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

Abstract

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

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

Introduction

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

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

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

In both North America and Europe, increased prevalence of infection and virulence are often associated to specific life history stages, whereas mass mortality events are linked to breeding aggregations (Cunningham *et al*., 1996; Brunner *et al*., 2004; Whittington *et al*., 2010; Hoverman *et al*., 2011; Price *et al*., 2014). Amphibians are notable for seasonal activity patterns dictated by the relationships between an ectothermic physiology and environmental factors such temperature, rainfall, humidity and sunlight (Duellman & Trueb, 1994). In temperate regions, the majority of amphibian species migrate to breeding sites, forming high density and typically transient adult aggregations. Because in most of these species' reproduction involves the production of single, or at best few egg clutches, and females migrate to breeding sites more or less synchronously, aggregations may form and dissipate in a matter of days or a few weeks.

One exception to this broad rule is the European newts, including those of the genus *Lissotriton* Bell, 1839. While adult *Lissotriton* do aggregate in water to reproduce, females produce eggs singly for periods well over a month and replenish sperm reserves throughout this period, requiring both sexes to persist at breeding sites, and with eggs hatching while reproduction is ongoing (Griffiths, 1997; Caetano & Leclair, 1999; Brockes & Kumar, 2005). As a result, *Lissotriton* newts show a peculiar phenology, with males, females and larvae using the aquatic environment both at different and overlapping times.

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

Material and Methods

Sites

Serra da Estrela is the highest mountain (1993 m a.s.l.) in Portugal's mainland territory. It is part of the Iberian Sistema Central, being located in the eastern part of

123 north-central Portugal (Daveau, 1971; Mora *et al*., 2001), and comprising the largest
124 protected area in Portugal: Serra da Estrela Natural Park (PNSE). Disease outbreaks
125 causing mass mortality emerged in the area of Folgosinho in the early autumn of 2011
126 (Rosa *et al*., 2017) in a 255 m^2 artificial pond of spring water with constant flow
127 (40°29'37.09"N, 7°31'47.61"W, 1079 m a.s.l.). We monitored another spring water
128 artificial pond at a similar elevation where *Ranavirus* outbreaks have never been
129 recorded but where infection occurs: the 50 m^2 artificial pond in the Sazes area
130 (40°20'39.70"N, 7°42'52.63"W; 985 m a.s.l.). The two ponds are about 23 km apart, both
131 approximately 1.2-1.7 m deep, located in mountain slopes with the same orientation
132 (facing west), and have the same amphibian assemblage composition breeding regularly
133 (Laurentino *et al*., 2016; Rosa *et al*., 2017).

134 *Batrachochytrium dendrobatidis*, a fungal pathogen commonly associated with
135 amphibian die-offs in Iberia and elsewhere, has been present in both sites at least since
136 2010, but no signs of mass mortality associated with chytridiomycosis were recorded in
137 Bosca's newt before (Rosa *et al*., 2013) or during the study (Rosa *et al*., 2017).

138 **Host phenology**

139 Bosca's newt, *Lissotriton boscai* (Lataste, 1879) is endemic to the western half of
140 the Iberian Peninsula and relatively common in suitable habitats (Pleguezuelos *et al*.,
141 2002; Loureiro *et al*., 2008). This species is subject to increasing anthropogenic threats,
142 including loss of breeding pools through drainage for agriculture and urbanization,
143 introduction of non-native predators, and also local outbreaks of emerging pathogens
144 (Soares *et al*., 2003; Arntzen *et al*., 2009; Rosa *et al*., 2017).

145 The breeding migration of *L. boscai* does not involve mass movements (Caetano
146 & Leclair, 1999), and at around 1000 m elevation locations in Serra da Estrela males start

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

157 **Survey and disease screening**

158 Newts were sampled in the two sites four times (seasons) per year for 5 years,
159 from 2011 to 2015, with the exception of summer 2014 through to winter 2015. We
160 sampled for 2 to 3 days during each sampling event (capture effort: 2 persons/ 2 hour/
161 site) and newts were captured using dip nets. The largest count per site within the same
162 season was considered the peak abundance for that season and used for analyses. To
163 assess the disease status of each living individual, a small piece of tail tissue or toe was
164 clipped, and from dead specimens a piece of liver was collected. All tissue samples were
165 stored in 70% ethanol for *Ranavirus* assay (St-Amour & Lesbarrères, 2007). Before
166 release, we applied the antiseptic/analgesic Bactine (Bayer, USA) to the clipped tail/ toe
167 (Martin & Hong, 1991). The clipping mark prevented the animals from being re-sampled
168 within a season.

169 DNA was extracted from tissue samples using the DNEasy Tissue Kit (Qiagen,
170 Hilden, Germany). *Ranavirus* was detected by PCR using the MCP4 and 5 primers

targeting the viral MCP gene (CMTV ORF 16L; major capsid protein; AFA44920) as described by Mao *et al*. (1996). All PCR assays were run with a negative control (HPLC water) and positive control (DNA extracted from an infected and confirmed ranavirus-positive tadpole) served as controls for the PCR runs. We have previously reported the results of sequence analysis for products generated from Bosca's newt as PNTRV (Stöhr *et al*., 2015; Rosa *et al*., 2017).

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

Population Viability Analyses

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

To simulate the effects of ranavirosis-induced mortality on population stability we tested different "harvest" rates (see below) for larvae and adults (the life stages predominantly making use of the aquatic environment), while holding subadult stage abundance constant (0). Models were constructed considering different scenarios of

sex-biased mortality. Stochastic simulation of demographic (and epidemiological) processes was carried out with 10000 iterations and extinction risk was measured as the proportion of populations going extinct within 20 years. We considered extinction to have occurred when only one sex remained. Seven scenarios were considered, testing specific hypotheses generated from field observations:

Scenario 1: no *Ranavirus* outbreaks;

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

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

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

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

Scenario 6: annual *Ranavirus* outbreaks over the total time of simulation (20 years) with a biased effect on females; at-risk stages were larvae and >3-year-olds; disease

outbreak was a constant annual offtake of 40% of larvae and 55% of all mature individuals (90% females and 10% males);

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

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

Statistical analysis

Density was calculated by dividing the highest number of individuals captured in a single day per life stage per sampling season by the area of the aquatic habitat (*n*/sampled area). We used a binary logistic regression to assess the effect of season (season*year) and sex (alone and over time) on the response variable (prevalence of infection). Sex ratio was expressed as the proportion males/(males + females). Comparisons of sex ratios were performed using a Generalized Linear Model (site*time as fixed effects) assuming a binomial error distribution with a logit link function. Post-hoc pairwise comparisons were performed using Bonferroni correction. We used the

nonparametric Kruskal-Wallis test to ascertain the differences of extinction probabilities between PVA scenarios. Post-hoc testing was then performed through Dunn-Bonferroni tests to ascertain which pairs of groups differed significantly. Statistical analysis was carried out with software IBM SPSS 20.0 (IBM corp. Chicago, USA).

Results

Ranavirus and mortality

Ranavirus with disease and associated mass mortality was first observed in November (autumn) 2011, where 92.3% (48/52) of Bosca's newts found at Folgosinho were dead. The same scenario occurred annually at about the same time of the year (late summer/early autumn) throughout our field surveys (Figs. 1, 2). Prevalence of infection in live animals broadly mapped with disease dynamics. Prevalence over the two seasons immediately preceding outbreaks or during outbreaks (summer/autumn) consistently averaged out to >75%, while winter/spring averages were significantly lower and, in 2013, close to zero (Fig. 2 and Table S1: Wald X^2 = 7.446; *df* = 1*; p* = 0.006). Sex-specific prevalences averaged across the entire study (males 34.2%, and females 31.2%), with sex not having a significant effect on infection prevalence over time (Wald X^2 = 0.008; *df* = 1*; p* = 0.928). The majority of dead and dying adult and larval Bosca's newts tested positive for *Ranavirus* (96%). Sick and dead/ moribund animals exhibited all gross signs typical of lethal ranavirosis (see Rosa *et al*., 2017). Mortality was not recorded during springtime but some positives were detected (e.g., 5%, *n* = 20 in 2013). However, when occasional visits were made to the pond early in the year, dead individuals were observed in the water (> 50 in January and > 10 in March 2012). In contrast, no outbreaks of ranavirosis or mass mortality events were ever recorded at

Sazes. Despite virus presence being recorded at the site since 2012 (in salamanders) with detection in Bosca's in 2014 (2/12, 16.7% prevalence), no animals have shown signs of disease (Rosa *et al*., 2017). At Folgosinho, the adult newt population suffered a decline of 45.5% between 2011 and 2012 and of 68.8% between 2011 and 2013. In spring 2014 the decline of the Folgosinho population was of 95.5% when compared to the 2011 numbers, before the *Ranavirus* outbreak.

Sex ratio

Populations of newts from both sites showed no differences in the sex ratio in springtime 2011, before the first outbreak of ranavirosis (Fig. 3, Table 1 and S2). At Sazes, where no outbreaks of disease were detected, there was no significant change in newt sex ratios (spring: Wald X^2 = 3.342; *df* = 4; *p* = 0.502; autumn: Wald X^2 = 2.706; *df* = 2; *p* = 0.258; Fig. 3, Table 1 and S2) over the period of this study.

During the first outbreak in Folgosinho, the majority of the adults (91.7%) found dead were females. Females also comprised the majority of dead animals (64.4% across all life stages) throughout the course of the study (Fig. S1). In the following years we noted a reversal in the sex ratio, which shifted from 25% males in late spring 2011 to over 60% in the subsequent years (Fig. 3). There was a significant effect of time after the first outbreak on the sex ratio in Folgosinho in springtime (Wald X^2 = 38.075; *df* = 3; $p < 0.001$), with a marked difference between the first year and subsequent years (Table 1and S2). The effect was detectable in the first autumn (2011), such that the proportion of males found in the autumn across years did not vary significantly (Wald X^2 = 0.638; *df* = 2; *p* = 0.727; see Table 1). Larvae comprised a smaller proportion of dead individuals over the entire study (28%) than adults (70%; Fig. S1).

Risk assessment and population viability

In all scenarios including disease outbreaks, there was a rapid decline in abundance to approximately 15% of the pre-outbreak population size, paralleling values observed in the field (95.5% decline until 2014 and 70.6% by 2015 compared to 2011; Fig. 2). Increasing persistence of annual mortality events caused by ranavirosis and altering the sex bias in probability of mortality had no strong effect on patterns of population declines over the first five years of the simulations. The effect of a 5-year period of female-biased mortality was not significantly different from the effect of mid- and long-term persistence of unbiased mortality (Fig. 4; Fig. S2; Table 3). However, both the persistence of declines and post-decline recovery rates were significantly affected by sex-biased mortality, as well as by sex-unbiased mortality (K-W: $X^2 = 55.163$; $df = 6$; $p < 0.001$; Table 3). Specifically, and perhaps unsurprisingly, population recoveries began as soon as annual mortality events ceased. However, the rate of recovery was significantly slower when ranavirosis events lasted ten years when compared to populations where disease ceased after five years (Fig. 4; Fig. S2). Rates were further depressed when mortality was sex-biased: for example, five years after the last outbreak, population size estimated in scenario #3 was 1.8 times greater than for projection #2 (Fig. 4; Fig. S2). In scenarios where disease persisted, a high likelihood of population extirpation was predicted, greater so when mortality was sex-biased (98%, versus 88% when risk of mortality was equal across the sexes). This difference in risk reflected a difference in rate of decline after the 5-year period: median time to extinction was 11 years when mortality was female biased versus 16 years (Table 2).

Discussion

Seasonality of mortality events is not uncommon in ranavirus epidemics and is often invoked as a covariate that affects viral growth dynamics (Pfennig *et al*., 1991;

313 Dowell, 2001; Rojas *et al*., 2005; Gray *et al*., 2007, 2009; Price *et al*., 2018). Our data
314 suggest seasonality can also affect disease dynamics indirectly through the breeding
315 phenology of amphibians, e.g., by eliciting sex-specific mortality schedules. Sex-biased
316 mortality is not uncommon across different vertebrate groups (e.g., Müller *et al*., 2005;
317 Sperry & Weatherhead, 2009), and when skewed towards females is expected to lead to
318 accelerated population declines. For example, Grüebler *et al*. (2008) showed that
319 female-biased mortality of whinchats could lead to a 1.7 times faster local population
320 decline. In support of this, we recorded a significant decline in abundance of adult newts
321 at Folgosinho (Portugal), but also a reversal of the sex ratio of the breeding population
322 and concurrent disproportionate reduction in female abundance (Fig. 2, 3). Although we
323 cannot determine whether population decline was a simple direct effect of adult (and
324 larval) mortality or if it was also affected by a reduction in recruitment due to a
325 decreasing availability of breeding females, our models show that either of the two
326 mechanisms is enough to drive the rate of decline we observed at Folgosinho.
327 Although recovery after declines due to ranavirosis can occur (Greer *et al*.,
328 2008), ranaviruses do meet the conditions required to cause host extinction (Miller *et*
329 *al*., 2011). A study by Earl & Gray (2014) modelled the likelihood of extinction of a closed
330 population of wood frogs (*Lithobates sylvaticus*) and showed that extinction could
331 theoretically occur as quickly as 5 years if ranavirosis persisted over that time span. Our
332 analyses and models suggest that even if the recurring ranavirosis outbreaks do not
333 drive the host to complete extinction, they can reduce population size to a point where
334 stochastic events can eliminate host populations (de Castro & Bolker, 2005). Even if
335 extirpation is avoided, recovery may not occur (Schock & Bollinger, 2005; Teacher *et al*.,
336 2010). This is also a key finding of our modelling, where we obtained a >88% probability

337 that extinction will occur within a median of 11 to 16 years if outbreaks occur yearly for
338 10 years, even if they are totally controlled after that.

339 More importantly, we found that female-biased mortality did not exacerbate
340 *Ranavirus*-driven population decline in the short-term, but impaired population recovery
341 once the lethal effect of disease was removed from the system. With that in mind, and
342 assuming the possibility of eventual conservation intervention/mitigation actions based
343 in our projections, this finding suggests that pathogen mitigation does not imply
344 population recovery. As a result of reduced recruitment rates, our models predict a
345 slower population growth, which could be offset if the female population is augmented.

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

352 Previous reports have shown that other populations of *L. boscai* have been
353 affected by ranavirosis outbreaks throughout the northern half of the species
354 distribution range (Soares *et al*., 2003; Price *et al*., 2014; Rosa *et al*., 2017). Moreover,
355 increasing threats to ponds by wildfires, desiccation and other human activities, even
356 within protected areas like Serra da Estrela (ICNB, 2008; Vicente *et al*., 2013), raise
357 additional concerns from the population management point of view. Vulnerability of
358 these freshwater habitats raises challenges for the conservation of newts and other
359 species affected by *Ranavirus*. However, declines due to ranavirosis are not
360 deterministic, as newts at Sazes have experienced low-level infections over a similar
361 time span and also exhibit similar sex-specific breeding phenologies, but prevalence has

yet to reach saturation and newt mass mortality does not occur. Locations like Sazes offer the opportunity to characterize the factors allowing host-*Ranavirus* coexistence. This is important, as unlike the situation with *Batrachochytrium dendrobatidis* (see Bosch *et al*., 2015; Garner *et al*., 2016), no successful treatment or mitigation measure for ranavirosis has been published.

Supporting Information

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

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CAPTIONS FOR TABLES

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

Folgosinho	2011	2012	2013
2012	**< 0.01**		
2013	**< 0.01**	1.00	
2014	††	††	††
2015	**< 0.01**	1.00	1.00

Sazes	2011	2012	2013	2014
2012	1.00			
2013	1.00	1.00		
2014	1.00	1.00	1.00	
2015	1.00	1.00	1.00	1.00

Sites	year	Bonferroni adj. *p* value
Folgosinho*Sazes	2011	1.00
	2012	**< 0.01**
	2013	**0.02**
	2015	**< 0.01**

†† No data were considered for Folgosinho site in spring 2014.

599 **Table 2** PVA outputs from simulation of disease outbreaks in a population of *Lissotriton*
600 *boscai* under seven different disease mortality offtakes. Adult (55%) and larvae mortality
601 (40%) is constant across scenarios #2–#7 (varying in time and sex offtake) but absent in
602 #1. PE: probability of population extinction after 20 yrs; GR: stochastic growth rate; N:
603 mean population size after 20 yrs; TE: time to first population extinction; SD: standard
604 deviation. Grey shading highlights scenarios with sex-biased mortality.

Scenario	PE	GR ± SD	N ± SD	Median TE
#1. no *Ranavirus* 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

605

606 **Table 3** Dunn-Bonferroni test between pairs of different scenarios generated from PVA,
607 to look at significant differences in probability of extinction. Adjusted *p* value < 0.05 was
608 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

609

CAPTIONS FOR FIGURES

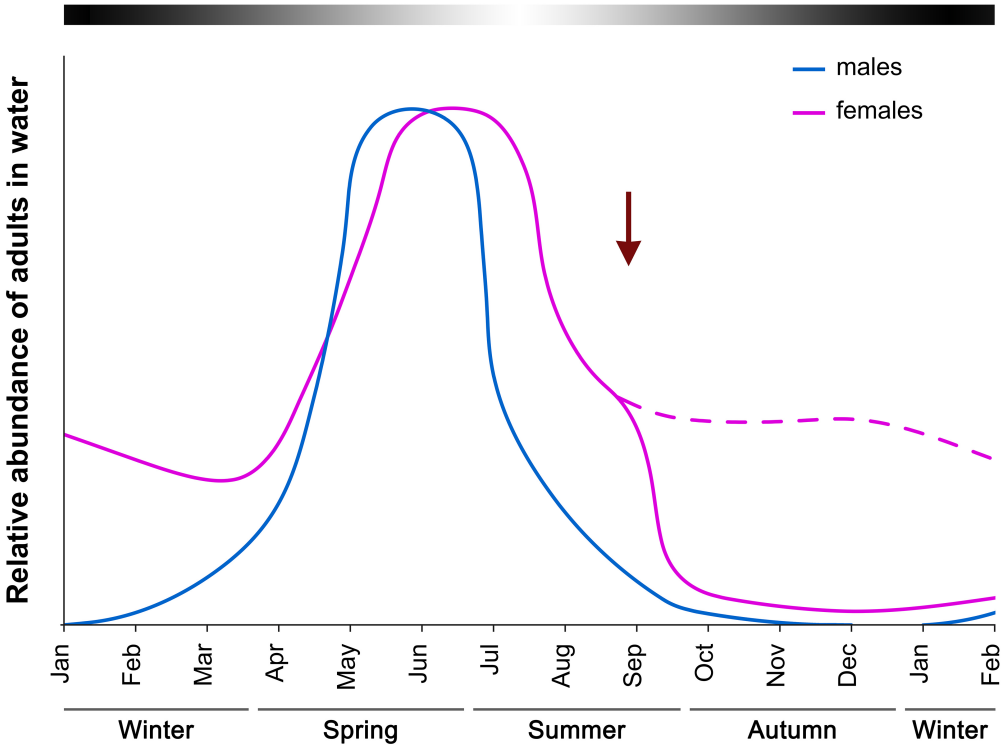
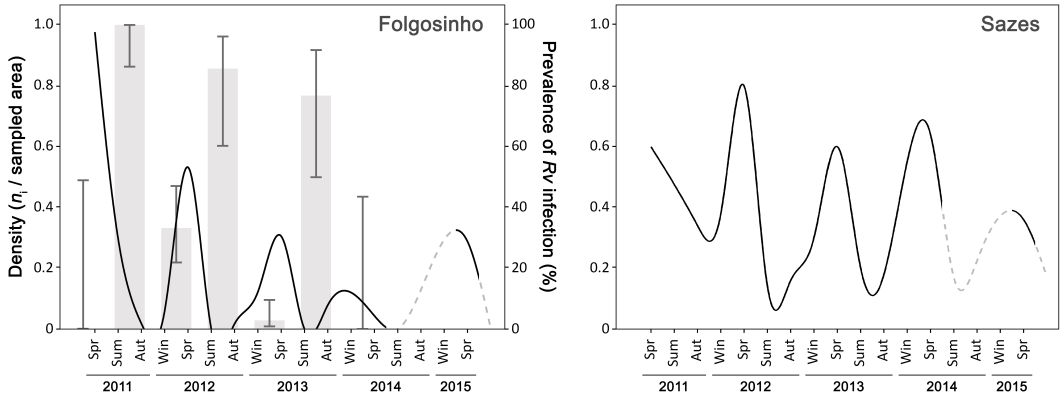
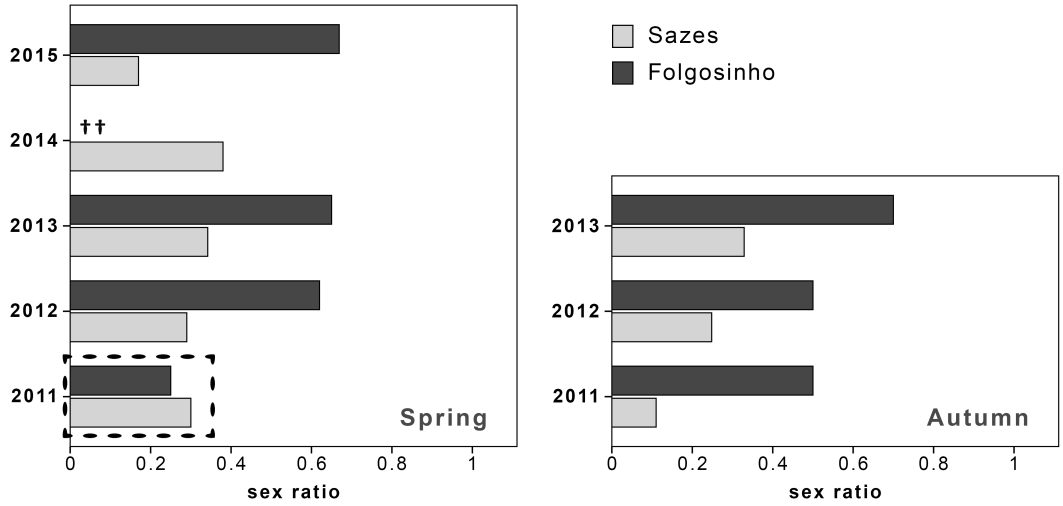


Figure 1 Schematic phenology of the host species, Bosca's newt (*Lissotriton boscai*) in Serra da Estrela (Portugal) in ponds located at about 800–1100 m of elevation showing a representation of expected relative proportion of males and females in the water throughout the year. Arrow indicates time of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



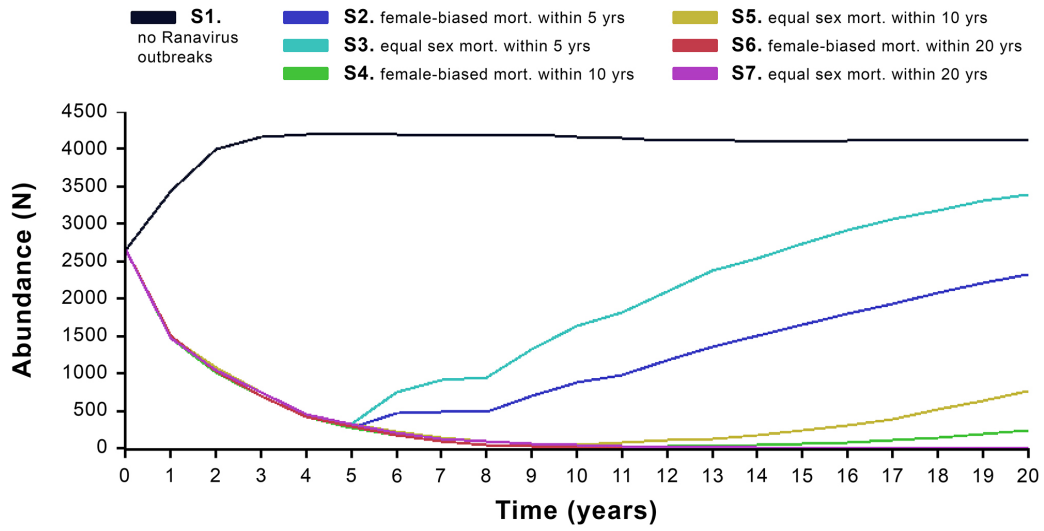
622

623 **Figure 2** Density of adult Bosca's newt (*Lissotriton boscai*) in two sites in Serra da Estrela
624 (Portugal) over five years (spline interpolation with data missing between summer 2014
625 and winter 2015): Folgosinho shows the density of newts with yearly outbreaks of
626 ranaviruses, while Sazes shows a natural population fluctuation in an area where
627 outbreaks have not been recorded. Prevalence of *Ranavirus* infection in Bosca's newts is
628 also provided for Folgosinho pond (grey bars) as the total proportion of infected
629 individuals by two grouped seasons (summer+autumn and winter+spring), where error
630 bars indicate the 95% confidence intervals.



631

632 **Figure 3** Comparative sex ratio of adult Bosca's newts (*Lissotriton boscai*) in two sites in
633 Serra da Estrela (Portugal) over five years. Folgosinho shows the sex ratio of a
634 population facing yearly outbreaks of ranavirosis after spring 2011, while Sazes shows
635 the expected sex ratio of a population where outbreaks have not been recorded. Sex
636 ratio is expressed as the proportion of males/(males + females) in two different seasons:
637 spring and autumn. Dashed rectangle highlights the sex ratio in both populations before
638 the first outbreak of ranavirosis, when there was no difference between them (*L. boscai*
639 $p > 0.05$). †† No data for Folgosinho site in spring 2014.



640

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