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2 **Manuscript title:** Sex-biased disease dynamics increase extinction risk by impairing
3 population recovery

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19

20 Running head: Sex-biased disease dynamics and extinction risk

21

22 **Sex-biased disease dynamics increase extinction risk by impairing**
23 **population recovery**

24 **Abstract**

25 The periodicity of life-cycle events (phenology) modulates host availability to pathogens
26 in a repeatable pattern. The effects of sexual differences in host phenology have been
27 little explored in wildlife epidemiological studies. A recent series of ranaviruses outbreaks
28 led to serious declines of Boscas' newt populations at Serra da Estrela (Portugal). The
29 peculiar phenology of this species, in which a large number of females remain in the
30 aquatic habitat after the breeding season, turns it into a suitable model to test how sex-
31 biased mortality can affect host population persistence in the context of infectious
32 diseases. We investigated how the phenology of Bosca's newt (i.e. biased number of
33 females) mediated the impact of *Ranavirus*. We then evaluated the risk of extinction of
34 the population under different scenarios of sex-biased mortality using a population
35 viability analysis. Two newt populations (one subject to yearly outbreaks and a
36 comparative site where outbreaks have not been recorded) were tracked for trends over
37 time following emergence of ranaviral disease, allowing us to assess the differential
38 impact of the disease on both sexes. In addition to a significant decline in abundance of
39 adult newts, our data suggest that phenology can affect disease dynamics indirectly,
40 leading to reduction in females and a reversal of the sex ratio of the breeding population.
41 Our models suggest that female-biased mortality does not exacerbate *Ranavirus*-driven
42 population declines in the short-term, but is likely to have a deleterious impact during the
43 recovery process once the lethal effect of disease is removed from the system.

44

45

46 **Keywords.** Bosca's newt; emerging infectious diseases; host-pathogen dynamics;
47 phenology; *Lissotriton boscai*; population viability analysis; *Ranavirus*

48

Introduction

49 Variation in virulence amongst host species or populations (e.g., Daszak *et al.*,
50 2000; Filotas & Hajek, 2004) can be mediated by environmental factors (Dowell, 2001;
51 Roberts & Wiedmann, 2003; Schmeller *et al.*, 2014; Raffel *et al.*, 2015), pathogen type or
52 genotype (Farrer *et al.*, 2011; Price *et al.*, 2014), or host community structure (Begon,
53 2008). However, within-population host heterogeneity may also produce differences in
54 susceptibility to a pathogen as well as recovery, altering the population-level impact of
55 disease, and driving ecological phenomena such as pathogen amplification or dilution
56 (Schmidt & Ostfeld, 2001; Keesing *et al.*, 2006; Searle *et al.*, 2011). There are evolutionary
57 (immunogenetic) explanations for variability of host susceptibility, but ecological factors
58 are more commonly invoked as drivers behind, for example, seasonal patterns of disease
59 prevalence (Altizer *et al.*, 2006; Grassly & Fraser, 2006; Brunner *et al.*, 2015). Among
60 these, differences in life-history strategies have been associated with changing rates of
61 pathogen transmission or growth, with phenology potentially playing an important role in
62 host-pathogen dynamics (Visser *et al.*, 2010; Searle *et al.*, 2011). Severe consequences
63 may arise for the host population when pathogen phenology is synchronized with a
64 particularly vulnerable phase of the host life cycle. Nevertheless, there are few empirical
65 studies that explore the relationship between phenology and infectious disease dynamics.

66 Ranaviruses are emerging pathogens of fish, reptiles and amphibians that have
67 caused mass host mortality in North and South America, Australia, Europe and Asia
68 (Schock *et al.*, 2008; Chinchar *et al.*, 2009; Teacher *et al.*, 2010; Chinchar *et al.*, 2011;
69 Chinchar & Waltzek, 2014; Price *et al.*, 2014, 2017; Rosa *et al.*, 2017). Although ranavirosis
70 has been reported for decades, lethal forms of the disease appear to be increasing in
71 incidence and affecting new host populations (Gray *et al.*, 2009; Price *et al.*, 2014, 2016),
72 but impacts on host populations are inconsistent. For example, annual ranavirosis

73 epizootics in North American amphibian populations do not appear to be causing
74 population declines (Brunner *et al.*, 2004; Greer *et al.*, 2005; Sutton *et al.*, 2015),
75 contrasting with the pattern in Europe, where catastrophic host population or amphibian
76 community declines have been recorded (Teacher *et al.*, 2010; Kik *et al.*, 2011; Price *et al.*,
77 2014; Miaud *et al.*, 2016; Rosa *et al.*, 2017). The range of European hosts seems to be, at
78 least in part, determined by pathogen genotype (Price *et al.*, 2014; Price, 2015), but single
79 host species population-level (and presumably community-level) responses may be
80 influenced by other ecological factors (Teacher *et al.*, 2010; North *et al.*, 2015; Rosa *et al.*,
81 2017). European amphibian populations experiencing extensive, persistent and recurring
82 mass mortality may, or may not, undergo population declines, despite ongoing episodes
83 of high mortality being expected to decrease the estimated time to population extinction
84 (Lafferty & Gerber, 2002; Teacher *et al.*, 2010; Price *et al.*, 2016).

85 In both North America and Europe, increased prevalence of infection and
86 virulence are often associated to specific life history stages, whereas mass mortality
87 events are linked to breeding aggregations (Cunningham *et al.*, 1996; Brunner *et al.*, 2004;
88 Whittington *et al.*, 2010; Hoverman *et al.*, 2011; Price *et al.*, 2014). Amphibians are
89 notable for seasonal activity patterns dictated by the relationships between an
90 ectothermic physiology and environmental factors such temperature, rainfall, humidity
91 and sunlight (Duellman & Trueb, 1994). In temperate regions, the majority of amphibian
92 species migrate to breeding sites, forming high density and typically transient adult
93 aggregations. Because most of these species' reproduction involves the production of
94 single, or at best few egg clutches, and females migrate to breeding sites more or less
95 synchronously, aggregations may form and dissipate in a matter of days or a few weeks.
96 One exception to this broad rule is the European newts, including those of the genus
97 *Lissotriton* Bell, 1839. While adult *Lissotriton* do aggregate in water to reproduce, females

98 produce eggs singly for periods well over a month and replenish sperm reserves
99 throughout this period, requiring both sexes to persist at breeding sites, and with eggs
100 hatching while reproduction is ongoing (Griffiths, 1997; Caetano & Leclair, 1999; Brockes
101 & Kumar, 2005). As a result, *Lissotriton* newts show a peculiar phenology, with males,
102 females and larvae using the aquatic environment both at different and overlapping
103 times.

104 Yearly outbreaks of ranavirosis in Serra da Estrela Natural Park (Portugal) due to
105 Portuguese Newt and Toad Ranavirus (PNTRV; Stöhr *et al.*, 2015) have devastated
106 amphibian populations at some locations, changing host community composition and
107 structure (Rosa *et al.*, 2017). In this study, Bosca's newt populations were tracked over
108 time following emergence of ranavirosis, allowing us to assess the differential impact of
109 disease on adults of both sexes. Making use of this host-pathogen system, we explored
110 how sex-based phenological differences can affect host persistence under hyper-virulent
111 recurring episodes of disease. More specifically, we 1) investigated how the phenology of
112 Bosca's newt mediates the impact of a generalist pathogen; and 2) evaluated the risk of
113 extinction of a population under different scenarios of pathogen-mediated sex-biased
114 mortality.

115 **Material and Methods**

116 **Sites**

117 Serra da Estrela is the highest mountain (1993 m a.s.l.) in Portugal's mainland
118 territory. It is part of the Iberian Sistema Central, being located in the eastern part of
119 north-central Portugal (Daveau, 1971; Mora *et al.*, 2001), and comprising the largest
120 protected area in Portugal: Serra da Estrela Natural Park (PNSE). Disease outbreaks

121 causing mass mortality emerged in the area of Folgoso in the early autumn of 2011
122 (Rosa *et al.*, 2017) in a 255 m² artificial pond of spring water with constant flow
123 (40°29'37.09"N, 7°31'47.61"W, 1079 m a.s.l.). We monitored another spring water
124 artificial pond at a similar elevation where *Ranavirus* outbreaks have never been recorded
125 but where infection occurs: the 50 m² artificial pond in the Sazes area (40°20'39.70"N,
126 7°42'52.63"W; 985 m a.s.l.). The two ponds are about 23 km apart, both approximately
127 1.2-1.7 m deep, located in mountain slopes with the same orientation (facing west), and
128 have the same amphibian assemblage composition breeding regularly (Laurentino *et al.*,
129 2016; Rosa *et al.*, 2017).

130 *Batrachochytrium dendrobatidis*, a fungal pathogen commonly associated with
131 amphibian die-offs in Iberia and elsewhere, has been present in both sites at least since
132 2010, but no signs of mass mortality associated with chytridiomycosis were recorded in
133 Bosca's newt before (Rosa *et al.*, 2013) or during the study (Rosa *et al.*, 2017).

134 **Host phenology**

135 Bosca's newt, *Lissotriton boscai* (Lataste, 1879) is endemic to the western half of
136 the Iberian Peninsula and relatively common in suitable habitats (Pleguezuelos *et al.*,
137 2002; Loureiro *et al.*, 2008). This species is subject to increasing anthropogenic threats,
138 including loss of breeding pools through drainage for agriculture and urbanization,
139 introduction of non-native predators, and also local outbreaks of emerging pathogens
140 (Soares *et al.*, 2003; Arntzen *et al.*, 2009; Rosa *et al.*, 2017).

141 The breeding migration of *L. boscai* does not involve mass movements (Caetano
142 & Leclair, 1999), and at around 1000 m elevation locations in Serra da Estrela males start
143 migrating to the ponds in November, although most wait until February. Mating takes
144 place in April-June and larvae are present between May-December, although a few might

145 overwinter. The adults start leaving the ponds in June, but some individuals, mostly
146 females, stay in the water year-round (Fig. 1). This sex-specific pattern of breeding site
147 occupation is similar to what has been described in other populations (e.g., Caetano &
148 Leclair, 1999). In Serra de Sintra, Malkmus (1980-81) also recorded aquatic activity by this
149 species throughout the year, with male abundance as low as 10% between August and
150 December. After metamorphosis, juveniles are predominantly terrestrial until sexual
151 maturity and rarely encountered in ponds before this (Lizana *et al.*, 1989).

152 **Survey and disease screening**

153 Newts were sampled four times (seasons) per year for 4 years, from 2011 to 2015,
154 with the exception of summer 2014 through to winter 2015. We sampled for 2 to 3 days
155 during each sampling event (capture effort: 4 persons/ hour/ site) and newts were
156 captured using dip nets. The largest count per site within the same season was considered
157 the peak abundance for that season and used for analyses. To assess the disease status of
158 each living individual, a small piece of tail tissue or toe was clipped, and from dead
159 specimens a piece of liver was collected. All tissue samples were stored in 70% ethanol
160 for *Ranavirus* assay (St-Amour & Lesbarrères, 2007). Before release, we applied the
161 antiseptic/analgesic Bactine (Bayer, USA) to the clipped tail/ toe (Martin & Hong, 1991).
162 The clipping mark prevented the animals from being re-sampled within a season.

163 DNA was extracted from tissue samples using the DNEasy Tissue Kit (Qiagen,
164 Hilden, Germany). *Ranavirus* was detected by PCR using the MCP4 and 5 primers targeting
165 the viral MCP gene (CMTV ORF 16L; major capsid protein; AFA44920) as described by Mao
166 *et al.* (1996). All PCR assays were run with a negative control (HPLC water) and positive
167 control (DNA extracted from an infected and confirmed ranavirus-positive tadpole) served

168 as controls for the PCR runs. We have previously reported the results of sequence analysis
169 for products generated from Bosca's newt as PNTRV (Stöhr *et al.*, 2015; Rosa *et al.*, 2017).

170 To prevent cross-contamination and the spread of pathogens across sites, we
171 sterilized tools between samples and wore disposable vinyl gloves to handle animals,
172 changing between specimens. Other field equipment used during the surveys, including
173 hiking boots, was periodically immersed in a 1% solution of Virkon® (Antec International
174 Ltd., Sudbury, Suffolk, UK) according to the protocol suggested by Phillott *et al.* (2010).

175 **Population Viability Analyses**

176 Population viability analyses (PVA) were used to evaluate the risk of extinction
177 and probability of population recovery under different scenarios of *Ranavirus* outbreaks.
178 Models were built using VORTEX v. 10.0, which is primarily used to model the probability
179 of extinction of small populations and the relative effects of differing actions and/or
180 perturbations, allowing for individual age- and sex-structured demographic models
181 (<http://www.vortex10.org>; Lacy *et al.*, 2005). A detailed software description and all
182 demographic parameters used in our models are described in the Supporting Information.

183 To simulate the effects of ranaviriosis-induced mortality on population stability we
184 tested different "harvest" rates (see below) for larvae and adults (the life stages
185 predominantly making use of the aquatic environment), while holding subadult stage
186 abundance constant (0). Models were constructed considering different scenarios of sex-
187 biased mortality. Stochastic simulation of demographic (and epidemiological) processes
188 was carried out with 10,000 iterations and extinction risk was measured as the proportion
189 of populations going extinct within 20 years. We considered extinction to have occurred
190 when only one sex remained. Seven scenarios were considered, testing specific
191 hypotheses generated from field observations:

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192 *Scenario 1: no Ranavirus outbreaks;*

193 *Scenario 2: annual Ranavirus outbreaks within the first 5 years of simulation with a biased*
194 *effect on females; at-risk stages were larvae and >3-year-olds; disease outbreak*
195 *caused constant annual offtake of 40% of larvae and 55% of all mature individuals*
196 *(90% females and 10% males; estimation of annual offtake based on Rosa et al.*
197 *(2017) and this study);*

198 *Scenario 3: same as Scenario 2, but with Ranavirus outbreaks affecting both males and*
199 *females equally: constant annual offtake of 40% of larvae, 55% of all mature*
200 *individuals (equal numbers of males and females);*

201 *Scenario 4: annual Ranavirus outbreaks within the first 10 years of simulation with a*
202 *biased effect on females; at-risk stages were larvae and >3-year-olds; disease*
203 *outbreak caused constant annual offtake of 40% of larvae and 55% of all mature*
204 *individuals (90% females and 10% males);*

205 *Scenario 5: same as Scenario 4, but with Ranavirus outbreaks affecting both males and*
206 *females equally: constant annual offtake of 40% of larvae, 55% of all mature*
207 *individuals (equal numbers of males and females).*

208 *Scenario 6: annual Ranavirus outbreaks over the total time of simulation (20 years) with*
209 *a biased effect on females; at-risk stages were larvae and >3-year-olds; disease*
210 *outbreak was a constant annual offtake of 40% of larvae and 55% of all mature*
211 *individuals (90% females and 10% males);*

212 *Scenario 7: same as for Scenario 6, but with Ranavirus outbreaks affecting both males and*
213 *females equally: constant annual offtake of 40% of larvae, 55% of all mature*
214 *individuals (equal numbers of males and females).*

215 Scenarios #2 and #3 were set to simulate what we observed in our system but also in
216 other Iberian CMTV-like *Ranavirus* systems, where the pathogen has been present in the
217 community for at least 5 years (Price *et al.*, 2014). Scenarios #4 to #7 reflected mid- to
218 long-term pathogen persistence in the population as long as there are suitable hosts and/
219 or environmental conditions, as suggested by Teacher *et al.* (2010), and where *Ranavirus*
220 decreases in virulence in the host species, which we modelled as the end of the epidemic
221 after 5 or 10 years. Additionally, the two cut-off points assume the possibility of eventual
222 conservation intervention/mitigation actions that prevent ongoing mortality. We
223 compared female-biased mortality as this reflected what we observed in nature (see
224 Results).

225 **Statistical analysis**

226 Density was calculated by dividing the highest number of individuals captured in
227 a single day per life stage per sampling season by the area of the aquatic habitat ($n/area$).
228 We used a binary logistic regression to assess the effect of season (season*year) and sex
229 (alone and over time) on the response variable (prevalence of infection). Sex ratio was
230 expressed as the proportion males/(males + females). Comparisons of sex ratios were
231 performed using a Generalized Linear Model (site*time as fixed effects) assuming a
232 binomial error distribution with a logit link function. Post-hoc pairwise comparisons were
233 performed using Bonferroni correction. We used the nonparametric Kruskal-Wallis test to
234 ascertain the differences of extinction probabilities between PVA scenarios. Post-hoc
235 testing was then performed through Dunn-Bonferroni tests to ascertain which pairs of
236 groups differed significantly. Statistical analysis was carried out with software IBM SPSS
237 20.0 (IBM corp. Chicago, USA).

238

Results

239 **Ranavirus and mortality**

240 *Ranavirus* with disease and associated mass mortality was first observed in
241 November (autumn) 2011, where 92.3% of Bosca's newts found at Folgoso were dead.
242 The same scenario occurred annually at about the same time of the year (late
243 summer/early autumn) throughout our field surveys (Figs. 1, 2). Prevalence of infection
244 in live animals broadly mapped with disease dynamics. Prevalence over the two seasons
245 immediately preceding outbreaks or during outbreaks (summer/autumn) consistently
246 averaged out to >75%, while winter/spring averages were significantly lower and, in 2013,
247 close to zero (Fig. 2: Wald $\chi^2 = 18.325$; $df = 1$; $p < 0.001$). Sex-specific prevalences averaged
248 across the entire study (males 34.2%, and females 31.2%), with sex not having a significant
249 effect on infection prevalence over time (Wald $\chi^2 = 0.078$; $df = 1$; $p = 0.779$). The majority
250 of dead and dying adult and larval Bosca's newts tested positive for *Ranavirus* (96%). Sick
251 and dead/moribund animals exhibited all gross signs typical of lethal ranavirosis (see Rosa
252 *et al.*, 2017). Mortality was not recorded during springtime but some positives were
253 detected (e.g., 5%, $n = 20$ in 2013). However, when occasional visits were made to the
254 pond early in the year, dead individuals were observed in the water (> 50 in January and
255 > 10 in March 2012). In contrast, no outbreaks of ranavirosis or mass mortality events
256 were ever recorded at Sazes. Despite virus presence being recorded at the site since 2012
257 (in salamanders) with first detection in Bosca's in 2014 (16.7% prevalence), no animals
258 have shown signs of disease (Rosa *et al.*, 2017). At Folgoso, the adult newt population
259 suffered a decline of 45.5% between 2011 and 2012 and of 68.8% between 2011 and
260 2013. In spring 2014 the decline of the Folgoso population was of 95.5% when
261 compared to the 2011 numbers, before the *Ranavirus* outbreak.

262 **Sex ratio**

263 Populations of newts from both sites showed no differences in the sex ratio in
264 springtime 2011, before the first outbreak of ranaviriosis (Fig. 3 and Table 1). At Sazes,
265 where no outbreaks of disease were detected, there was no significant change in newt
266 sex ratios (spring: Wald $X^2 = 3.328$; $df = 4$; $p = 0.505$; autumn: Wald $X^2 = 1.075$; $df = 2$; $p =$
267 0.584 ; Fig. 3; Table 1) over the period of this study.

268 During the first outbreak in Folgoso, the majority of the adults (91.7%) found
269 dead were females. Females also comprised the majority of dead animals (64.4% across
270 all life stages) throughout the course of the study (Fig. S1). In the following years we noted
271 a reversal in the sex ratio, which shifted from 25% males in late spring 2011 to over 60%
272 in the subsequent years (Fig. 3). There was a significant effect of time after the first
273 outbreak on the sex ratio in Folgoso in springtime (Wald $X^2 = 45.209$; $df = 3$; $p < 0.001$),
274 with a marked difference between the first year and subsequent years (Table 1). The
275 effect was detectable in the first autumn (2011), such that the proportion of males found
276 in the autumn across years did not vary significantly (Wald $X^2 = 0.638$; $df = 2$; $p = 0.727$;
277 see Table 1). Larvae comprised a smaller proportion of dead individuals over the entire
278 study (28%) than adults (70%; Fig. S1).

279 **Risk assessment and population viability**

280 In all scenarios including disease outbreaks, there was a rapid decline in
281 abundance to approximately 15% of the pre-outbreak population size, paralleling values
282 observed in the field (95.5% decline until 2014 and 70.6% by 2015 compared to 2011; Fig.
283 2). Increasing persistence of annual mortality events caused by ranaviriosis and altering
284 the sex bias in probability of mortality had no strong effect on patterns of population
285 declines over the first five years of the simulations. The effect of a 5-year period of female-

286 biased mortality was not significantly different from the effect of mid- and long-term
287 persistence of unbiased mortality (Fig. 4; Fig. S2; Table 3). However, both the persistence
288 of declines and post-decline recovery rates were significantly affected by sex-biased
289 mortality, as well as by sex-unbiased mortality (K-W: $\chi^2 = 55.163$; $df = 6$; $p < 0.001$; Table
290 3). Specifically, and perhaps unsurprisingly, population recoveries began as soon as annual
291 mortality events ceased. However the rate of recovery was significantly slower when
292 ranavirus events lasted ten years when compared to populations where disease ceased
293 after five years (Fig. 4; Fig. S2). Rates were further depressed when mortality was sex-
294 biased: for example, five years after the last outbreak, population size estimated in
295 scenario #3 was 1.8 times greater than for projection #2 (Fig. 4; Fig. S2). In scenarios where
296 disease persisted, a high likelihood of population extirpation was predicted, greater so
297 when mortality was sex-biased (98%, versus 88% when risk of mortality was equal across
298 the sexes). This difference in risk reflected a difference in rate of decline after the 5-year
299 period: median time to extinction was 11 years when mortality was female biased versus
300 16 years (Table 2).

301

Discussion

302 Seasonality of mortality events is not uncommon in ranavirus epidemics and is
303 often invoked as a covariate that affects viral growth dynamics (Pfennig *et al.*, 1991;
304 Dowell, 2001; Rojas *et al.*, 2005; Gray *et al.*, 2007, 2009; Price *et al.*, 2018). Our data
305 suggest seasonality can also affect disease dynamics indirectly through the breeding
306 phenology of amphibians, e.g., by eliciting sex-specific mortality schedules. Sex-biased
307 mortality is not uncommon across different vertebrate groups (e.g., Müller *et al.*, 2005;
308 Sperry & Weatherhead, 2009), and when skewed towards females is expected to lead to
309 accelerated population declines. For example, Gruebler *et al.* (2008) showed that female-

310 biased mortality of whinchats could lead to a 1.7 times faster local population decline. In
311 support of this, we recorded a significant decline in abundance of adult newts at
312 Folgoso (Portugal), but also a reversal of the sex ratio of the breeding population and
313 concurrent disproportionate reduction in female abundance (Fig. 2, 3). Although we
314 cannot determine whether population decline was a simple direct effect of adult (and
315 larval) mortality or if it was also affected by a reduction in recruitment due to a decreasing
316 availability of breeding females, our models show that either of the two mechanisms is
317 enough to drive the rate of decline we observed at Folgoso.

318 Although recovery after declines due to ranavirus can occur (Greer *et al.*, 2008),
319 ranaviruses do meet the conditions required to cause host extinction (Miller *et al.*, 2011).
320 A study by Earl & Gray (2014) modelled the likelihood of extinction of a closed population
321 of wood frogs (*Lithobates sylvaticus*) and showed that extinction could theoretically occur
322 as quickly as 5 years if ranavirus persisted over that time span. Our analyses and models
323 suggest that even if the recurring ranavirus outbreaks do not drive the host to complete
324 extinction, they can reduce population size to a point where stochastic events can
325 eliminate host populations (de Castro & Bolker, 2005). Even if extirpation is avoided,
326 recovery may not occur (Schock & Bollinger, 2005; Teacher *et al.*, 2010). This is also a key
327 finding of our modelling, where we obtained a >88% probability that extinction will occur
328 within a median of 11 to 16 years if outbreaks occur yearly for 10 years, even if they are
329 totally controlled after that.

330 More importantly, we found that female-biased mortality did not exacerbate
331 *Ranavirus*-driven population decline in the short-term, but impaired population recovery
332 once the lethal effect of disease was removed from the system. With that in mind, and
333 assuming the possibility of eventual conservation intervention/mitigation actions based
334 in our projections, this finding suggests that pathogen mitigation does not imply

335 population recovery. As a result of reduced recruitment rates, our models predict a slower
336 population growth, which could be offset if the female population is augmented.

337 What is not considered in our study, though, is the effect of other possible
338 reservoir hosts on both declines and recovery. Our study species exists in a multi-host
339 community system (Rosa *et al.*, 2017), as are other CMTV-affected sites in Western
340 Europe (see Kik *et al.*, 2011; Price *et al.*, 2014; Miaud *et al.*, 2016). Given the broad host
341 range and the potential for ranaviruses to persist in the environment, we are likely
342 underestimating the risk of extinction for Bosca's newts.

343 Previous reports have shown that other populations of *L. boscai* have been
344 affected by ranaviruses outbreaks throughout the northern half of the species distribution
345 range (Soares *et al.*, 2003; Price *et al.*, 2014; Rosa *et al.*, 2017). Moreover, increasing
346 threats to ponds by wildfires, desiccation and other human activities, even within
347 protected areas like Serra da Estrela (ICNB, 2008; Vicente *et al.*, 2013), raise additional
348 concerns from the population management point of view. Vulnerability of these
349 freshwater habitats raises challenges for the conservation of newts and other species
350 affected by *Ranavirus*. However, declines due to ranaviruses are not deterministic, as
351 newts at Sazes have experienced low-level infections over a similar time span and also
352 exhibit similar sex-specific breeding phenologies, but prevalence has yet to reach
353 saturation and newt mass mortality does not occur. Locations like Sazes offer the
354 opportunity to characterize the factors allowing host-*Ranavirus* coexistence. This is
355 important, as unlike the situation with *Batrachochytrium dendrobatidis* (see Bosch *et al.*,
356 2015; Garner *et al.*, 2016), no successful treatment or mitigation measure for ranaviruses
357 has been published.

358

359 **Supporting Information**

360 A detailed PVA software description and all demographic parameters used in our models
361 are described in the Supporting Information, and can be found online at <https://XXXXX>

362

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CAPTIONS FOR TABLES

580 **Table 1** Pairwise comparisons of springtime sex ratios between two populations of
 581 *Lissostriton boscai* over time at Serra da Estrela (Portugal) using a Generalized Linear Model
 582 (site*year). Folgoso: yearly outbreaks of *Ranavirus* record (2011 represent the sex
 583 ratio before the first outbreak); Sazes: no *Ranavirus* outbreak recorded. A *p* value < 0.05
 584 was considered significant and highlighted in bold after Bonferroni correction.

Folgoso	2011	2012	2013	Sazes	2011	2012	2013	2014
2012	< 0.01			2012	1.00			
2013	< 0.01	1.00		2013	1.00	1.00		
2014	††	††	††	2014	1.00	1.00	1.00	
2015	< 0.01	1.00	1.00	2015	1.00	1.00	1.00	1.00

Sites	year	Bonferroni adj. <i>p</i> value
	2011	1.00
Folgoso*Sazes	2012	< 0.01
	2013	0.02
	2015	< 0.01

585 †† No data were considered for Folgoso site in spring 2014.

586 **Table 2** PVA outputs from simulation of disease outbreaks in a population of *Lissotriton*
587 *boscai* under seven different disease mortality offtakes. Adult (55%) and larvae mortality
588 (40%) is constant across scenarios #2–#7 (varying in time and sex offtake) but absent in
589 #1. PE: probability of population extinction after 20 yrs; GR: stochastic growth rate; N:
590 mean population size after 20 yrs; TE: time to first population extinction; SD: standard
591 deviation. Grey shading highlights scenarios with sex-biased mortality.

Scenario	PE	GR \pm SD	N \pm SD	Median TE
#1. no <i>Ranavirus</i> outbreaks	0.001	0.206 ± 0.606	4104.95 ± 1551.87	0
#2. female biased mortality within 5 yrs	0.132	-0.026 ± 0.661	2325.63 ± 2112.10	0
#3. equal sex mortality within 5 yrs	0.009	0.046 ± 0.677	3390.51 ± 1916.13	0
#4. female biased mortality within 10 yrs	0.580	-0.293 ± 0.634	237.41 ± 714.17	12
#5. equal sex mortality within 10 yrs	0.164	-0.149 ± 0.687	769.65 ± 1246.74	0
#6. female biased mortality within 20 yrs	0.984	-0.516 ± 0.393	0.12 ± 0.84	11
#7. equal sex mortality within 20 yrs	0.880	-0.452 ± 0.472	0.67 ± 2.44	16

592

593 **Table 3** Dunn-Bonferroni test between pairs of different scenarios generated from PVA,
594 to look at significant differences in probability of extinction. Adjusted p value < 0.05 was
595 considered significant and highlighted in bold.

Scenario	1	2	3	4	5	6
2	< 0.01					
3	1.00	0.26				
4	< 0.01	1.00	< 0.01			
5	0.03	1.00	1.00	0.31		
6	< 0.01	1.00	< 0.01	1.00	0.10	
7	< 0.01	1.00	0.22	1.00	1.00	1.00

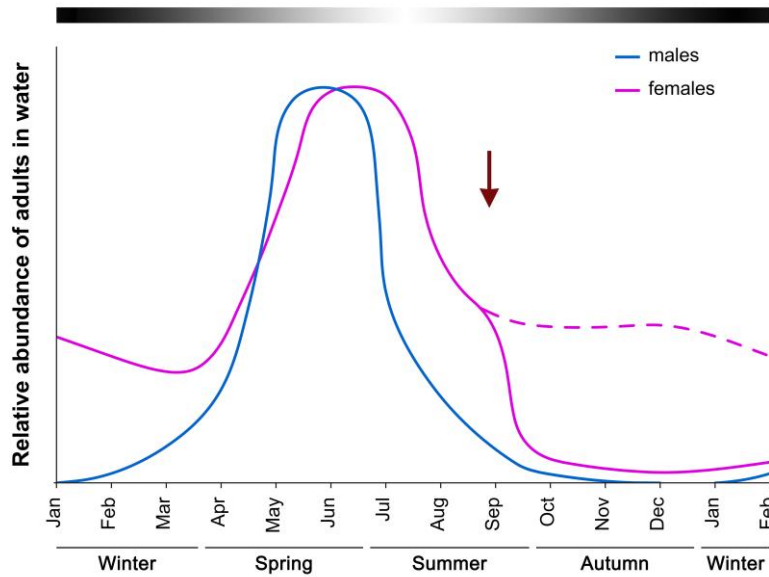
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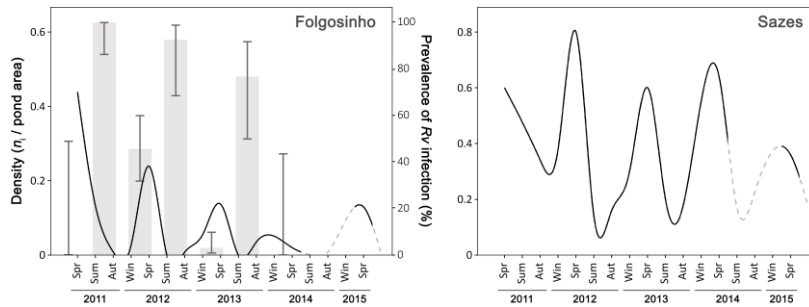
CAPTIONS FOR FIGURES

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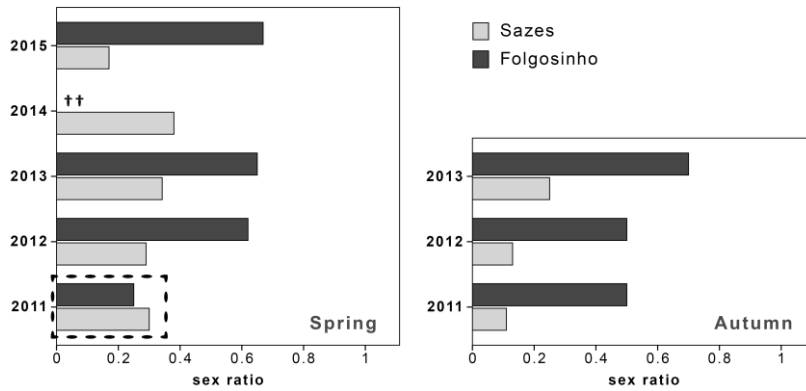
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601 **Figure 1** Schematic phenology of the host species, Bosca's newt (*Lissotriton boscai*) in
602 Serra da Estrela (Portugal) in ponds located at about 800–1100 m of elevation showing a
603 representation of expected relative proportion of males and females in the water
604 throughout the year. Arrow indicates time of the first outbreak of ranavirosis (2011) and
605 dashed line illustrates the expected relative abundance of females in the absence of
606 outbreak (based on relative data from previous years and other similar ponds, collected
607 as part of ongoing amphibian monitoring studies; Rosa *et al.*, 2013, 2017; Laurentino *et*
608 *al.*, 2016). Top bar represents the temperature throughout the year from cool (dark) to
609 warm (light).



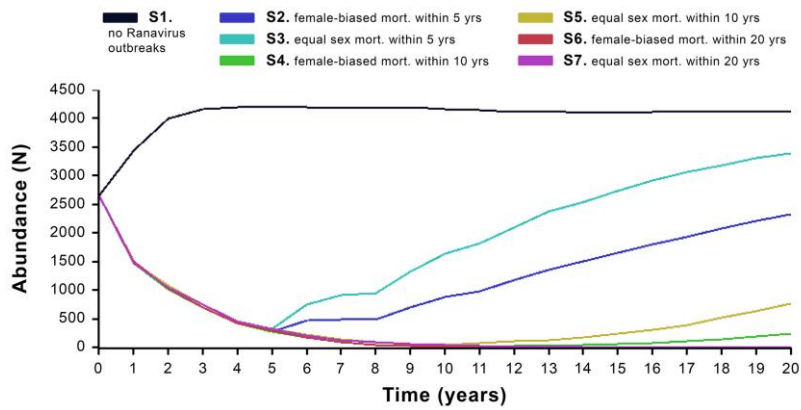
610

611 **Figure 2** Density of adult Bosca's newt (*Lissotriton boscai*) in two sites in Serra da Estrela
 612 (Portugal) over five years (spline interpolation with data missing between summer 2014
 613 and winter 2015): Folgosinho shows the density of newts with yearly outbreaks of
 614 ranaviruses, while Sazes shows a natural population fluctuation in an area where
 615 outbreaks have not been recorded. Prevalence of *Ranavirus* infection in Bosca's newts is
 616 also provided for Folgosinho pond as the total proportion of infected individuals by two
 617 grouped seasons (summer/autumn and winter/spring). Error bars indicate the 95%
 618 confidence intervals.



619

620 **Figure 3** Comparative sex ratio of adult Bosca's newts (*Lissotriton boscai*) in two sites in
 621 Serra da Estrela (Portugal) over five years. Folgoso shows the sex ratio of a population
 622 facing yearly outbreaks of ranavirosis after spring 2011, while Sazes shows the expected
 623 sex ratio of a population where outbreaks have not been recorded. Sex ratio is expressed
 624 as the proportion of males/(males + females) in two different seasons: spring and autumn.
 625 Dashed rectangle highlights the sex ratio in both populations before the first outbreak of
 626 ranavirosis, when there was no difference between them (*L. boscai* $p > 0.05$). ++ No data
 627 for Folgoso site in spring 2014.



628

629 **Figure 4** 20-year population projection for Bosca's newt (*Lissotriton boscai*) in VORTEX
 630 under seven different disease scenarios with no management: S1. No *Ranavirus*
 631 outbreaks; S2. Female-biased mortality during 5 years; S3. Equal sex mortality during 5
 632 years; S4. Female-biased mortality during 10 years; S5. Equal sex mortality during 10
 633 years; S6. Female-biased mortality during 20 years; S7. Equal sex mortality during 10
 634 years.