LIVERPOOL
JOHN MOORES
UNIVERSITY

LJMU Research Online

Clare, FC, Halder, JB, Daniel, O, Bielby, J, Semenov, MA, Jombart, T, Loyau, A, Schmeller, DS, Cunningham, AA, Rowcliffe, M, Garner, TWJ, Bosch, J and Fisher, MC

 Climate forcing of an emerging pathogenic fungus across a montane multi-host community

http://researchonline.ljmu.ac.uk/10457/

Article

Climate forcing of an emerging fungal pathogen across a multihost community

Frances C. Clare[1,3]*, Julia B. Halder[3], Olivia Daniel[2], Jon Bielby[1], Mikhail A. Semenov[4], Thibaut Jombart[3], Adeline Loyau[5,6,7], Dirk S. Schmeller[5,6], Andrew A. Cunningham[1], Marcus Rowcliffe[1], Trenton W. J. Garner[1], Jaime Bosch[8], Matthew C. Fisher [3]*

1. Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, UK

2. Department of Life Sciences, Silwood Park Campus, Imperial College London SL5 9PU, UK

3. Department of Infectious Disease Epidemiology, Imperial College London W2 1PG, UK

4. Computational and Systems Biology, Rothamsted Research, West Common, Harpenden, Hertfordshire, AL5 2JQ

5. Université de Toulouse; UPS, INPT; EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement); 118 route de Narbonne, 31062 Toulouse, France

6. Helmholtz Centre for Environmental Research- UFZ, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany

7. Helmholtz Centre for Environmental Research - UFZ, Department of System Ecotoxicology, Permoserstr. 15, 04318 Leipzig, Germany

8. Departamento de Biodiversidad y Biologia Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, Jose Gutierrez Abascal, 2 28006 Madrid, Spain

Corresponding authors:

*Frances C. Clare, Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, UK; +44 2074496626; frances.clare@ioz.ac.uk

*Matthew C. Fisher, Dpt. Infectious Disease Epidemiology, Norfolk Place, London W2 1PG; +44 2073727733 matthew.fisher@imperial.ac.uk

Summary

Changes in the timings of seasonality as a result of anthropogenic climate change are predicted to occur over the coming decades. While this is expected to have widespread impacts on the dynamics of infectious disease through environmental forcing, empirical data is lacking. Here, we investigated whether seasonality affected susceptibility to infection by the emerging fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) across a montane community of amphibians that are suffering declines and extirpations as a consequence of this infection. We found a robust temporal association between the timing of the spring thaw and *Bd* infection in two host species, where we show that an early onset of spring forced high prevalences of infection. A third highly susceptible species (the midwife toad *Alytes obstetricans*) maintained a high prevalence of infection independent of seasonality. Our data show that perennially overwintering midwife toad larvae act as a year-round reservoir of infection with seasonality determining the extent to which infection spills over into sympatric species. We used future temperature projections based on global climate models to demonstrate that the timing of spring thaw in this region will advance markedly by the 2050's, indicating that climate change will further force the severity of infection. Our findings of the effect of seasonality on multi-host infection dynamics show that the community-level impact of fungal infectious disease on biodiversity will need to be re-evaluated in the face of climate change.

Introduction

Climate change is likely to influence infectious disease dynamics, with many pathogens, especially those with complex life cycles or those infecting ectothermic hosts, predicted to increase in severity or range as the earth continues to warm (1-3). *Batrachochytrium dendrobatidis* (*Bd*), one of two fungi known to cause amphibian chytridiomycosis, is associated with the decline and extinction of amphibians worldwide (4, 5). In common with other infections, it is widely assumed that climate change has facilitated epizootics of chytridiomycosis, thus allowing *Bd* to establish in naïve ecosystems (6-8).

A link between climatic variables, host phenology (the timing of recurring natural phenomena) and the population level impact of *Bd* has been investigated by both *in situ* (6, 7), and *ex situ* (8-10) studies. However, the hypothesis that climate change dictates *Bd* infection dynamics in nature has not been proven. This is because the key *in situ* studies to date have confounded two variables, pathogen introduction and environmental variation (6, 7), and the required longitudinal epidemiological studies in established diseased ecosystems have not yet been undertaken (11, 12). Further, *Bd* is a generalist pathogen (13), exhibiting broad variation in its ability to infect and cause disease across species (14). Within multi-species amphibian assemblages, different host species exhibit a range of responses to pathogen exposure. These responses include resistance to infection (diluters of infection), infection tolerance (reservoirs of infection), and variation in susceptibility to lethal disease (15, 16). Yet, studies to date have focussed on the most readily infected species assuming homogeneous host response over time, and data that addresses the medium to long-term temporal impact of *Bd* across all members of a host community in concert with local climatic data, is absent.

Climate variation is pronounced in montane systems, and it is in these environments that epizootics of chytridiomycosis predominantly occur (17-20) presenting an opportunity to untangle the complex relationship between disease dynamics and climate. One such environment, the Pyrenean mountain range, contains many lakes housing multi-species amphibian assemblages within an expanding epizootic of *Bd* infection (20). Across the core outbreak region of the Western Pyrenees, the midwife toad (*Alytes obstetricans*) experiences annual mass mortality due to chytridiomycosis. Two other anuran species also commonly breed in this area, the common toad (*Bufo spinosus,* previously known as *Bufo bufo* (21)) and the common frog (*Rana temporaria*).

54 Across Europe, the prevalence of *Bd* within affected common toads is usually low (14),

55 with the majority of individuals tolerating infection (22). In comparison, the common

56 frog is considered to be resistant to *Bd* infection (14, 23), and until now there has been no

57 evidence of disease in this species. As a result of the differences that these three species

58 exhibit in their response to exposure to *Bd*, they constitute an ideal study-system for

59 exploring the temporal trends in pathogen infection at a community level. To

60 understand the inter-relationship between infection, community and climate, we

61 investigated seasonality in this montane ecosystem and studied how this affects

62 infection dynamics at the present, and when extrapolated into the future using

63 downscaled outputs of global climate change models.

Results and Discussion

65 We monitored amphibians at a key remote infected site, Lac Arlet (altitude 1,986 m absl), in

66 the French Pyrenees over seven years. Across this period we found a robust temporal link

67 between disease dynamics (Fig. 1a & b) and the timing of spring across this community of

68 amphibians (Fig. 1c & d). We uncovered a significant effect of the onset of spring on the

69 prevalence of *Bd* infection in both *B. spinosus* (slope = -0.1000; z = -4.715, d.f. = 149, $p<0.001$;

70 adjusted $R^2=0.19$), and *R. temporaria* (slope = -0.039, z = -4.356, d.f. = 207, $p<0.001$; adjusted

71 $R^2=0.08$), with early spring onset resulting in a higher prevalence of *Bd* infection (Fig. 1d).

72 The highly susceptible species, *A. obstetricans*, maintained a high prevalence of infection

73 throughout the study independent of the onset of spring for both overwintering tadpoles

74 and metamorphs ($p=0.09$). The timing of the end of season showed little variation between

75 years (Fig. 1c; Table 1), with the corollary that the length of the amphibian activity period

76 was increased in years with an early spring onset.

77 The fact that species previously resistant to infection and disease alters in different

78 environmental / climatic contexts illustrates the importance of understanding community-

79 level dynamics when considering the impacts of infections. Our data show that a species

80 thought to be highly resistant to infection and disease can suffer mortality (Fig. 2a); prior to

81 the current study, *R. temporaria* has rarely been found to be infected with *Bd* (14) and has

82 never been observed to suffer chytridiomycosis despite widespread surveillance across

83 Europe. However, we detected widespread infection in this species, along with mortality

84 due to chytridiomycosis, when seasonal onset was early. We also found concurrent temporal

85 changes in the prevalence of *Bd* infection in the more susceptible species, *B. spinosus*, which

86 became locally extinct in 2013 (Fig. 1a & b). We believe that the abrupt decline of *B. spinosus*

87 was disease-driven, due to the high prevalence of infection and widespread mortality

88 detected in metamorphs in the years leading to its disappearance. The number of *A.*

89 *obstetricans* OW larvae has also declined steadily at Lac Arlet during the seven-year course of

90 our study alongside high recorded mortality (Fig 2b; Table 2), showing that mortality due to

91 chytridiomycosis is leading to synchronous multi-species declines across this site.

92 Our data suggest that the susceptible and highly parasitised *A. obstetricans* are acting as the

93 key biological reservoir of *Bd* in this system, and that seasonality is determining the extent to

94 which infection spills over into sympatric species. At the altitude of Lac Arlet, *A. obstetricans*

95 tadpoles over-winter for a number of years before completing development. The tadpole

96 stage of the majority of amphibians rarely suffer mortality or visible effects of *Bd* infection

97 (24, 25) and these life-history traits result in over-wintering tadpoles playing a leading a role

98 in the maintenance of *Bd* across seasons, as has been proposed previously for other systems

99 (19, 26). The yearly changes in infection prevalence that we detected in hosts that are less

100 susceptible to infection could imply that the density of one generally heavily infected

101 species, *A. obstetricans,* drives infection levels within this system. We detected the highest

102 abundance of *A. obstetricans* metamorphs in 2010 and, in that year we also saw the highest

103 proportional mortality, with almost half of all *A. obstetricans* individuals encountered being

104 dead. It is possible that the sharp increase in prevalence of infection in 2010 that we detected

105 in both *B. spinosus* and *R. temporaria* was a direct result of the observed increase in the

106 number of infected *A. obstetricans* in that year. However, there was no significant association

107 between the number of deceased *A. obstetricians* mortalities seen each year and the

108 prevalence of infection in either species. Further, the prevalence of infection in both *B.*

109 *spinosus* and *R. temporaria* continued to increase the following year (Fig.1a), despite a

110 decrease in the abundance of *A. obstetricans*. This contraindicates the hypothesis that the

111 density of *A. obstetricans* is the sole driver of force of infection upon sympatric species.

112 To assess the impact of climate change on onset of spring, the optimal time period over

113 which mean air temperature is most closely related to time of spring thaw was estimated

114 (supplementary Fig. 1). For 2007-2015, mean temperature over days 83-153 (a 70 day period

115 centred on day 118 of the year) was found to be the best predictor of onset of spring,

116 explaining 73% of the variance. For every 1 degree increase in the mean temperature over

117 this time, onset of spring is estimated to be 10 days earlier (Fig. 2c).

5

118   We then used the LARS-WG weather generator as a downscaling technique to generate

119   local-scale climate scenarios for the site, based on projections from global climate models

120   from the CMIP5 multi-model ensemble under the representative concentration pathway

121   (RCP) 8.5 (greenhouse gas emissions continue rising over 21st[t] Century) (27) and modelled

122   using local weather data (Fig. 2c). To capture uncertainty in the CMIP5 climate projections

123   we selected two global climate models, GISS-E2 and HadGEM2, with low and high climate

124   sensitivities respectively, which thus predict lesser and greater amounts of warming for the

125   region. The predictions all indicate that an early onset of spring in the western Pyrenees will

126   become commonplace by the 2050s due to global warming (Fig. 2d). Over days 83-153 of the

127   year, the mean daily temperature is projected to be around 5.5°C by GISS-E2 and 5.8°C by

128   HadGEM2 in the 2050s (medians of 100 years plausible weather generated by LARS-WG)

129   (Fig. 2d). This corresponds, under the current relationship, with an onset of spring around

130   day 130-133, comparable to that seen in 2011. By the 2090s, the median GISS-E2 scenario for

131   this time is 6.5°C, potentially indicating an onset of spring around day 122. The median

132   HadGEM2 scenario is 7.8°C; since this is out of the range of observed temperatures (with the

133   rest of the year also being substantially warmer) we make no estimated projection of onset of

134   spring under this scenario. LARS-WG output across the winter months for the 2090s for

135   HadGEM2 suggests that daily mean temperature will rarely drop below 0°C (daily

136   minimum temperature may be below freezing for 3 months, compared to currently around 6

137   months), which may lead to a short, punctuated, or non-existent duration of ice cover

138   (supplementary Fig. 2). If the trends that we have established here continue, increases in

139   amphibian activity periods are predicted to correspond to increases in infection levels across

140   these species, which could have profound effects on populations of hosts we currently

141   consider to be resistant or tolerant of infection.

142   The mechanisms underpinning the seasonal forcing of infection that we observe are

143   unknown. A broad range of biotic and abiotic factors are influenced by seasonality, and it is

144   probable that many factors acting on both the host and the pathogen are driving the

145   seasonal prevalence of infection in the amphibians studied. On one hand, abiotic drivers

146   include increased temperature volatility causing a reduction in temperature acclimation of

147   host resistance to infection (28), and patterns of disease are known to be also exacerbated as

148   hosts are moved outside of their temperature norms (9). On the other hand, biotic factors

149   such as the density and species complement of aquatic microfauna present at our research

150   site are known to modulate the risk of infection (29). Aquatic microfauna have seasonal

151   peaks and dips in abundance throughout the spring and summer corresponding to the local

152   availability of nutrients (30), therefore, changes in seasonality may affect the abundance of

153   the microfauna, impacting the removal of *Bd* zoospores and force of infection. When

154   complex biotic and abiotic drivers such as these interact, nonlinear responses are expected

155   and the uncertainty associated with predicting future trends in this, and other montane,

156   systems, will likely be high. However, regardless of the underlying mechanisms, our

157   findings robustly show that seasonality drives synchronous infection dynamics in *B. spinosus*

158   and *R. temporaria*, despite previous research indicating a clear difference in susceptibility to

159   *Bd* infection between these species (14, 31). More broadly, we have shown that failing to

160   include environmental information may undermine our understanding of how pathogens

161   spread and persist within host communities. Identifying differences in infection levels across

162   species under different environmental conditions is therefore central to understanding

163   disease ecology within multi-host communities, especially where changes in climate are

164   predicted to exacerbate the impact of emerging infectious disease leading to further losses of

165   biodiversity.

166

Methods

168   The study was conducted at Lac Arlet (Longitude: 0°36'54.12"W, Latitude: 42°50'24.20"N) in

169   the Pyrenean National Park between 2007 and 2014 (with dates of thaw up to 2015). This

170   lake sits at an altitude of 1986 m and encompasses an area of 2.7ha (Fig. 3).

171   *Prevalence and intensity of Bd infection*

172   All three species of amphibian present at Lac Arlet were sampled: emerging metamorphs of

173   *B. spinosus* and *R. temporaria* and two life stages of *A. obstetricans*, over-wintered (OW)

174   tadpoles and recent metamorphs; OW tadpoles are those which have spent at least one

175   winter in the lake before completing metamorphosis. To sample the animals, sterile rayon-

176   tipped swabs (MWE medical wire) were gently rotated (10 times per individual) over the

177   mouthparts of OW tadpoles, and used to swab the hind legs, feet and pelvic patch (five

178   swipes per area, with the swab rotated between each area) of each recent metamorph. All

179   swabs were stored in dry tubes at 4°C until processing. Sampling of tadpoles was conducted

180   each year in July. Metamorphs were sampled in August during the height of metamorphic

181   emergence. In 2007, toe clips were taken from metamorphic *A. obstetricans* instead of skin

182 swabs; a 2-3mm clip was cut from a single hind toe using a sterile scalpel blade. These toe

183 clips were fixed in 70% ethanol.

184 We followed the protocol of Boyle et al. (32), to quantify *Bd* prevalence and intensity of

185 infection, as assessed by quantitative PCR (qPCR). To avoid inhibition, all extractions were

186 diluted 1:10 prior to qPCR; therefore results were multiplied by 10 in order to determine the

187 true value. We defined infection intensity as the number of *Bd* zoospore genomic

188 equivalents (GE) per swab. All samples were run in duplicate, and a sample was assigned a

189 positive reading if both wells amplified and an average estimate of 0.1GE or above was

190 produced when comparing the sample to the curve generated by the standards. Samples

191 were repeated up to three times if only one well amplified, after which time they were

192 assigned a negative reading if both wells failed to amplify.

193 *Population counts*

194 Counts of newly emerged live and dead metamorphic *A. obstetricans* were conducted twice

195 during each metamorph emergence period each year from 2010 to 2014. Searches were

196 carried out around the entire circumference of the lake, from the shallows of the lakes (<0.5

197 metres in depth) to a 1.5m distance from the water's edge onto dry land, including under

198 movable rocks. Any deceased metamorphs found were removed so as not to be counted a

199 second time on subsequent visits. We assumed that live animals would either disperse from

200 the water's edge within a few days of emergence or die, and would therefore not be counted

201 again in subsequent live estimates. Approximate counts (<100; 100-1000; >1000) were made

202 for both newly emerged *R. temporaria* and *B. spinous* metamorphs each year, during the

203 above surveying time. This measure was also applied to *A. obstetricans* OW larvae by

204 scanning a 2m area of the water, from the water's edge.

205 *Diagnosis of chytridiomycosis in* **R. temporaria**

206 Owing to the high prevalence of *Bd* that we observed infecting *R. temporaria*, we decided to

207 investigate whether individuals were suffering chytridiomycosis due to *Bd* infection,

208 something which has not been shown before. Recently deceased, fresh *R. temporaria*

209 metamorphs found around the edge of the lake were collected and fixed in 10% neutral

210 buffered formalin. After fixation, the lower half of the each body (including pelvic region)

211 and the front legs were processed for histopathological examination. Three levels per section

212 were prepared, using a standard Haematoxylin & Eosin stain. Histological sections were

213   examined microscopically to determine the presence or absence of the disease

214   chytridiomycosis.

Determining length of season

216   Lac Arlet water temperature was measured every half an hour throughout the study period

217   using a data logger (HOBO Water Temperature Pro v2 Data Logger - U22-001). The logger

218   was positioned two meters from the shore, approximately half a meter below the water

219   surface, attached to a large rock with non-perishable wire. For each year, the date of the

220   onset of spring and the date of the presumed end of the amphibian active season was

221   recorded. The onset of spring was defined as the first day of the year (00:00 – 23:59) with a

222   mean water temperature above 1°C, which remained so until the winter. The end of the

223   active season was defined as the first day in the second half of the year when the mean

224   water temperature dropped below 5°C, as European amphibians will often enter hibernation

225   around this temperature (33). This allowed the length of the active season (total number of

226   possible 'active days' for amphibians) to be calculated by subtracting the Julian date of the

227   onset of spring from the Julian date of the end of season.

Establishing the relationship between air temperature and lake thaw (onset of spring)

229   To project the impact of rising temperatures, we first quantified a linear relationship

230   between air temperature and time of thaw, which corresponds to onset of spring for this

231   ecological system. This is consistent with findings from other studies showing air

232   temperature to be highly informative for thawing of many lakes (34), even if temperatures

233   are measured some distance from the lake. To establish this relationship for this site, daily

234   weather data (maximum and minimum temperatures, precipitation) were obtained from the

235   nearest meteorological station to Lac Arlet, Canfranc Los Arañones, 13km south-east of Lac

236   Arlet. This is situated at 1160 metres above sea level, 826 metres below Lac Arlet. Hence, a

237   correction of -5.29 °C was applied to the air temperatures to account for tropospheric

238   temperature decrease with altitude, in accordance with previous studies (35). These adjusted

239   temperature data along with daily precipitation for 1995-2015 were also used as baseline

240   input for LARS-WG.

241   To find the optimal time period over which these air temperatures are most predictive for

242   ice thawing time, and to quantify that relationship, linear regressions were performed

243   between air temperature and thaw dates for 2007-2015 inclusive. Mean daily air temperature

244   was calculated over time periods of lengths varying from 21-81 days in 10-day increments,

245   centred on days 30-170 of the year. The time period with the best predictive power for onset

246   of spring, as judged by R-squared value and standard model checking plots of the linear

247   regression, was chosen. Analysis was performed in R version 3.2.3.

248   We used the LARS-WG weather generator as a downscaling technique (36) to generate local-

249   scale climate scenarios, based on climate projections from global climate models (GCMs)

250   from the CMIP5 multi-model ensemble used in the latest IPCC Assessment Report 5 (AR5)

251   (27). To capture uncertainty in the CMIP5 climate projections we selected two GCMs with

252   low, (GISS-E2), and high, (HadGEM2), climate sensitivities (37), which thus predict lesser

253   and greater degrees of warming for this region. This allowed us to quantify uncertainty in

254   predictions of the onset of spring under climate change. We generated 100 years of daily

255   plausible weather for the periods 2050s and 2090s under Representative Concentration

256   Pathway (RCP) 8.5. The mean temperatures across the time period ascertained as most

257   predictive for lake thaw were calculated from each of the 100 years of synthetic daily

258   weather, for each GCM and time period combination.

259   *Statistical analysis*

260   All statistical analyses were carried out using the statistical software package 'R', version

261   3.2.3. All *Bd* DNA values (GE) were rounded to the nearest whole number and treated as

262   count data. Any value of 0.1 to 0.9 was assigned a value of 1. Negative binomial regression

263   models (function glm.nb from the R-package MASS) were used to look for any differences in

264   the intensity of infection (GE values) between years and species. Likelihood ratio tests were

265   used to assess the significance of predictor variables and of differences between factor levels

266   within predictors. Where more than three factor levels remained significant, Tukey post-hoc

267   tests (function glht from the package multcomp) were applied to allow pairwise

268   comparisons. Fisher's Exact Test was used to compare differences in the prevalence of

269   infection in each of *A. obstetricans* metamorphs and tadpoles, over the years 2007 (2008 for

270   tadpoles) to 2014; in *B. spinosus* compared to *R. temporaria* over the years 2008 to 2012 and

271   between each of *B. spinosus* and *R. temporaria* compared to *A. obstetricans* metamorphs over

272   the years 2008-2014 (2008 -2012 for *B. spinosus*). Pearson Correlation tests were used to

273   determine whether there was a correlation between the prevalence of infection in both *B.*

274   *spinosus* and *R. temporaria* and, 1) the mean infection intensity (GE) in *A. obstetricans*

275   metamorphs, 2) the number of dead *A. obstetricans* metamorphs encountered each year.

276 Using the temperature data we were able to determine the date of the spring onset for all

277 years apart from 2010, due to a failure of the datalogger resulting in missing data. However,

278 a strong association was seen between the onset of spring and the date at which the first *A.*

279 *obstetricans* metamorph was seen in the years excluding 2010 (t= 7.724; *p* = 0.005, adjusted R^2

280 = 0.94). We used this strong association to predict the onset of spring in 2010 and included

281 the predicted value in all further analyses. The onset of season (mean Julian days=150, SD

282 =20) varied more than the end of season (mean Julian days=302, SD =4), therefore the onset

283 of spring is the measure which primarily dictates season length. For this reason, we used

284 spring onset as a proxy to assess changes in season length. Generalized Linear Models

285 (GLM) using a binomial response (logistic regression) were used to determine if there was a

286 relationship between the prevalence of infection in all three species and the onset of spring

287 each year. Likelihood ratio tests were used to assess the significance of these effects. A

288 generalized adjusted R^2 was calculated to assess the predictive power of each model (38).

Acknowledgments

References

297 1.   Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, et al. Ecology -

298 Climate warming and disease risks for terrestrial and marine biota. Science.

299 2002;296(5576):2158-62.

300 2.   Epstein P. The ecology of climate change and infectious diseases: comment. Ecology.

301 2010;91(3):925-8.

302 3.   Rodo X, Pascual M, Doblas-Reyes FJ, Gershunov A, Stone DA, Giorgi F, et al. Climate

303 change and infectious diseases: Can we meet the needs for better prediction? Climatic

304 Change. 2013;118(3-4):625-40.

305 4.   Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, et al. Status

306 and trends of amphibian declines and extinctions worldwide. Science. 2004;306(5702):1783-6.

307   5.   Fisher MC, Garner TWJ, Walker SF. Global Emergence of *Batrachochytrium*

308   *dendrobatidis* and Amphibian Chytridiomycosis in Space, Time, and Host. Annual Review of

309   Microbiology. 2009;63:291-310.

310   6.   Pounds AJ, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, et

311   al. Widespread amphibian extinctions from epidemic disease driven by global warming.

312   Nature. 2006;439:161-7.

313   7.   Bosch J, Carrascal LM, Duran L, Walker S, Fisher MC. Climate change and outbreaks

314   of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? P R Soc B.

315   2007;274(1607):253-60.

316   8.   Voyles J, Johnson LR, Briggs CJ, Cashins SD, Alford RA, Berger L, et al. Temperature

317   alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen

318   associated with the global loss of amphibians. Ecol Evol. 2012;2(9):2241-9.

319   9.   Ribas L, Li MS, Doddington BJ, Robert J, Seidel JA, Kroll JS, et al. Expression

320   Profiling the Temperature-Dependent Amphibian Response to Infection by *Batrachochytrium*

321   *dendrobatidis*. Plos One. 2009;4(12):-.

322   10.   Garner TWJ, Rowcliffe JM, Fisher MC. Climate change, chytridiomycosis or

323   condition: an experimental test of amphibian survival. Global Change Biol. 2011;17(2):667-

324   75.

325   11.   Rohr JR, Raffel TR. Linking global climate and temperature variability to widespread

326   amphibian declines putatively caused by disease. P Natl Acad Sci USA. 2010;107(18):8269-74.

327   12.   Rohr JR, Raffel TR, Romansic JM, McCallum H, Hudson PJ. Evaluating the links

328   between climate, disease spread, and amphibian declines. P Natl Acad Sci USA.

329   2008;105(45):17436-41.

330   13.   Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, et al.

331   Mapping the Global Emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid

332   Fungus. Plos One. 2013;8(2).

333   14.   Balaz V, Voros J, Civis P, Vojar J, Hettyey A, Sos E, et al. Assessing Risk and

334   Guidance on Monitoring of *Batrachochytrium dendrobatidis* in Europe through Identification

335   of Taxonomic Selectivity of Infection. Conservation Biology. 2014;28(1):213-23.

336   15.   Bielby J, Bovero S, Angelini C, Favelli M, Gazzaniga E, Perkins M, et al. Geographic

337   and taxonomic variation in *Batrachochytrium dendrobatidis* infection and transmission within

338   a highly endemic amphibian community. Divers Distrib. 2013;19(9):1153-63.

339   16.   Doddington BJ, Bosch J, Oliver JA, Grassly NC, Garcia G, Schmidt BR, et al. Context-

340   dependent amphibian host population response to an invading pathogen. Ecology.

341   2013;94(8):1795-804.

342   17.   Bosch J, Martínez-Solano I, García-París M. Evidence of a chytrid fungus infection

343   involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas

344   of central Spain. Biol Conserv. 2001;97:331-7.

345   18.   Whiles MR, Lips KR, Pringle CM, Kilham SS, Bixby RJ, Brenes R, et al. The effects of

346   amphibian population declines on the structure and function of Neotropical stream

347   ecosystems. Front Ecol Environ. 2006;4(1):27-34.

348   19.   Briggs CJ, Knapp RA, Vredenburg VT. Enzootic and epizootic dynamics of the

349   chytrid fungal pathogen of amphibians. P Natl Acad Sci USA. 2010;107(21):9695-700.

350   20.   Walker SF, Bosch J, Gomez V, Garner TWJ, Cunningham AA, Schmeller DS, et al.

351   Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in

352   Iberia. Ecol Lett. 2010;13(3):372-82.

353   21.   Recuero E, Canestrelli D, Voros J, Szabo K, Poyarkov NA, Arntzen JW, et al.

354   Multilocus species tree analyses resolve the radiation of the widespread *Bufo bufo* species

355   group (Anura, Bufonidae). Mol Phylogenet Evol. 2012;62(1):71-86.

356   22.   Garner TW, Walker S, Bosch J, Leech S, Rowcliffe M, Cunningham AA, et al. Life

357   history trade-offs influence mortality associated with the amphibian pathogen

358   *Batrachochytrium dendrobatidis*. Oikos. 2009(118):783-91.

359   23.   Bielby J, Fisher MC, Clare FC, Rosa GM, Garner TWJ. Host species vary in infection

360   probability, sub-lethal effects, and costs of immune response when exposed to an amphibian

361   parasite. Sci Rep-Uk. 2015;5.

362   24.   Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ. Investigating the population-

363   level effects of chytridiomycosis: An emerging infectious disease of amphibians. Ecology.

364   2005;86(12):3149-59.

365   25.   Bradley GA, Rosen PC, Sredl MJ, Jones TR, Longcore JE. Chytridiomycosis in native

366   Arizona frogs. J Wildlife Dis. 2002;38(1):206-12.

367   26.   Rachowicz LJ, Vredenburg VT. Transmission of *Batrachochytrium dendrobatidis* within

368   and between amphibian life stages. Dis Aquat Organ. 2004;61(1-2):75-83.

369   27.   Stocker T. Climate change 2013: the physical science basis: Working Group I

370   contribution to the Fifth assessment report of the Intergovernmental Panel on Climate

371   Change.: Cambridge University Press; 2014.

372   28.   Raffel TR, Romansic JM, Halstead NT, McMahon TA, Venesky MD, Rohr JR. Disease

373   and thermal acclimation in a more variable and unpredictable climate. Nat Clim Change.

374   2013;3(2):146-51.

375   29.   Schmeller DS, Blooi M, Martel A, Garner TWJ, Fisher MC, Azemar F, et al.

376   Microscopic Aquatic Predators Strongly Affect Infection Dynamics of a Globally Emerged

377   Pathogen. Curr Biol. 2014;24(2):176-80.

378   30.   Scheffer M, Rinaldi S, Kuznetsov YA, vanNes EH. Seasonal dynamics of Daphnia

379   and algae explained as a periodically forced predator-prey system. Oikos. 1997;80(3):519-32.

380   31.   Bosch J, Rincon PA. Chytridiomycosis-mediated expansion of *Bufo bufo* in a montane

381   area of Central Spain: an indirect effect of the disease. Divers Distrib. 2008;14(4):637-43.

382   32.   Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. Rapid quantitative detection

383   of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time

384   Taqman PCR assay. Dis Aquat Organ. 2004;60(2):141-8.

385   33.   Reading CJ. The effect of winter temperatures on the timing of breeding activity in

386   the common toad *Bufo bufo*. Oecologia. 1998;117(4):469-75.

387   34.   Thompson R, Ventura M, Camarero L. On the climate and weather of mountain and

388   sub-arctic lakes in Europe and their susceptibility to future climate change. Freshwater Biol.

389   2009;54(12):2433-51.

390   35.   Thompson R, Price D, Cameron N, Jones V, Bigler C, Rosen P, et al. Quantitative

391   calibration of remote mountain-lake sediments as climatic recorders of air temperature and

392   ice-cover duration. Arct Antarct Alp Res. 2005;37(4):626-35.

393   36.   Semenov MA, Stratonovitch P. Use of multi-model ensembles from global climate

394   models for assessment of climate change impacts. Clim Res. 2010;41(1):1-14.

395   37.   Semenov MA, Stratonovitch P. Adapting wheat ideotypes for climate change:

396   accounting for uncertainties in CMIP5 climate projections. Clim Res. 2015;65:123-39.

397   38.   Heinzl H, Mittlbock M. Pseudo R-squared measures for Poisson regression models

398   with over- or underdispersion. Comput Stat Data An. 2003;44(1-2):253-71.

399

400   **Supplementary Information:** see appended supplement

401

402   **Table 1:** The timing, in Julian days, of the start of spring and the end of season

403

Year	Start of spring	End of season	Days of activity
2008	162	302	140
2009	150	NA	NA
2010	137	298	161
2011	120	298	178
2012	145	302	157
2013	181	308	127
2014	160	309	149

404

405   **Table 2:** Visual estimates of amphibian abundance + <100; ++100-1000; +++ >1000

406

Year	*Ao* OW tadpoles	*Rt* Mets	*Bb* Mets
2008	+++	+++	+++
2009	+++	+++	+++
2010	+++	+++	++
2011	+++	+++	++
2012	++	+++	+
2013	+	+++	0
2014	+	+++	0

407

408

Figure Legends

Figure 1 a. Temporal change in prevalence of infection for *Alytes obstetricans* (Ao), *Bufo spinosus* (*Bs*) and *Rana temporaria* (*Rt*); **b.** Temporal change in intensity of infection; **c.** Seasonal changes in water temperature and timing of spring onset in Lac Arlet; **d.** Relationship between spring onset and the prevalence of *Bd* infection across all species

Figure 2 a. Histology slide showing a section from the hind legs of a deceased *R. temporaria* metamorph demonstrating clear evidence of the disease chytridiomycosis. The two long arrows point to two of many sporangia full of zoospores, and the two short arrows point to empty sporangia cases (once zoospores have burst out), both embedded with the upper skin layers; **b.** Counts of live and dead *A. obstetricans metamorphs* over time with the number of dead (black) and alive (white) A. *obstetricans* metamorphs encountered. No 'alive' counts were made for years 2008 - 2009; **c.** Relationship between air temperature and the onset of spring at Lac Arlet; **d.** Future predictions of air temperatures at Lac Arlet

Figure 3 a. Lac Arlet showing the position of temperature datalogger (red arrow). **b.** Mass mortalities of midwive toads *Alytes obstetricans* caused by *Batrachochytrium dendrobatidis* lineage *Bd*GPL at Lac Arlet