An emerging viral pathogen truncates population age structure in a European amphibian and may reduce population viability

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Infectious diseases can alter the demography of their host populations, reducing their viability even in the absence of mass mortality. Amphibians are the most threatened group of vertebrates globally, and emerging infectious diseases play a large role in their continued population declines. Viruses belonging to the genus *Ranavirus* are responsible for one of the deadliest and most widespread of these diseases. To date, no work has used individual level data to investigate how ranaviruses affect population demographic structure. We used skeletochronology and morphology to evaluate the impact of ranaviruses on the age structure of populations of the European common frog (*Rana temporaria*) in the United Kingdom. We compared ecologically similar populations that differed only in their historical presence or absence of ranavirosis (the acute syndrome caused by ranavirus infection). Our results suggest that ranavirosis may truncate the age structure of *R. temporaria* populations. One potential explanation for such a shift might be increased adult mortality and subsequent shifts in the life history of younger age classes that increase reproductive output earlier in life. Additionally we constructed population projection models which indicated that such increased adult mortality could heighten the vulnerability of frog populations to stochastic environmental challenges.

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Abstract

1   Infectious diseases can alter the demography of their host populations, reducing their viability even

2   in the absence of mass mortality. Amphibians are the most threatened group of vertebrates

3   globally, and emerging infectious diseases play a large role in their continued population declines.

4   Viruses belonging to the genus *Ranavirus* are responsible for one of the deadliest and most

5   widespread of these diseases. To date, no work has used individual level data to investigate how

6   ranaviruses affect population demographic structure. We used skeletochronology and morphology

7   to evaluate the impact of ranaviruses on the age structure of populations of the European common

8   frog (*Rana temporaria*) in the United Kingdom. We compared ecologically similar populations

9   that differed only in their historical presence or absence of ranavirosis (the acute syndrome caused

10 by ranavirus infection). Our results suggest that ranavirosis may truncate the age structure of *R.*

11 *temporaria* populations. One potential explanation for such a shift might be increased adult

12 mortality and subsequent shifts in the life history of younger age classes that increase reproductive

13 output earlier in life. Additionally we constructed population projection models which indicated

14 that such increased adult mortality could heighten the vulnerability of frog populations to

15 stochastic environmental challenges.

Introduction

16   The emergence of infectious diseases can truncate of the age structure of host populations (Jones

17   et al. 2008a, Lachish et al. 2009, Ohlberger et al. 2011, Fitzpatrick et al. 2014). Within age-

18   structured populations, such truncation is primarily caused by compensatory changes in the vital

19   rates (rates of growth, fecundity and survival) of younger age classes which occur in response to

20   elevated levels of extrinsic adult mortality (death of adult animals attributable to external factors

21   such as disease or predation; Stearns 1992; Roff 1993). To maximise individual fitness within an

22   environment of high extrinsic adult mortality, selection for increased juvenile survival and

23   developmental rates, decreased size and age at sexual maturity, and ultimately an increased adult

24   life span (decreased intrinsic adult mortality) will occur (Stearns et al. 2000). This theory has been

25   empirically borne out in a number of systems in response to several different sources of mortality,

26   including predation (Reznick et al. 1990), over-harvesting (Olsen et al. 2004), and experimentally

27   induced adult mortality (Stearns et al. 2000).

28   Changes to population demography can have profound impacts on the growth and stability of

29   infected populations (Saether and Bakke 2000). This phenomenon has recently been demonstrated

30   by Scheele *et al.* (2016), who documented high adult mortality and associated truncation of

31   population age structure in alpine tree frog (*Litoria verreauxii alpina*) populations infected with

32   the lethal fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Populations with truncated age

33   structure were shown to be more vulnerable to decline due to stochastic recruitment failure than

34   Bd-free populations (Scheele et al. 2016). These results highlight an important, yet relatively

35   unexplored mechanism by which infectious diseases can negatively affect their hosts. Infectious

36   diseases are emerging at a faster rate and threatening a larger range of species than at any prior

37   point in history (Jones et al. 2008b). Therefore, it is imperative to better understand demographic

38 shifts associated with disease, and their consequence for population viability, particularly in

39 species of conservation concern.

40 Worldwide, amphibians are the most imperilled class of vertebrates (Wake and Vredenburg 2008).

41 Aside from threats such as habitat loss (Cushman 2006), over-harvesting (Xie et al. 2007) and

42 climate change (Foden et al. 2013), one major driver of amphibian declines is the emergence of a

43 suite of infectious diseases (Daszak et al. 1999). One of the most widespread and deadly of these

44 diseases, ranavirosis, is caused by viral pathogens belonging to the genus *Ranavirus* (Chinchar

45 2002, Green et al. 2002). Ranaviruses are globally distributed and are capable of infecting and

46 killing a wide range of species from 3 classes of ectothermic vertebrates (Cunningham et al. 1996,

47 Whittington et al. 2010, Marschang 2011). Clinical ranavirosis is often characterised by severe

48 dermal ulcerations, as well as haemorrhaging and lesions affecting the internal organs

49 (Cunningham et al. 1996, Bayley et al. 2013). At the population level, acute incidences of

50 ranavirosis often manifest in mass mortality events, with up to 90% mortality reported in some

51 instances (Green et al. 2002). Evidence from the United Kingdom shows that following an episode

52 of mass mortality due to ranavirosis European common frog (*Rana temporaria*) populations can

53 decline in size by more than 80%. Though population recovery has been observed in some

54 instances, often population size remains supressed, and some populations decline to local

55 extinction (Teacher et al. 2010). Campbell et al. (2018) demonstrated that ranaviruses are

56 potentially more widespread than previously thought, and present within the environment even

57 when resident frog populations show no overt signs of disease (Campbell et al. 2018). These

58 findings suggests that whether or not an outbreak of ranavirosis occurs may depend on currently

59 unknown biotic or abiotic factors of an environment, although there is evidence for a role of the

60   cutaneous or environmental microbiome (Campbell et al. 2018), secondary host species, and

61   chemical usage (North et al. 2015). Susceptibility to ranavirosis outbreaks varies among species,

62   but a number of ecological risk factors, including life history strategy, have been identified

63   (Hoverman et al. 2011). To date, no work has evaluated the impact of ranavirosis on the

64   demographic structure of host populations using individual age data.

65   In this study, we used a unique comparative field system born out of the Frog Mortality Project

66   (FMP; see Teacher et al. 2010; Price et al. 2016 for details) to study the impacts of ranaviral disease

67   history on the demographic structure of wild European *R. temporaria* populations in the United

68   Kingdom. Unlike in most other species of susceptible amphibian, ranavirosis disproportionately

69   kills adult *R. temporaria*, rather than tadpoles (Duffus et al. 2013). We determined population age

70   structure using skeletochronology (age determination by counting skeletal growth rings) to test the

71   hypothesis that a positive history of ranavirosis truncates the age structure of *R. temporaria*

72   populations. Additionally we investigated potential mechanisms for such age truncation by using

73   morphometric data to explore if frogs originating from populations with a positive history of

74   ranavirosis display reduced body size or evidence of more rapid growth compared to their

75   counterparts from ostensibly disease-free populations. Finally, to examine the potential effect of

76   the demographic impacts of ranavirosis, we used population projection modelling to simulate the

77   dynamics of hypothetical *R. temporaria* populations under a range of stochastic environmental

78   scenarios that impacted recruitment, adult survival, or both. Based on previous evidence

79   (Ohlberger et al. 2011, Rouyer et al. 2012, Scheele et al. 2016), we hypothesised that age structure

80   truncation would heighten the vulnerability to *R. temporaria* populations to environmental

81   stochasticity.

Methods

Ethics statement

82 This project was approved by the ethics boards of both the University of Exeter and Zoological

83 Society of London and conducted under UK Home Office project license 80/2466. All field

84 sampling was conducted under the personal Home Office license 30/10730 issued to Lewis

85 Campbell.

Field Sampling

86 Evidence suggests that ranaviruses may be more ubiquitous within amphibian populations in the

87 UK than previously thought, being detectable in populations with no history of ranavirosis

88 (Campbell et al. 2018). We therefore used a history of ranavirosis related mortality (or lack of),

89 rather than detectable ranavirus burden, to differentiate between populations that are impacted by

90 ranaviruses and those which are not. Candidate *R. temporaria* populations were identified using

91 the FMP database of privately owned field sites that have experienced at least one *R. temporaria*

92 mass mortality event due to ranavirosis and a complimentary database of fields sites thought to

93 have not experienced a ranavirosis outbreak, based upon close observation by field site owners.

94 Briefly, ranavirosis-positive field sites were classified as such if they had experienced a mass

95 mortality event between 1997 and 1998 and ongoing discovery of frog carcasses with symptoms

96 consistent with ranavirosis, suggesting continued disease. In order to be classified as disease-free,

97 field sites must have been owned or occupied by the same people at all times since 1997 and have

98 contained a well-monitored *R. temporaria* population for at least as long. Owners/occupiers of

99 disease-free field sites must not have observed any signs or symptoms of ranavirosis at any time.

100 See Teacher *et al.* 2010 for more detailed field site selection criteria. Field site proprietors were

101   contacted to establish their willingness to be involved in this study. Five ranavirosis populations

102   were successfully recruited and matched with five populations that have remained disease-free

103   (Fig. 1). None of our ranavirosis-positive populations had experienced a mass mortality event

104   within the last decade.

105   Field sites were surveyed during the spring breeding season of 2015, with each site sampled on a

106   single day. Sampling involved opportunistic searching for frogs within and around the perimeter

107   of the breeding ponds. Captured frogs were placed into plastic holding tanks before sampling,

108   which took place in situ. To ensure sampling effort between populations was as equal as possible,

109   searching took place during a one hour, mid-morning time window.

110   Snout to vent length was measured using 0.1mm scale callipers and the distal portion of the 1st

111   digit of a hind limb was clipped using surgical scissors. A topical disinfectant that contained an

112   analgesic (Bactine; WellSpring Pharmaceutical, Florida, USA) was applied to the surgical area

113   prior to the procedure. Toe clips were placed into individual 1.5 ml micro-centrifuge tubes

114   containing 1 ml of 70% ethanol. Following sampling, all animals were released at point of capture.

115   The number of individuals sampled in each population varied between 4 (Witham) and 61

116   (Mitcham and Palmer's Green) with a mean of 30 animals sampled per site (Tables S1). All

117   captured frogs were considered to be part of the breeding population, as juvenile frogs rarely return

118   to breeding ponds (Wilbur 1980, Verrell 1985), and all sampled individuals were found to be over

119   the minimum known age of sexual maturity for the study species.

Age determination

120 The age of each frog was determined by skeletochronology, which has been calibrated and

121 demonstrated as a reliable method of determining the age of northern European *R. temporaria* in

122 several previous studies (Gibbons 1983, Gibbons and McCarthy 1986, Ryser 1996, Miaud et al.

123 1999). We followed the protocol for aging *R. temporaria* from Miaud *et al* (1999) with the

124 following modifications. The phalangeal bone was separated from soft tissues, decalcified with

125 5% nitric acid for 1.5 hours and washed with water over night. Cross sections (12 μm thick) were

126 then cut from the bone using a cryostat and stained using haematoxylin for 20 minutes. Lines of

127 arrested growth (LAG) were counted using a light microscope at 200x-400x magnification, 10-12

128 sections were analysed for each individual and two different researchers verified counts. Age at

129 sexual maturity was determined as the youngest age at which inter-LAG space reduced in size, as

130 juvenile inter-LAG space is significantly wider than post-sexual maturity (Sinsch 2015).

Statistical Analyses

Body size by age and age at sexual maturity.

131 We conducted all statistical modelling in R (R Core Team 2014). We used linear mixed effects

132 regression (lmer) models, implemented in the package lme4 (Bates et al. 2015), fitted with a

133 Gaussian error structure, and a stepwise simplification procedure to investigate the impact of

134 population ranavirosis history on the body size (SVL) of *R. temporaria*. Sex, ranaviral disease

135 history of the source population and their interaction were fitted as fixed effects and source

136 population alone as a random effect (Table 1). Since male and female frogs grow at different rates

137 (Gibbons 1983, Ryser 1996, Miaud et al. 1999), the datasets of each sex were analysed separately.

138 A separate lmer model was fitted to investigate the impact of ranavirosis history status of the source

139 population on age at sexual maturity. In the full model, age at maturity was fitted as the response

140   variable, ranaviral disease history of source population as a fixed effect and source population as

141   a random effect. As female *R. temporaria* mature later than males (Gibbons 1983, Miaud et al.

142   1999), the datasets of each sex were analysed separately.

Influence of ranavirus on population age structure

143   The impact of disease status on the age structure of *R. temporaria* populations was investigated

144   using a Bayesian ordinal mixed effects model in the package MCMCglmm (Hadfield 2010). We

145   fitted age class as an ordinal response variable (9 discrete classes, ages 2 – 10 years), disease status

146   of the source population as the fixed effect, and source population as a random effect. We used

147   uninformative priors for both the random effect (G) and residual variance (R) structures, but fixed

148   the residual variance at 1 as this quantity cannot be estimated in ordinal models. The model was

149   run for a total of 600,000 iterations with a burn-in period of 100,000 iterations and a thinning rate

150   of 500, giving a final sample of 1,000 draws from the posterior distributions. We assessed model

151   convergence using the Gelman-Rubin (G-R) statistic calculated from two independent chains

152   initiated with overdispersed starting values. All G-R values were <1.05, indicating convergence.

153   Mean probability of membership and associated 95% credible intervals for each age class were

154   calculated from the linear predictor, for each of the two disease history groups. Age structure plots

155   (Fig. 2) suggest that observed changes were similar for both sexes, so the dataset was not split by

156   sex.

Population Matrix Modelling

157   To investigate how changes in population age structure, as well as scenarios that can bring about

158   such changes, can impact the dynamics and stability of *R. temporaria* populations, we constructed

159   population matrix models. Comprehensive methodologies of our matrix modelling can be found

160   in our

161   lementary methods section (Methods S1). However, in brief, we created hypothetical *R.*

162   *temporaria* populations of 150 sexually mature female animals and projected these populations 20

163   years into the future based on two matrices which represented potential vital rates at ranavirosis-

164   positive *R. temporaria* population (increasing annual mortality in each adult age class) and a

165   disease-free *R. temporaria* population (uniform adult mortality). We populated these matrices

166   using the vital rates for *R. temporaria* published by Biek et al. (2002) except that we increased the

167   fecundity of sexually mature *R. temporaria* annually from 250 eggs per 2 year old adults to 650

168   eggs per year per 10 year old adults. This increase was done according to age by size data collected

169   for this study and the knowledge that fecundity in *R. temporaria* is tightly positively correlated

170   with body size (Gibbons and McCarthy 1986). To analyse the impact of these adjustments we

171   compared the sensitives of our adjusted matrices to one constructed with the unadjusted vital rates

172   of Biek et al. (2002). As previous evidence has shown that disease induced demographic shifts or

173   the changes in vital rates which may bring them about can increase susceptibility to environmental

174   change (Rouyer et al. 2012, Scheele et al. 2017) we then modelled our populations in

175   environmentally stochastic scenarios which incorporated processes which may result in annual

176   reproductive failure, mass mortality or both simultaneously.

Results

Body size by age and age at sexual maturity

177   We sampled 208 male and 66 female frogs, of which 103 males and 31 females were sampled at

178   ranavirosis-positive populations. A break-down of the number of frogs samples per each

179   population can be found in table S2. For both sexes, age had a significant effect on SVL (males;

180   df = 4, Chisq = 186.38, $p<0.001$, females; df = 4, Chisq = 47.16, $p<0.001$; Fig. S3; Table 1). Mean

181   age at sexual maturity of males from ranavirosis-positive populations (n=57) was 2.6 years (± SE

182   0.07) and from disease-free populations (n=59) it was 2.8 years (± SE 0.06). Mean age at sexual

183   maturity of females from ranavirosis-positive populations (n=19) was 3.2 years (± SE 0.13) and

184   for females from disease-free populations (n=17) it was 3.3 years (± SE 0.10), and all differences

185   were non-significant (Fig. S4; Table 1).

Influence of ranavirosis on population age structure.

186   The mean age of males from ranavirosis-positive populations was 4.8 years old (± SE 0.16)

187   compared to an average age of 6.3 years (± SE 0.13) at disease-free populations. Mean female age

188   at ranavirosis-positive populations was 5.3 years (± SE 0.28) compared to 6.6 years (± SE 0.26) at

189   disease-free populations

190   Disease history had a significant effect on population age structure (effect size -1.43, 95% credible

191   intervals -2.37, -0.38, p=0.008; Fig. 2). We found populations with a positive history of ranavirosis

192   to be dominated by younger *R. temporaria*. We calculated the difference in posterior probabilities

193   of belonging to an age class based upon disease history status by subtracting the posterior

194   probability of a frog of age *X* being encountered at ranavirosis-positive population from the

195   posterior probability of a frog of age *X* being found at a disease-free population. The resulting

196   difference values show that adults aged 2 - 5 years old are more likely to be encountered in positive

197   disease history populations, and those aged 6 − 10 years old are more likely to be observed at

198   populations where no disease has been recorded (Fig. 3). Differences in age distributions were

199   strongly supported for all age classes (95% credible intervals of difference do not cross zero)

200   except for six year olds. Although six-year-old frogs were more likely to be observed in disease-

201   free populations, the 95% credible intervals incorporated 0 (mean difference = -14.41, 95% CI   -

202   30.45 to 3.96; Fig. 3).

Population projection modelling

203   When projected based upon a matrix with constant annual adult survival, both disease-free and

204   ranavirosis-positive population vectors showed similar population dynamics. Population growth

205   initially spiked due to an influx of pre-mature age classes into the population. This initial growth

206   was followed by a period of attenuating oscillation, before reaching equilibrium at around the

207   fifteenth year (Fig. S5). Projected populations starting with a truncated age structure obtained

208   smaller population sizes per each year, however this difference was non-significant (Fig. S5,

209   ANOVA; df= 1, F= 0.935, p= 0.34). Projecting either starting population vector using a matrix

210   incorporating reduced annual adult survival resulted in the same pattern of population growth but

211   further reduced population sizes per year, with the lowest annual population sizes obtained by an

212   age truncated population projected with decreased annual adult survival, though this reduction was

213   again non-significant (Fig. S5; ANOVA; df = 3, F= 0.99, p= 0.39).

214   Elasticity analysis showed that both disease-free and ranavirosis-positive matrices were similarly

215   sensitive to the same matrix elements. However, the decreased annual adult survival incorporated

216   into our ranavirosis-positive matrix rendered that matrix marginally more sensitive to fluctuations

217   in survival of larval, juvenile and two year old life stages. Sensitivity to survival of all subsequent

218   adult age classes was higher in our disease-free population matrix (Fig. S6). Similarly, our

219   ranavirosis-positive matrix was more sensitive to changes in the fecundity of 2, 3 and 4 year old

220   breeding adults, whereas sensitivity to changes in fecundity of older adult age classes was higher

221   in our disease-free population matrix (Fig. S7). In concordance with the matrix constructed using

222   the unadjusted vital rates of Biek et al. 2002, both of our altered matrices were most sensitive to

223   fluctuations in the summed vital rates of post metamorphic *R. temporaria* life stages, though larval

224   survival demonstrated the highest or second highest individual elasticity in all matrices (Fig. S6).

225   The most noticeable dissimilarity between our adjusted matrices and the unadjusted matrix was

226   that matrix sensitivity was distributed more evenly between the fecundity of all adult age classes

227   in the former, whereas the unadjusted matrix was extremely sensitive to the fecundity of 2 year

228   old breeding adults (Fig. S7).

229   Our stochastic projection models showed that disease-free populations subject to a 10% probability

230   of recruitment failure per year were still able to consistently attain population sizes near carrying

231   capacity much more often than ranavirosis-positive populations subjected to the same scenario,

232   which attained a more variable array of population sizes (Fig. 4, Table 2). The inclusion of

233   potential ranavirosis mass mortality events further destabilised ranavirosis-positive populations

234   and under a scenario when both adult mass mortality and reproductive failure could occur within

235   the same year populations were driven locally extinct in 58% of model iterations (Fig. 4, Table 2).

Discussion

The impact of disease on population demographics

236   Our results illustrate that a history of ranavirosis can have a significant effect on the age structure

237   of a population. We found that older *R. temporaria* (aged 6-10 years) are significantly less likely

238   to be found within populations with a positive history of ranavirosis than they are in disease-free

239   populations. Since none of our sampled populations have experienced a significant mass mortality

240   event within the lifetime of any of the sampled frogs (~10 years), this suggests a pattern of

241   attritional mortality in ranavirosis-positive populations, rather than the sudden and catastrophic

242   loss of any particular age classes. Attritional mortality of adults is consistent with our knowledge

243   of ranavirosis in *R. temporaria*. First, *R. temporaria* are highly philopatric (Brabec et al. 2009),

244   and our study populations occupy permanent, urban or semi-urban garden ponds (Teacher et al.

245   2010, Price et al. 2016). Ranaviruses have been shown to persist in such water bodies (Nazir et al.

246   2012), particularly in the presence of secondary host species (Hoverman et al. 2011, North et al.

247   2015). Second, mortality due to ranavirosis is annually recurrent (Daszak et al. 1999, Teacher et

248   al. 2010), and unlike all other host-ranavirus systems, infection in the UK primarily affects adult

249   life stages of *R. temporaria* (Cunningham et al. 1996, Duffus et al. 2013). Third, adaptive immune

250   response to ranaviruses are apparently limited in *R. temporaria* (Price et al. 2015, Campbell et al.

251   2018). Consequently, it is possible that adult mortality within populations with a history of

252   ranavirosis is maintained in such a way that an individual is more likely to become infected and

253   succumb to ranavirosis the more often it returns to spawn. Such a scenario would explain the

254   decreased likelihood of observing individuals older than 5 years of age in populations with a

255   history of ranavirosis.

256   Concurrently, we found that a significantly higher number of 2 – 5 year old frogs were captured at

257   disease-free populations than at populations with a history of ranavirosis. Life history theory

258   predicts that the first compensatory response to high adult mortality should come in the survival

259   rates of lower age classes (Stearns 1992). We lack any data on the immature age classes present at

260   our study populations and the snapshot nature of our study means we cannot draw inference on

261   whether the survival rates of younger adult age classes are increased in positive disease history

262   populations. However, it is plausible that such an increase could result in the observed increased

263   abundance of younger animals.

264   Empirical studies conducted on populations of other vertebrates subjected to persistent infectious

265   diseases have detected a reduction in the age or size at sexual maturity within diseased populations

266   (Jones et al. 2008a, Lachish et al. 2009, Ohlberger et al. 2011, Fitzpatrick et al. 2014). As such,

267   we hypothesised that *R. temporaria* originating from populations where ranavirosis causes

268   increased adult mortality would reach sexual maturity at an earlier age than frogs from disease-

269   free populations. We also hypothesised that a trade-off in the allocation of resources to early

270   reproduction, away from growth, would cause frogs from ranavirosis-positive populations to attain

271   a lower body size per age than those from disease-free populations. However, we found that a

272   positive history of ranavirosis is not associated with a significant impact on either age at sexual

273   maturity or body size throughout life. These findings are in contrast with the findings of Scheele

274   et al. (2017) who found that an infectious disease (Bd) reduced the size and age at sexual maturity

275   of *L. v. alpina.*

276   The age at which *R. temporaria* mature is intrinsically linked to attaining a minimum body length

277   needed to successfully reproduce (Ryser 1996, Miaud et al. 1999). The age at which this length is

278   attained has been shown to be heavily influenced by several environmental factors such as photo-

279   period and altitude (Miaud et al. 1999). All frogs in our study were found to mature at either 2, 3

280   or 4 years of age and this is consistent with the findings of other similar studies on *R. temporaria*

281   (Gibbons 1983, Ryser 1996, Miaud et al. 1999). In fact, no previous study has found male or

282   female *R. temporaria* to reach maturity younger than 2 years of age and post-sexual maturity there

283   is little detectable trade-off between growth and fecundity in response to sub-prime environments

284   (Lardner and Loman 2003). This evidence suggests that the life history strategy of *R. temporaria*

285   may already be optimised to generate maximum reproductive fitness in light of other

286   environmental factors and that scope for further plasticity in traits such as age at sexual maturity

287   and subsequent growth rate in response to diseases may be minimal.

288   Given the apparent lack of compensatory change in the onset of sexual maturity an alternative

289   explanation for the fact that we encountered 2- and 3-year-old breeding frogs at ranavirosis-

290   positive populations but not disease-free populations may be behavioural changes that enhance the

291   life time reproductive success of individuals in the face of high adult mortality. Participation in

292   spawning events in exposed aquatic environments is associated with a significant mortality risk to

293   an individual, caused either by exposure to predation or the act of mating itself (Beebee 1996).

294   Additionally, smaller *R. temporaria* present in breeding populations are easily outcompeted by

295   larger individuals, who are better able to secure a mate and achieve amplexus (Gibbons 1983). The

296   loss of larger, more competitive individuals at ranavirosis-positive field sites may release smaller

297   *R. temporaria* from this intraspecific competitive pressure. Increased opportunity for less

298   competitive individuals to participate successfully in reproductive events may result in animals

299   that would normally defer breeding until subsequent years or larger body sizes attempting to

300   reproduce earlier. Additionally, in environments of high adult mortality the number of lifetime

301   reproductive events is potentially limited. In such environments lifetime reproductive success is

302   likely higher when an individual exploits all possible chances to produce offspring. Thus smaller,

303   less competitive individuals may attempt to breed earlier than they would do in the absence of

304   disease induced mortality, irrespective of the odds of being out competed by older/larger frogs.

305   Any or all of the potential processes outlined above could lead to the observed truncation of age

306   structures in *R. temporaria* populations with a history of ranavirosis. While our results provide

307   strong evidence of such truncation, they are based on data collected from adult *R. temporaria*

308   sampled during one breeding season. While they highlight a potentially important and as yet

309   unexplored facet of the relationship between ranaviruses and their amphibian hosts, we are unable

310   to draw conclusions as to the mechanisms which drive observed changes to population age

311   structure. The impact of demographic shifts has been demonstrated in a number of study systems

312   (e.g. Jones *et al.* 2008a; Lachish *et al.* 2009; Ohlberger *et al.* 2011; Rouyer *et al.* 2012; Scheele *et*

313   *al.* 2016) and as such the relationship between ranaviruses and population demographics

314   undoubtedly warrants further investigation. A long-term, mark-recapture study within the same

315   populations used here would likely prove a critical first step in elucidating the mechanisms which

316   bring about shifts in *R. temporaria* population age structure.

The potential impact of disease on the viability of populations

317   Previous investigations have shown that age structure truncation, associated with an emerging

318   pathogen, severely reduces the viability of host populations, particularly under variable

319   environmental conditions (Scheele et al. 2016). We used population matrix models to probe the

320   potential impacts of a truncated age structure, and the demographic processes which may bring

321   about such changes, on the viability of *R. temporaria* populations in the UK. Additionally, we

322   introduced environmental stochasticity into our models in order to explore the combined impact

323   of these two challenges simultaneously.

324   When modelled in the absence of increased adult mortality due to ranavirosis, starting population

325   vectors representing both a disease-free population and a ranavirosis-positive population

326   (truncated age structure) exhibited minimal difference in their dynamics. This finding suggests

327   that the level of age structure truncation we document is unlikely to reduce the viability of *R.*

328   *temporaria* populations. The same was true when both population age structures were modelled in

329   the presence of increasing adult mortality due to ranavirosis, suggesting that even under such

330   conditions the relatively high survival of pre-metamorphic, juvenile and young adult age classes

331   is enough to maintain population viability. This suggestion is supported by the elasticity of our

332   population matrices which demonstrated that the survival of tadpoles, juveniles and early adult life

333   stages to have higher predicted impact on the population growth dynamics of our populations than

334   the survival of older adult age classes. This result is supportive of the findings of other

335   investigations of amphibian population dynamics (Biek et al. 2002, Earl and Gray 2014).

336   However, under environmentally stochastic scenarios, where recruitment was reduced,

337   populations that were subjected to increasing adult mortality due to ranavirosis faired much worse

338   than ranavirosis-free populations. These results are consistent with previous observations of age

339   structure truncation increasing the vulnerability of populations to environmental stochasticity

340   (Ohlberger et al. 2011, Rouyer et al. 2012, Scheele et al. 2016). Body size, age and fecundity are

341   positively correlated in *R. temporaria* (Gibbons and McCarthy 1986), as well as in many other

342   species (eg; Blueweiss *et al.* 1978; Honěk 1993; Trippel 1993; Sand 1996; Penteriani, Balbontin

343   & Ferrer 2003). The relative absence of the oldest and largest breeding animals from disease

344   positive populations means that per capita fecundity will be reduced and annual recruitment rates

345   lowered. Such changes likely heighten the impacts of any events that result in failed recruitment

346   or further adult mortality.

347   It is important to note that our matrix models are approximations of the populations that they

348   represent, and the results of our models are therefore demonstrative of potential additional impacts

349   of ranavirosis, rather than definitively demonstrating the existence of such impacts. Although our

350   simulations are based on published literature, they necessarily incorporate several assumptions. A

351   key assumption in our models is the number of eggs produced per each individual, per each adult

352 year of age. Although we have strong grounds to suggest that fecundity of female *R. temporaria*

353 increases with age and size, the number of eggs produced by each frog of a given age or size is

354 unknown in our study populations. If a larger number of eggs are produced than represented by

355 our matricies, it may be that the impact of stochastic recruitment failure upon our populations is

356 reduced and vice versa. Similarly, we reduce the survival of each adult age class into the next by

357 5% for each year of development post-sexual maturity. Whilst we found no impact of the amount

358 by which we reduced this annual survival parameter on the dynamics of our modelled populations,

359 in reality survival may not decrease annually in such a uniform manner. Further investigation of

360 the life history traits of our study populations would allow for more robust parameterisation of our

361 models and the ability to more accurately predict the impact of ongoing ranavirosis within them.

362 Despite these limitations, our population models corroborate previous empirical data collected

363 from within our study system. A long-term study of population sizes has shown that following an

364 outbreak of ranavirosis, characterised by a mass mortality event, UK *R. temporaria* populations

365 follow three possible trajectories. These are: 1) complete recovery to post outbreak population

366 levels, 2) persistence at a largely reduced population size, or 3) local extinction (Teacher et al.

367 2010). Our simulated *R. temporaria* populations were projected to population sizes that

368 incorporate all of these possible outcomes, dependant on the levels of environmental stochasticity

369 to which a population was subjected. Importantly, our simulations suggest that the fate of a

370 population subjected to ranavirosis may depend heavily upon the stability of its environment,

371 providing a potential explanation as to why some populations appear to persist with endemic

372 infectious disease, while others are driven to local extirpation. Although we have demonstrated the

373 potential importance of environmental variation on the within population disease dynamics of

374 ranaviruses, it is not thought to play a role in the wider context of disease spread (Price et al. 2016).

The importance of variations in fecundity in population models

375 Variations in adult fecundity due to body size are often not included in population projection

376 modelling (Briggs *et al.* 2005; but see Zambrano *et al.* 2007). However, our results highlight the

377 importance of considering age or body size specific changes in fecundity in population modelling,

378 particularly when considered threats disproportionately impact certain age classes. Ensuring

379 matrix models of populations represent the life history of the study species as closely as possible

380 is essential, especially when seeking to inform conservation or policy decision making.

Conclusion

381 Our results highlight an increasing need to better understand the impact of disease on the

382 demography of host populations and the processes which can bring about such demographic shifts.

383 Further investigation of this relationship, possibly via a long-term mark-recapture study on the

384 same populations used here could help elucidate the exact mechanisms responsible for age

385 structure truncation in *R. temporaria* populations. This work also further suggests that the

386 emergence of an infectious disease within a population can heighten its vulnerability to external

387 stressors. Although the theoretical stressor incorporated into our models was environmental

388 stochasticity, the same is likely to be true for all types of stressor including anthropogenic. This

389 result is timely given that we live in a time of unprecedented disease emergence and anthropogenic

390 change (Daszak et al. 2001).

Data accessibility

391 The raw data file and R scripts used for this work are available online via GitHub at the following

392 link;

393   https://github.com/zoolew/Ranavirus-FrogDemography

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Figure 1(on next page)

Map of sampled *R. temporaria* populations

Map of the locations of sampled populations within the southern United Kingdom. Field sites were drawn from the Frog Mortality Project database of populations known to have experienced mass mortality events due to ranavirosis and a complimentary database of populations known to have been ranavirosis-free since disease emergence in the 1990s. Populations = 1. Oxford; 2. Witham; 3. Palmer's Green; 4. Folkington Corner; 5. Ealing; 6. Chessington; 7. Mitcham; 8. Tadworth; 9. Southampton; 10. Poole.
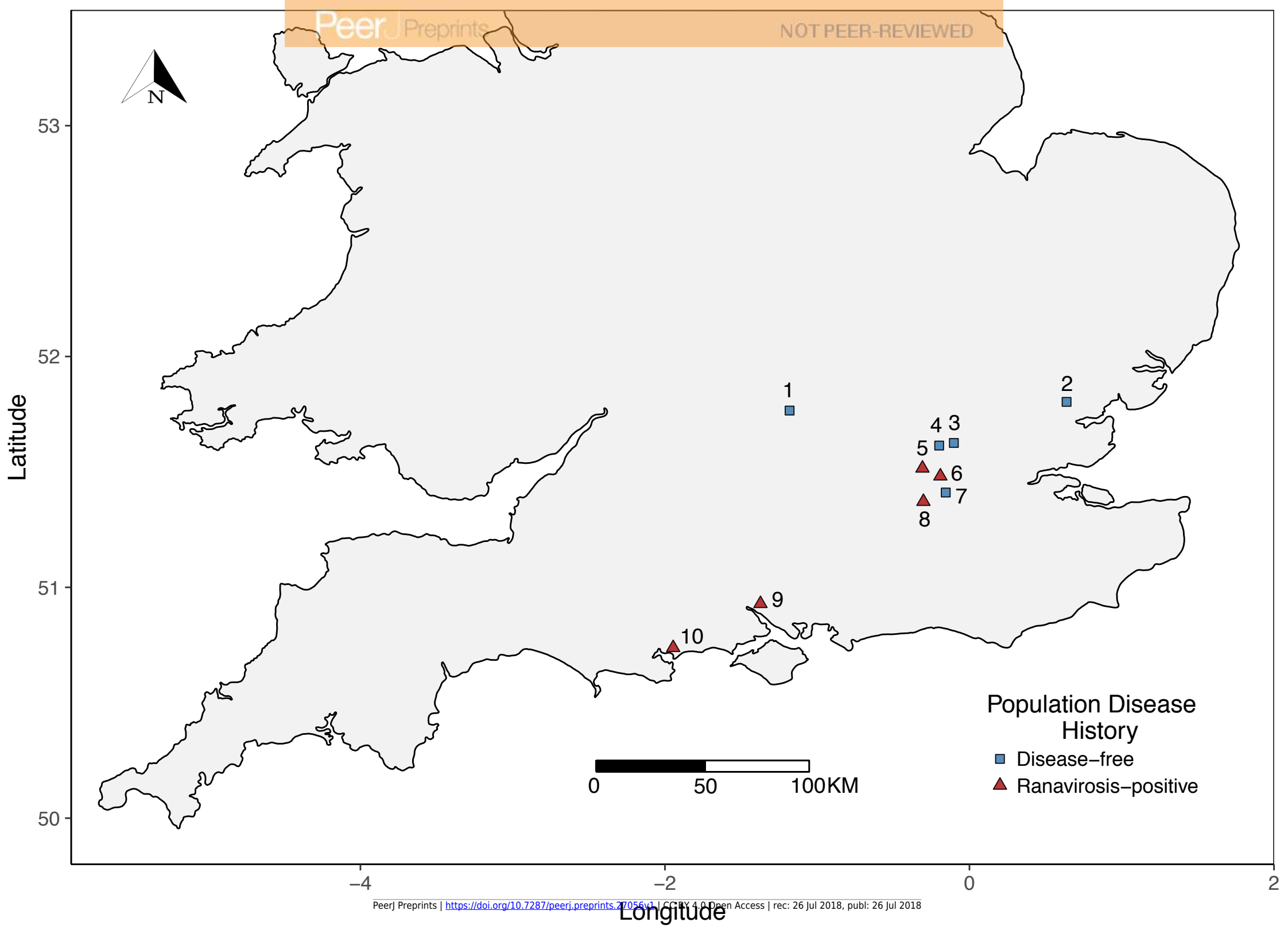
Figure 2(on next page)

Observed age structure across disease-free and ranavirosis-positive *R. temporaria* populations.

A)Histogram of raw counts of numbers of individuals observed per age class per disease history status type. **B)**Proportional stacked bar chart of the proportion of individuals found in populations of each disease history that was a given age, broken down by sex. Breeding populations with a positive history of ranavirosis are dominated by animals 5 years of age and younger. Disease-free populations are majorly comprised of animals 6 years of age and older.
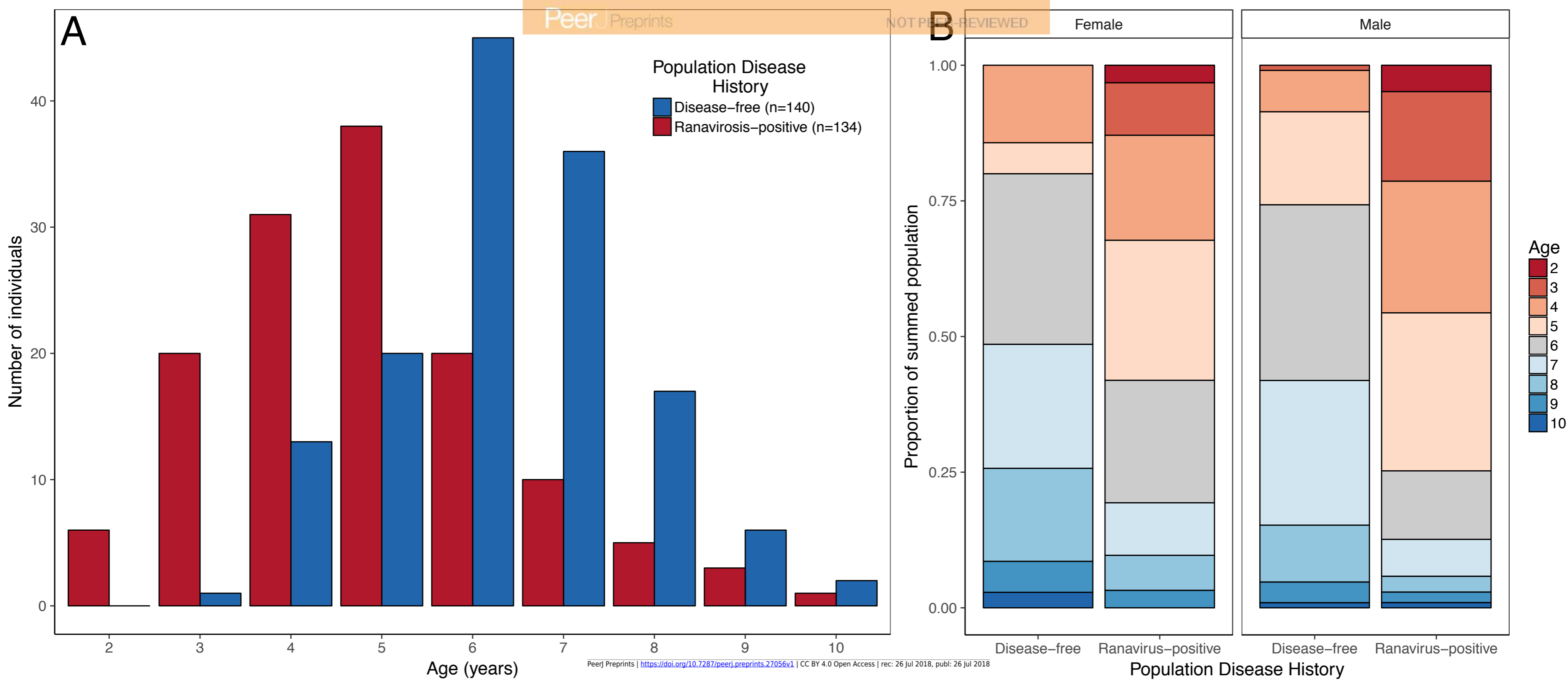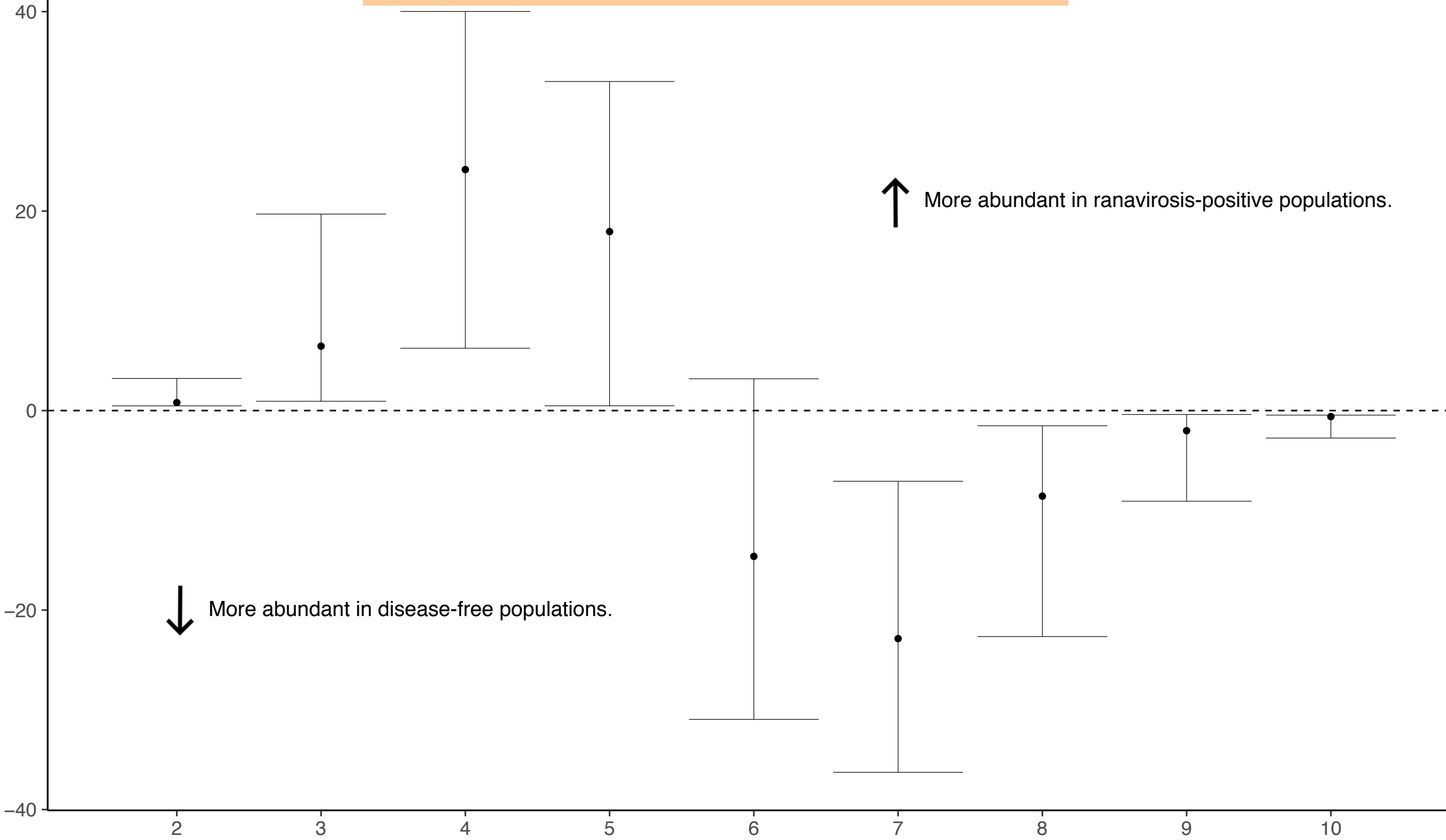
Figure 3(on next page)

Differences in posterior probabilities of belonging to a given age class between population groups of varying disease history.

The mean difference in the posterior probabilities of belonging to a given age class by population ranavirosis history. Values > 0 indicate that an age class is more likely to be observed in a ranavirosis-positive population and < 0 a disease-free population. An age with 95% (2.5% - 97.5%) credible intervals that do not span zero suggests that influence of disease history on that age class is significantly supported by our model. This is the case for all classes other than age 6 which although found to be observed more often in disease-free populations has credible intervals spanning 0.

Figure 4(on next page)

Frequency plot of the number of model iterations in which each modelled population reached a given population size under stochastic environmental conditions.

Frequency polygon of iterations in which the projected population hit a given size in stochastic projection modelling. The same starting population vector based on summed observed disease-free populations was used in all models. Disease-free = Simulated disease-free population under a 10% annual chance of complete reproductive failure. Ranavirosis-positive A = Simulated ranavirosis-positive population under a 10% annual chance of complete reproductive failure. Ranavirosis-positive B = Simulated ranavirosis-positive population under a 10% annual chance of reproductive failure AND a 10% annual chance of a recurrent adult mass mortality event in exclusive years. Ranavirosis-positive C = Simulated ranavirosis-positive population under identical conditions to Ranavirosis-positive B with addition of a 5% annual chance of complete recruitment failure and adult mass mortality in the same year.
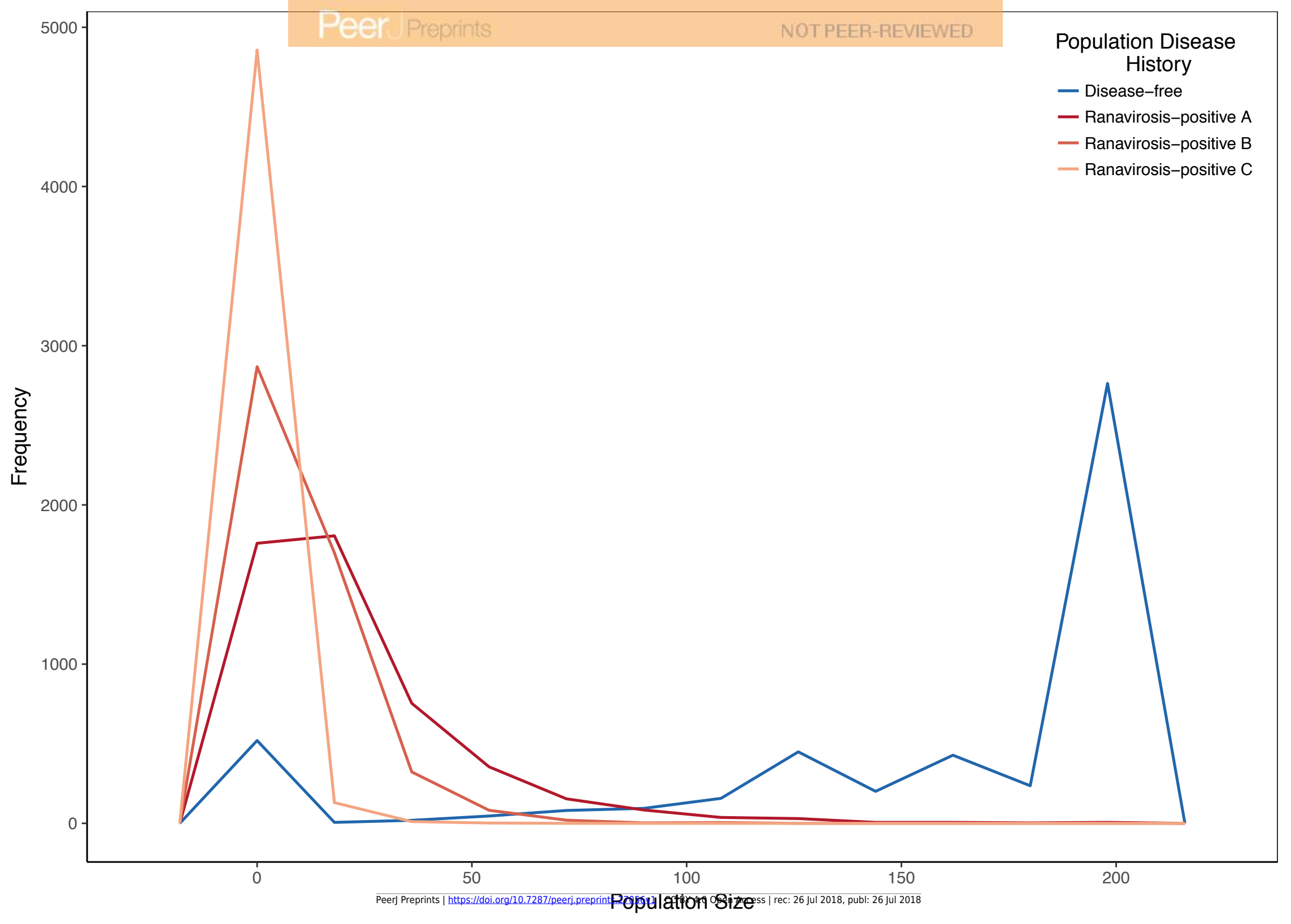
Table 1(on next page)

Summary of statistical model simplification procedure and results.

Model summaries of model simplification procedure to evaluate the effect of ranavirosis history on the body size and age at maturity of *R. temporaria* populations. All models contained only population of origin as a random effect. The p values presented here represent the significance of the parameter removed from the preceding model as calculated by a likelihood ratio test between models (anova in R.) svl = snout to vent length, agemat = age at sexual maturity and df = degrees of freedom of the model.

1

Male body size				
Fixed effects structure	**Removed fixed effect**	**df**	**Chi²**	***p***
svl~age * status		6		
svl~age + status	Age * Status	5	0.059	0.81
svl~age	Status	4	0.21	0.65
svl~1	Age	3	186.38	<0.001
Female body size				
Fixed effects structure	**Removed fixed effect**	**df**	**Chi²**	***p***
svl~age * status		6		
svl~age + status	Age * Status	5	0.94	0.33
svl~age	Status	4	2.43	0.12
svl~1	Age	3	47.16	<0.001
Male age at maturity				
Fixed effects structure	**Removed fixed effect**	**df**	**Chi²**	***p***
agemat ~ status		4		
agemat ~ 1	Status	3	0.99	0.32
Female age at maturity				
Fixed effects structure	**Removed fixed effect**	**df**	**Chi²**	***p***
agemat ~ status		4		
agemat ~ 1	Status	3	0.29	0.59

Table 2(on next page)

Summary of the number of projection model iterations in which a population reached a given size under stochastic environmental conditions.

The number of iterations per 5000 that each stochastic projection model reached a given population size. Disease-Free = Simulated disease-free population under a 10% annual chance of complete reproductive failure. Positive = Simulated ranavirosis positive population under a 10% annual chance of complete reproductive failure. Positive A = Simulated ranavirosis population under a 10% annual chance of reproductive failure and a 10% annual chance of a recurrent adult mass mortality event in exclusive years. Positive B = Simulated ranavirosis positive population under identical conditions to Positive A with addition of a 5% annual chance of complete recruitment failure and adult mass mortality in the same year. *K*= Imposed population carrying capacity of 200.

1

Model	Extinct	<50	50-100	100-150	150-199	K
Disease-Free	12	525	177	734	1319	2233
Positive	429	4062	443	48	15	3
Positive A	611	4300	87	2	0	0
Positive B	2918	2081	1	0	0	0