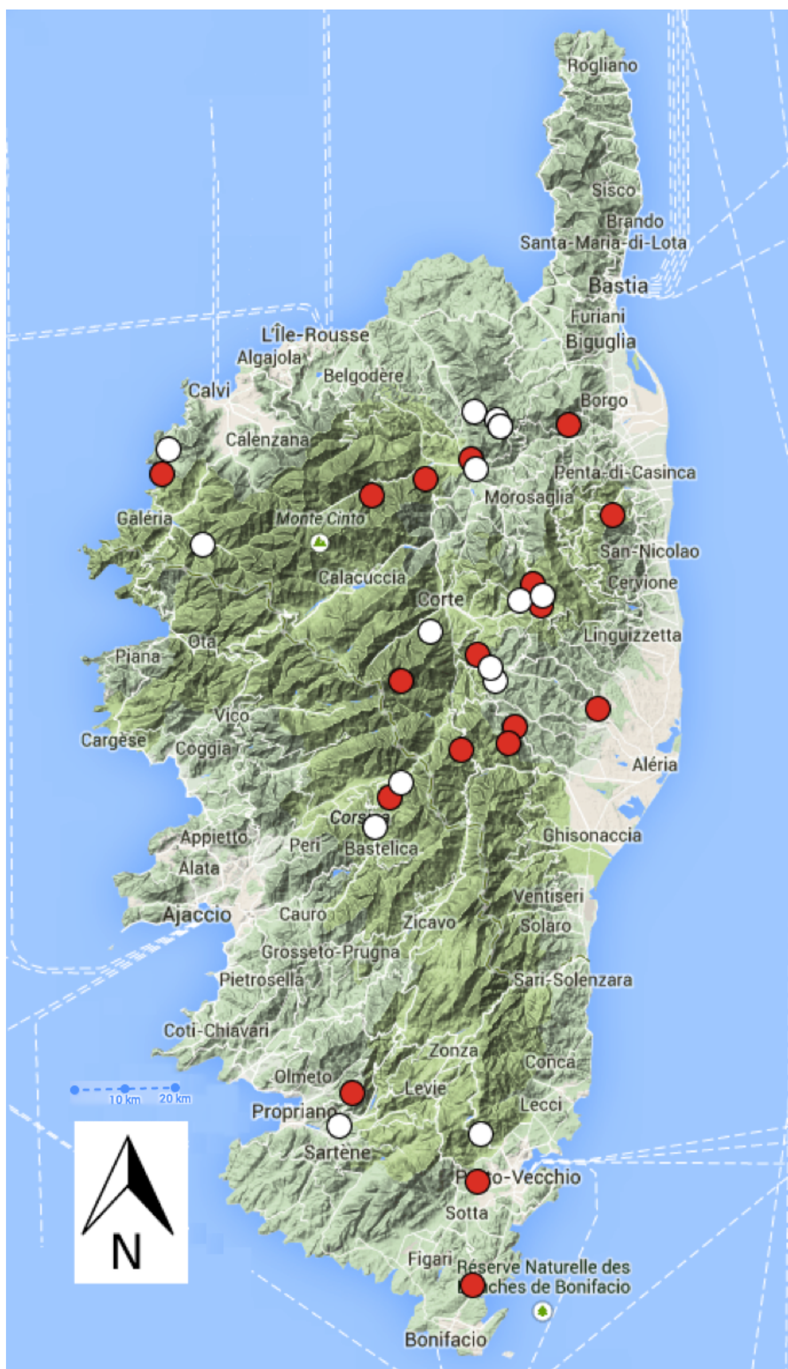

1235 Figure 3
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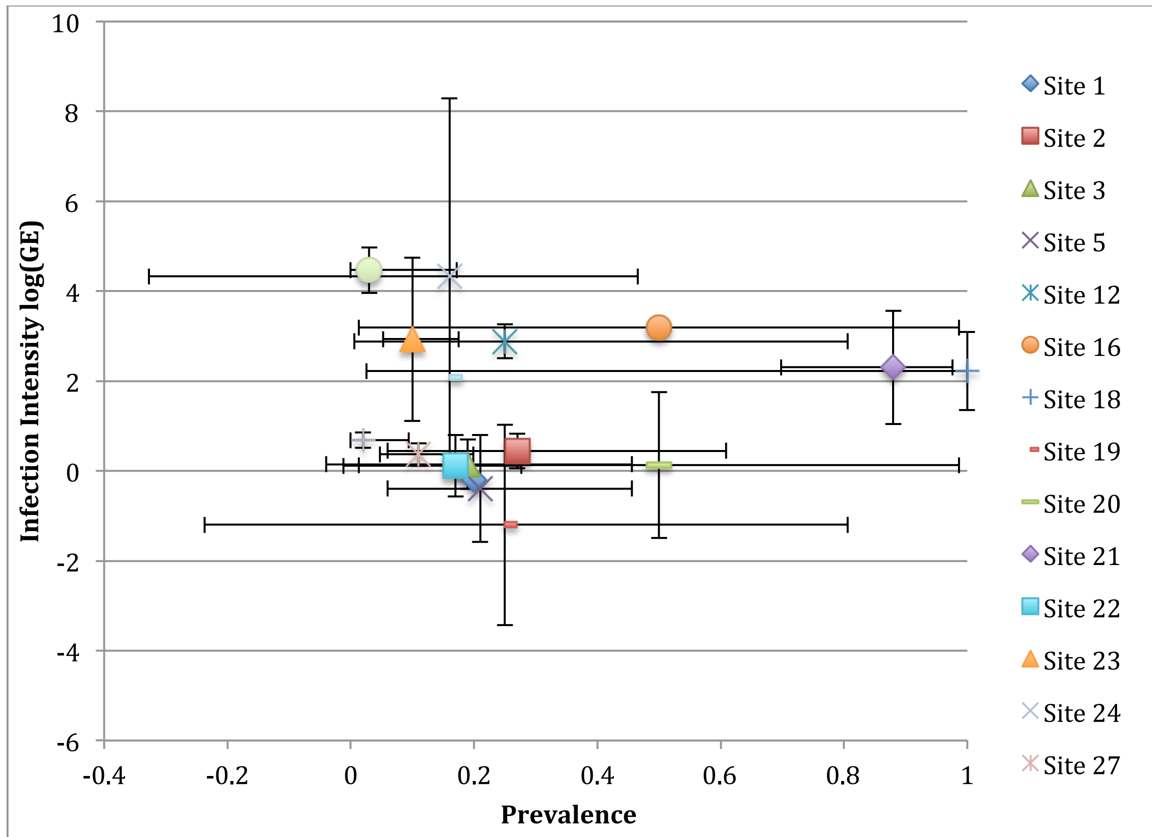

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1264 Figure 4
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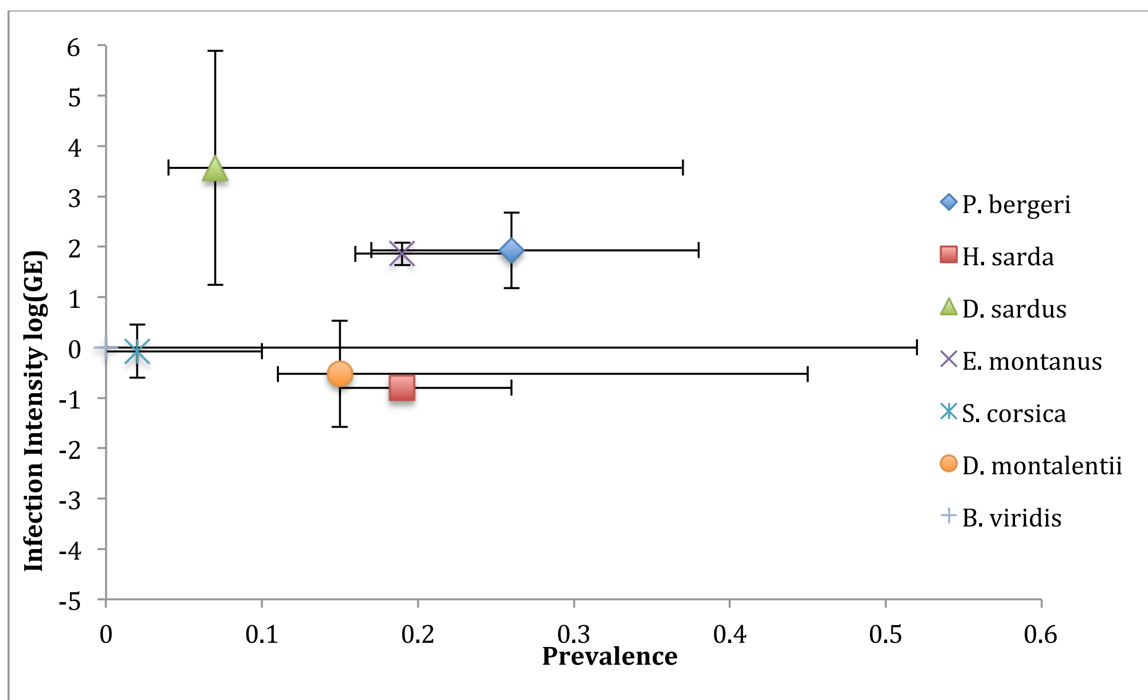

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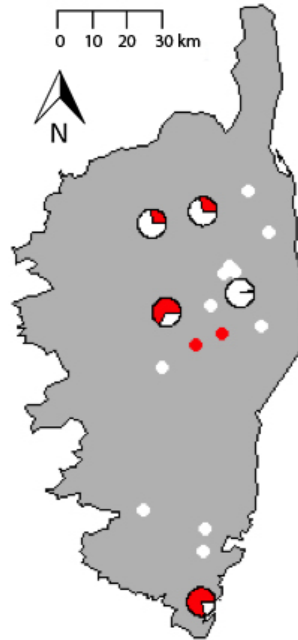
1297 Figure 5

1298 a) *D. sardus*



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1300 b) *P. bergeri*

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1302 c) *H. sarda*

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1304 d) *D. montalentii*

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1307 e) *S. corsica*

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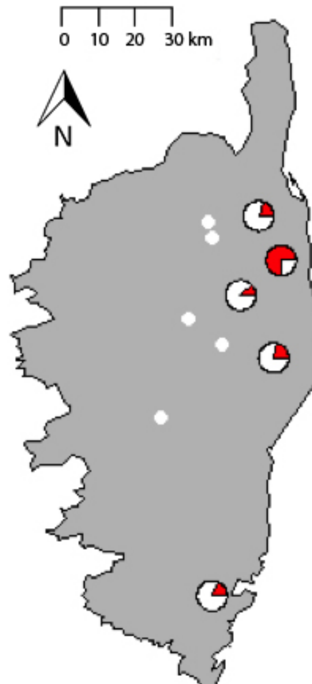
1309 f) *E. montanus*



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1311 g) *B. viridis*

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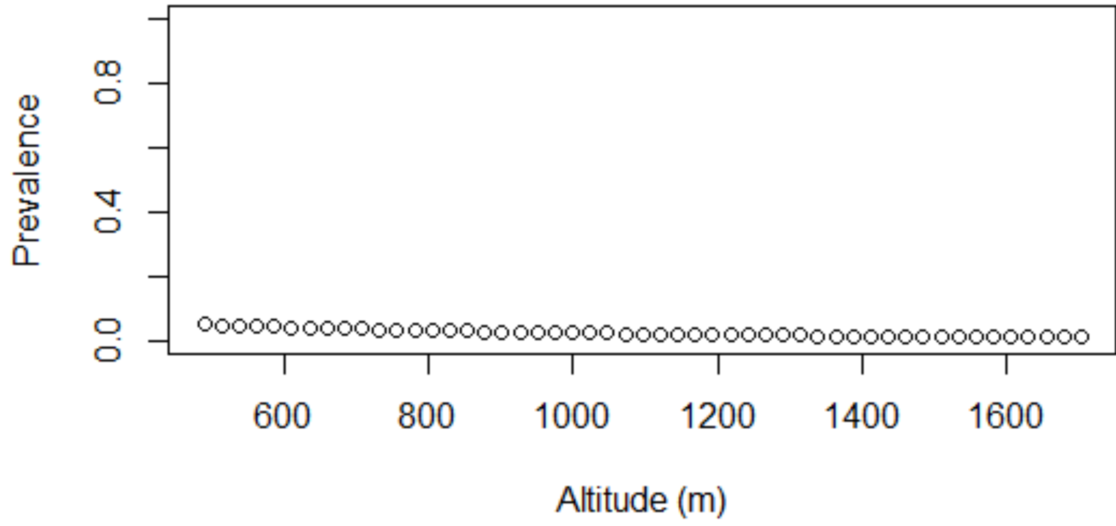
1339 Figure 6

1340 a) Stage 0 – Tadpoles



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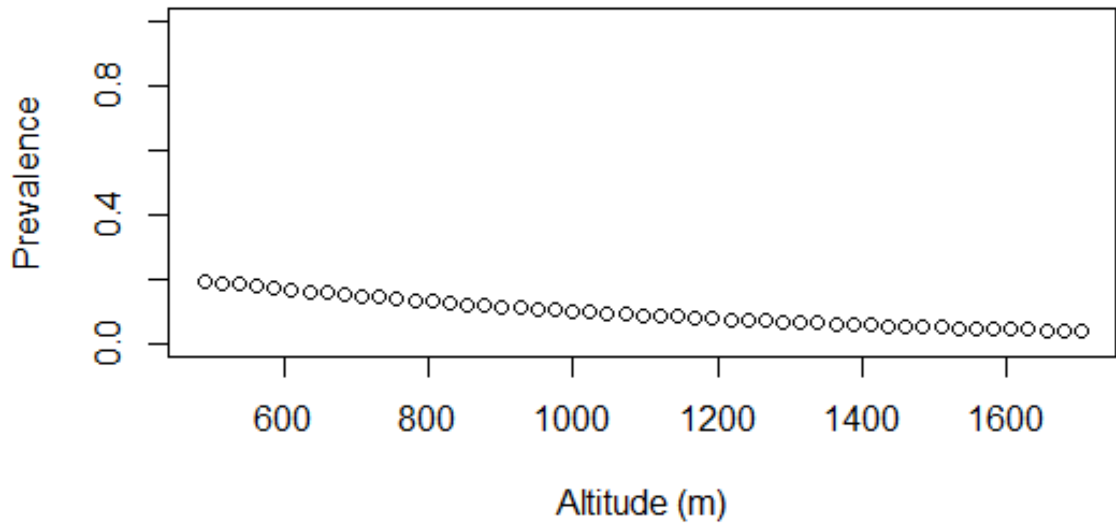
1342 b) Stage 1 - Adults



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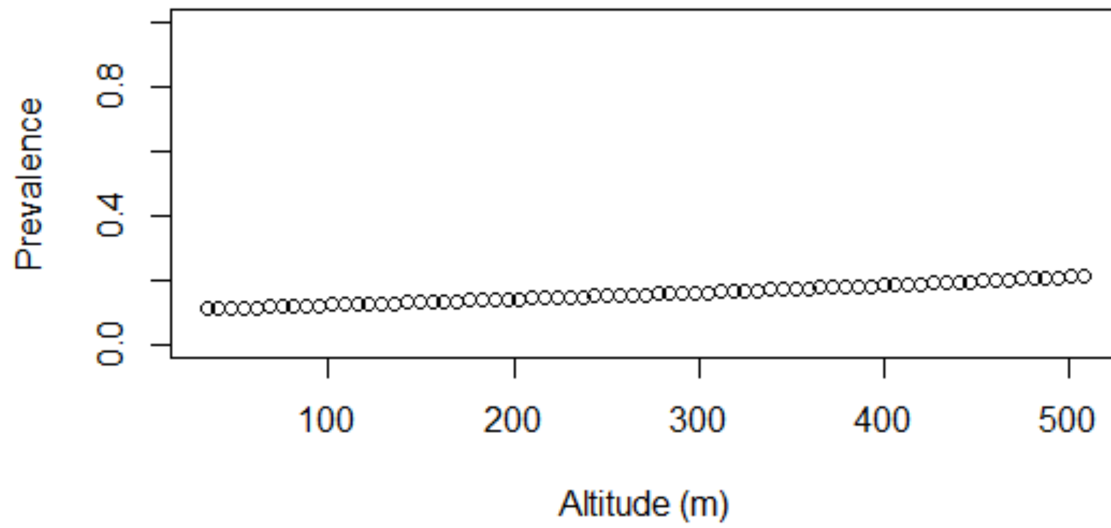
1352 Figure 7



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