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Genetic erosion and escalating extinction risk in frogs with increasing wildfire frequency

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Running Title: Fires increase genetic extinction risk

Summary

1. Wildfires are increasing in both frequency and intensity in many ecosystems, with climate change models predicting further escalations in fire-prone environments. Set against this background is the global decline of amphibians, with up to 40% of species facing extinction from multiple additive threats. Despite these disturbing figures, it is currently unclear how increasing fire frequency may impact the long-term persistence of frog populations.

2. Following a severe wildfire in south-eastern Australia in 2009, field surveys indicated healthy tree-frog populations. However, the 2009 fire had significant impacts on genetic diversity, including increased levels of inbreeding and declines in effective population size.

3. Using stochastic population modelling under a range of fire-frequency scenarios, we demonstrate that amphibian populations in fire-prone environments may be increasingly vulnerable to extinction, particularly where rates of immigration are low.

4. *Synthesis and applications.* This study of amphibian population genetics before and after a major wildfire emphasizes the importance of integrating both ecological and genetic data into population models. This will help managers make more appropriate conservation decisions regarding fire management of natural environments, especially those containing threatened populations. Priorities for agencies involved in planning controlled burns should consider carefully the timing of controlled burns, along with maintaining habitat connectivity.

Key-words: amphibians, extinction risk, field surveys, fire management, frogs, *Litoria*, population genetics, population modelling, wildfire

Introduction

In the past thirty years, wildfires have increased in both frequency and intensity in many ecosystems, particularly in environments already strongly influenced by fire such as the western United States (Westerling *et al.* 2006) and eastern Australia (Lindenmayer *et al.* 2011). In addition, climate models have predicted an increase in the frequency and severity of wildfires into the future (Flannigan, Stocks & Wotton 2000; Westerling *et al.* 2006; Pitman, Narisma & McAneney 2007). Like other ecological disturbances, wildfires can drive spatial and temporal variation in the abundance of species and the composition of communities (Banks *et al.* 2013). However, the impacts of wildfire on the genetic diversity of populations and species are poorly understood. As genetic diversity has a fundamental influence on individual fitness, population viability and species adaptability to environmental change (Banks *et al.* 2013), this limited understanding hampers our ability to predict how increasing fire frequency with anthropogenic climate change will influence the persistence of species.

Amphibians are believed to be particularly vulnerable to changing environmental conditions, with more than 70% of frog species predicted to be negatively impacted by climate change in some regions (Wake 2012). Although research into the impacts of anthropogenic climate change on amphibians has often focused on changes in temperature and rainfall that may lead to range shifts (Early & Sax 2011), associated changes in wildfire regimes may also have profound effects on species persistence in fire-prone environments. Specifically, impacts of wildfire on population connectivity and genetic diversity has been severely understudied in this context. Many studies have already shown a decline in the genetic diversity of frogs due to habitat fragmentation (Andersen, Fog & Damgaard 2004; Lesbarrères *et al.* 2006; Hale *et al.* 2013), however there are currently no empirical data on the impacts of fire on genetic diversity in amphibians using pre- and post-fire estimates. Results of the few studies on other faunal groups have been mixed, with one finding evidence of changes in allele frequencies but not of population bottlenecks post-fire (Suárez *et al.* 2012) and another finding both increasing and decreasing genetic diversity (Smith *et al.* 2014). Surprisingly, numerous studies estimating post-fire abundance using count and call surveys have indicated that frog species are resilient to fire (Bamford 1992; Ford *et al.* 1999; Greenburg & Waldrop 2008; Engbrecht & Lannoo 2012; Westgate, Driscoll & Lindenmayer 2012). These studies have concluded that wildfires may not be as important as other ecological disturbances for frogs (Westgate, Driscoll & Lindenmayer 2012).

Research on the biology of tree frogs in the Kinglake region of Victoria, south-eastern Australia (Fig. S1), has spanned more than fifty years (Littlejohn 1965; Smith *et al.* 2013a; Smith *et al.* 2013b), and we have been studying the population genetics of these frogs since 2007. In February 2009, this region was burnt by an extensive wildfire – the worst in Australian recorded history (Cordner, Woodford & Bassed 2011). Severe wildfires are not exceptional events in this area, with five significant fires in the last 100 years; climate change models predict that local wildfires will increase in severity and extent in the future, with potentially devastating effects (Pitman, Narisma & McAneney 2007). We investigated the effects of this wildfire on the population genetic structure of the tree frogs Southern brown tree frog *Litoria ewingii* and Victorian tree frog *Litoria paraewingi* in a study spanning seven years, two of which were pre-fire. We then used these empirical genetic data in stochastic population models of each species to determine how wildfire frequency may influence the effective population size and extinction risk of populations of common frog species in the context of anthropogenic climate change. We extended this model to predict the effects of increased fire frequency on a related but threatened species, *Litoria raniformis* (growling grass frog), which is federally listed as Vulnerable to Extinction.

Materials and methods

Study System

We focused this study on comprehensively studied populations of *Litoria ewingii* and *Litoria paraewingi*, two closely related pond-breeding hylid frogs in the Kinglake region of Victoria, Australia (~ -37.50°S, 145.42°E). Historical studies have shown that the morphology, life-histories, habitat requirements and breeding behaviours of these two species are almost identical (Watson, Loftus-Hills & Littlejohn 1971; Littlejohn 1982; Sherwin 1984). Both species are small (20-45 mm SVL), and breed during the southern winter in ponds, marshlands and streams. The two species are common in a variety of habitats, including farmland and forested areas (Watson, Loftus-Hills & Littlejohn 1971).

Field Surveys and Population Estimates

We conducted fieldwork between June and October over seven years (2007-2013), to coincide with the breeding season of *Litoria ewingii* and *L. paraewingi*. Each year, we sampled eleven ponds across the Glenburn transect (Fig. 1). Two sites were unburnt in 2009, the rest were burnt with varying degrees of intensity in early February 2009. We visited each site two to four times per year, spaced equally across the breeding season. During each visit, we made a five-minute acoustic recording (Smith *et al.* 2013a) once a breeding chorus had begun (approximately one hour after sunset) using a Rode microphone and a Marantz PMD671 Solid State recorder (Marantz, Kanagawa, Japan). Following this, we used a capture-sampling technique to locate frogs using acoustic cues and capture by hand by two trained personnel for a period of 30 minutes between 1830 h and 2200 h. A single toe clip was taken from each individual for subsequent genetic analysis before release at the site of capture.

We captured a total of 680 individuals from the *Litoria* genus over the course of the study (Table 1). Re-captures within a season were rare and not included in these counts. In the laboratory, we used the software Syrinx (Burt 2001) to create spectrograms, or visual representations of all acoustic recordings. We then used both acoustic and visual means of counting the number of breeding males calling in any one recording. Following the protocol of Smith, Oliver and Littlejohn (2012), we identified individuals via their individual call frequency, call structure (i.e. number of notes in the call), call rate and geographic position in the pond. We used the sum of these counts to estimate the number of breeding males throughout the season at each site in order to be consistent with capture counts. All work was done within Museum Victoria and University of Melbourne ethical guidelines.

Genetic Techniques and Analyses

We extracted DNA from tissue samples using two methods; samples from 2007-2009 were extracted using a standard chloroform: isoamyl alcohol procedure (Gemmell & Akiyama 1996); samples from 2010-2013 were extracted using a Qiaxtractor and a standard protocol (QIAGEN). We then used the PCR-RFLP assay protocol described in Smith, Hale, Kearney, *et al.* (2013b) to identify whether an individual was *L. ewingii* or *L. paraewingi* by amplifying and digesting mitochondrial DNA segments. This process also identified 35 individuals as *Litoria verreauxii,* a third and closely related species; these were removed from further analysis. We used PCR to amplify eight microsatellite markers that have been described for these species (Smith *et al.* 2011) using a previously established laboratory protocol (Smith *et al.* 2013b). We sent PCR products to Macrogen, Inc. (Seoul, Korea) for genotyping using an ABI 3730XL. We scored alleles using Geneious v. 6.1.2, screening manually to check for accuracy.

We conducted genetic analysis on each species separately. Alleles were confirmed to conform generally to Hardy-Weinberg equilibrium using GenePop 4.2 (Raymond & Rousset 1995). We used the program STRUCTURE v 2.3.4 (Pritchard, Stephens & Donnelly 2000) to confirm the species identity of each individual and identify any *Litoria ewingii-paraewingi* hybrid individuals (methods described in Smith *et al.* 2011; Smith *et al.* 2013b). All 80 individuals identified as hybrids were removed from subsequent analyses. To determine the level of population-wide inbreeding we used Genodive 2.0 (Meirmans & Van Tienderen 2004) to obtain G-indices (Gis - the mean observed heterozygosity in relation to expected heterozygosity within each site for a given year) and allelic richness (AR - the number of times an allele is found in the population divided by the sum of the count of all different alleles in the population) for the entire metapopulation each year from 2007-2013. We then used Genodive to estimate pairwise Fst values between each pond for each year. We used STRUCTURE to estimate an individual's membership in a population each year and also to estimate the number of distinct genetic groupings (populations) before and after the fire (Fig. 3). STRUCTURE uses a Bayesian approach to analyze microsatellite data sets without *a priori* assumptions of population structure. Each individual is placed in one out of a number (K) of estimated populations with a likelihood percentage based on their genetic profile. We varied K from 1 to 11 (number of ponds) for analyses of all populations and used admixed origins of populations. The burn-in was set at 100,000 with 100,000 runs, with each dataset run five times to check for consistency. The most likely value of K was determined by observing where values of $\Pr(X|K)$ plateaued (Evanno et al 2005). These values were compared to similar estimates made in the program Geneland (Guillot, Mortier & Estoup 2005; Guillot 2008), which also maps the probability of population membership geographically.

We used NeEstimator V2.01 to obtain an estimate of year-wide effective population size (Ne) from the microsatellite data (Do *et al.* 2014). We chose a Linkage Disequilibrium model with random mating, temporal estimation and molecular coancestry, in order to obtain the most accurate estimates for our system (Gilbert & Whitlock 2015). Unfortunately, sample sizes from each pond for each year were too small to gain accurate estimates of Ne for each subpopulation, therefore Ne was calculated for the entire metapopulation each year.

The effects of wildfire on population counts as observed in field surveys (Table 1) were then estimated using a Bayesian regression model in the program OpenBUGS version 3.3.2 (Spiegelhalter 2006), using year as a predictive variable. The models included uninformative priors as well as a random site effect to account for geographic location of each subpopulation. This model estimated the mean, standard deviation, and 95% credible intervals of model coefficients from 100,000 samples from the posterior distribution after discarding an initial 'burn-in' of 100,000 samples to check for convergence. Using these same parameters, we then used a Bayesian one-way ANOVA with pre-fire levels (from years 2007-2008) as a reference class and compared these with values obtained in the years immediately following fire (post-fire: 2009-2010) and in a recovery period (2011-2013) to analyze how allelic richness, Gis and Ne may have been affected immediately post-fire and during the recovery period.

Stochastic Population Models

We constructed stochastic population models for *Litoria ewingii*, *L. paraewingi* and *L. raniformis* in OpenBUGS (Spiegelhalter 2006; Appendix S1), using information on annual migration rate and effective population size (Ne) at equilibrium from the present study and a population-genetic study of *L. raniformis* in northern Melbourne. While *L. ewingii* and *L. paraewingi* are hybridizing sister species, we chose to model them separately as our results indicated important differences in annual migration rate. We also constructed a population model for *L. raniformis* as a representative threatened species within the genus that is also affected by wildfire. Data used in the model of *L. raniformis* were a combination of unpublished and published data, based on 10 microsatellite loci (Hale *et al.* 2013).

Small sample sizes meant that year-to-year estimates of Ne for *L. paraewingi* fluctuated substantially. Therefore, we estimated effective population size at equilibrium for the *L. ewingii* and *paraewingi* models from the average Ne for *L. ewingii* observed across the six non-fire years of our study (2007-8, 2010-13). Effective population size at equilibrium for *L. raniformis* was estimated from a detailed study of this species in northern Melbourne (C. Keely, unpublished data). We modelled the number of immigrants into the population each year as a Poisson function with $\lambda = 5$ (for the *L. ewingii* model) and $\lambda = 1$ (for the *L. paraewingi* and *L. raniformis* models), and ran each model for a period of 100 years. These values were drawn from the mean and variation in annual Nm values estimated by Genepop using the original microsatellite data. We estimated maximum population growth rate and the standard deviation in growth rate due to environmental stochasticity using a combination of published data, unpublished data and expert opinion on vital rates in the closely related *L. spenceri* (for the *L. ewingii* and *L. paraewingi* models) and *L. raniformis (*for the *L. raniformis* model).

The probability of fire was expressed as a Bernoulli function, with each fire leading to a 95% reduction in effective population size. This reduction was based on the change in Ne following fire observed in the present study. We ran the model under four fire-frequency regimes; an average of 1 fire in 100 years, 1 in 50 years, 1 in 25 years and 1 in 10 years. This range was based on estimates that fire frequencies will increase by 25% by 2050 and a further 20% by 2100 in southeastern Australia (Pitman, Narisma & McAneney 2007). Forests in our study region have experienced at least six significant fires in the last 100 years, in 1926, 1939, 1962, 1983, 2006 and 2009 (Collins 2008; Lindenmayer *et al.* 2011). Thus, with predicted increases in fire frequencies, an average fire frequency of 1 in 10 years represents a plausible, if extreme, scenario. For each fire frequency, we estimated the mean, standard deviation, and the 2.5th and 97.5th percentiles (to give a 95% credible interval) of expected minimum population size (expressed as a proportion of the initial effective population size (Ne) at equilibrium) and the probability of extinction over 100 years. We used 1,000 samples drawn from the posterior distribution after discarding 1,000 samples as an initial ‘burn-in’ and checking for convergence via visual inspection of the MCMC chain histories in OpenBUGS. These values are shown in Tables S2 and S3.

Results

Using traditional survey methods to estimate frog abundance, we found minimal impact of the 2009 wildfire on populations of *Litoria ewingii (LE)* and *L. paraewingi (LP)*. We used recordings of calling males from across the eleven ponds to estimate abundance during each breeding season (June-October) from 2007-2013. Estimates of maximum abundance based on acoustic recordings indicated a small effect of fire on breeding males (Table 1). There were approximately 12% fewer males recorded in each of the two years post-fire than in each of the two years pre-fire (mean effect of fire on abundance estimates = -2.25, 95% CIs = -6.33, 1.86). Immediately post-fire (2009), we detected only a ~4% decrease in the number of breeding males (mean difference in abundance estimates between 2008 and 2009 = -0.56, 95% CIs = -1.44, 0.32).

However, genetic data showed that both species were more inbred post-fire than pre-fire (Fig. 2). *LE* showed an increase in inbreeding of approximately 21% (mean increase in Gis post-fire = 0.042, 95% CIs = -0.049, 0.133), while *LP* showed an increase in inbreeding of approximately 139% (mean increase = 0.12, 95% CIs = -0.03, 0.26) over the two years post-fire, compared to the two years pre-fire (Fig. 2). Coupled with these increased levels of inbreeding, effective population sizes (Ne) declined post-fire. Effective population size of *LE* populations decreased by 98% immediately following the fire in 2009, and then showed steady recovery in subsequent years; that of *LP* populations decreased by 74% in 2009 followed by a further decrease of 99% from original pre-fire levels in 2010 (Table 2, Table 3, Figure 3), with populations not fully recovered at the completion of the study. See Table 2 for all other genetic diversity estimates for each year.

These patterns were confirmed with the post-hoc analyses by time period: statistics can be found in Table 3. The 95% credible intervals of the posterior probability distributions were wide (Table 3) due to pooling of data into one meta-population each year (See Appendix S2 for estimates using each pond as a population). However, results demonstrated that allelic richness in *LE* and *LP* declined by 2-7% after the fire and returned to pre-fire levels within the study period, although this decline was more pronounced in *LP* than in *LE* (Table 3).

Spatial population genetic analyses in Geneland demonstrated that the connectivity between populations decreased in the two years post-fire (*22*). STRUCTURE analyses supported these results, identifying that the most likely number of genetic populations increased from four (*LE)* and two (*LP)* before the fire, to seven (*LE*) and five (*LP*) after the fire (Fig. 3). In 2012-2013, STRUCTURE identified three populations of *LE* and two populations of *LP*, which is very similar to pre-fire population structures, however with markedly less admixture. It should be noted that Ne estimates may reflect population structure; in this case, the increased differentiation between populations may have contributed to the dramatic changes in Ne estimates observed post-fire.

Stochastic population modelling indicated an important effect of fire frequency, immigration rate and variation in the population growth rate due to environmental stochasticity on the persistence of populations over time, expressed as expected minimum population size and the probability of extinction over a 100-year period (Fig. 4; Table S1). For all three species, expected minimum population size decreased and the probability of extinction increased with increasing fire frequency. However, these effects differed substantially between species. For example, with an average fire frequency of 1 in 100 years, the expected minimum population size of *LE* was 0.33 of the effective population size at equilibrium (95% CI: 0.29-0.37), decreasing to 0.073 (0.063-0.083) with an average fire frequency of 10 in 100 years (Fig. 4a). The corresponding EMP sizes for *LR* were 0.046 (0.036-0.055) and 0.0041 (0.0018-0.0067), with those for *LP* intermediate between the two. The probability of extinction of the *LE* population was relatively low, but increased from 0.01 (0.00-0.04) to 0.12 (0.06-0.19) with increasing fire frequency from an average of 1 to 10 fires per 100 years. In contrast, the probability of extinction of the *LR* population increased from 0.40 (0.31-0.50) to 0.91 (0.86-0.96) with the same increase in average fire frequency (Fig. 4b; Table S2). The combination of more variable population-growth rate and a low immigration rate appear to make populations of *LR* the most vulnerable to increasing fire frequency of the three species modelled, while a lower immigration rate makes *LP* more vulnerable than *LE*.

Discussion

Impact of Wildfire: Field Surveys and Genetic Data

Acoustic and count surveys detected only a small effect of wildfire on the number of breeding males of our study species. This is consistent with a number of previous studies suggesting that frogs are resilient to fire, based on high post-fire count data (Keyser *et al.* 2004; Greenburg & Waldrop 2008; Engbrecht & Lannoo 2012; Hossack, Lowe & Corn 2013). Explanations for this resilience include the concurrent resilience of food items (e.g., freshwater invertebrates) (Dunham *et al.* 2007), behavioural adjustments (Engbrecht & Lannoo 2012) and increased immigration of individuals from nearby source populations (Greenburg & Waldrop 2008). In fact, it has been suggested that the warming of soils and other changes post-fire are advantageous to some anurans, resulting in increasing numbers post-fire (Hossack *et al.* 2009; Moorman, Russell & Greenberg 2011; Hossack, Lowe & Corn 2013). On the basis of count data we might conclude that our study species are also resilient to severe wildfire, and possibly well adapted to such recurring ecological disturbances.

However, genetic data showed that effective population sizes of both *L. ewingii (LE)* and *L. paraewingi (LP)* were lower, populations were more inbred, and there was a drop in allelic richness following the fire. While the genetic diversity of populations of both species recovered in the years post-fire, genetic diversity of *LP* populations had not recovered to pre-fire levels after five years. The 2009 fire caused not only a decrease in genetic diversity but also marked changes in the spatial structure of frog populations in the region. Previous studies that have attempted to quantify effects of fire on genetic patterns in vertebrates have found contrasting results (Schrey *et al.* 2011). Of those focusing on sampling before and after a specific fire, one study on chaffinches (*Fringilla teydea polatzeki)* found no impact on population inbreeding or genetic richness (Suárez *et al.* 2012), and another on lizards (*Amphibolurus norrisi* and *Ctenotus atlas*) a decrease in genetic diversity but no concurrent effects on gene flow or population genetic structure (Smith *et al.* 2014). While census data on the chaffinches indicated a major population decline, genetic results (collected only post-fire) indicated a healthy genetic diversity (Suárez *et al.* 2012). These results provide evidence that discrepancies in population estimates based on field-survey counts and genetics following wildfire, although a growing body of evidence suggests that genetic data may be more accurate and more useful from a conservation standpoint (Beissinger & Westphal 1998; Storfer 2003; Palsbøll, Bérubé & Allendorf 2007).

Estimates of population size made from ecological field surveys are often found to differ from those made using genetic data in studies where both methods are used (Palstra & Fraser 2012). Count data (using acoustic recordings or capture techniques) of amphibians in particular may be a poor reflection of actual population size (Brown *et al.* 2007; Elphick 2008; Corn *et al.* 2011).

In order to accurately quantify the effects of fire on populations in the long term, more data must be incorporated; the inclusion of genetic data in studies to estimate the effects of disturbances on population viability in the past has proven valuable (Storfer 2003; Palstra & Fraser 2012). The differing conclusions about the status of *Litoria* populations post-fire based on our field surveys and genetic data suggest four possible scenarios. First, while calling males may have recovered post-fire, females may have been more severely affected, resulting in a large sex-ratio bias and lower population sizes in subsequent years as reproduction declined: increased sampling of females would be required to test this. Second, while adults may have survived the fire, reproduction may have been affected by high egg/larval failures (Driscoll & Roberts 1997) or predation of eggs or larvae. Third, insulated populations may have had a high survival rate during the fire, but dispersal (i.e. movement between ponds) may have been impeded and inbreeding may have risen post-fire, resulting in the observed increase in spatial genetic structure (Fig. 3). Lastly, mortality due to fire could have been restricted to adult individuals, while tadpoles - present at any time of year - remained safe and matured to breed in the winter after the fires. This mortality bias would then mean that alleles present in the population in the year after the fire would likely be a diminished sample, representing only those present in the cohort that was at a larval stage at the time of the fire. Indeed, cohort-based survival and reproduction post-fire presenting with a similar genetic structure and differentiation pattern have been observed in other taxa (Pierson *et al.* 2013). Any of these scenarios may account for the low genetic diversity observed in the burnt populations of both species in the years following the fire.

Extinction Risk with Increasing Wildfire Frequency

While natural wildfire plays an important ecological role in southern Australia (Burrows 2008), climate models predict that current and future climatic changes will have unprecedented impacts on the frequency and severity of fires in this region (Hennessy *et al.* 2005). Stochastic disturbances may contribute to biodiversity in ecosystems where fire naturally – albeit infrequently – occurs (Morrison *et al.* 1996). However, it is difficult to predict how changing fire regimes might impact populations and species in the coming centuries, even those that have evolved in ecosystems that benefit from natural fire over the long-term (Westgate, Driscoll & Lindenmayer 2012). In the context of global change, it is important to consider how distinct human activities (e.g., changing climate, landscape modification) have synergistic impacts on natural systems. Understanding how predicted changes might contribute to a species' likelihood of local extinction or persistence is vital for making informed decisions about management and conservation of native wildlife in fire-prone areas of the world.

Stochastic models of population dynamics showed an important effect of fire frequency and on the persistence of populations over time, but these effects differed between species. These inter-specific differences are likely driven by the higher coefficient of variation in adult survival rate leading to a higher standard deviation in population growth rate in the more vulnerable *Litoria raniformis*, as well as a higher immigration rate in *L. ewingii* than in *L. raniformis* and *L. paraewingi* (expected immigration rate of 5 versus 1 individual per year). Our modelling indicated a probability of local extinction > 80% for *L. raniformis* and *L. paraewingi* (the two species with the lowest immigration rate) over 100 years if fire frequency reaches an average of 10 fires/100 years. .These results indicate that isolated populations of frogs with low rates of immigration are vulnerable to extinction under increasing fire frequencies expected in southeastern Australia with climate change.

Conclusions and Management recommendations

Previous studies have shown an intricate relationship between climate change, habitat loss and disease in the extinction risk of amphibians (Hof *et al.* 2011). Here, we provide evidence of an additional cause of extinction risk in amphibians with anthropogenic climate change. Our results support the disturbing suggestion that, at least in some environments, extinctions may result from climate-mediated causes other than increasing temperatures, long before the physiological inability to adapt to high temperatures becomes an issue (Cahill *et al.* 2012). The data from our models show that one of the most important factors for decreasing extinction risk due to increased wildfires is the ability of animals to move between habitat patches in fire-prone environments. We recommend that prescribed burning regimes in threatened species' habitats should incorporate data regarding genetic connectivity rather than relying only on count data when planning the timing and extent of controlled burns. Additionally, conservation efforts should focus on maintaining or re-establishing habitat connectivity (including vegetation and waterways) in fire-prone areas, so that animals - especially amphibians - can move across the landscape in order to re-establish genetically healthy populations after wildfires. Such actions will have a profound impact on the long-term persistence of frog populations in areas of increasing human-mediated disturbance.

Authors' Contributions: JJA, JM & KMP conceived the study, DAP, KLS, JH, RDB, SH & JM organized fieldwork and collected data, KLS, JH, DAP, SH, CCK & RDB performed genetic labwork and analyses, DAP performed statistics on the genetic data and wrote the first draft of the manuscript, KMP constructed and ran the population models, and all authors contributed substantially to revisions.

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Data accessibility

Data supporting the results of this article are available in the Figshare digital repository and can be accessed at https://dx.doi.org/10.4225/49/57DF2AEE61B76 (Potvin *et al.* 2016).**References:**

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Parameter estimates from the population models of *Litoria ewingii* under various fire frequencies and immigration rates.

Table S2. Parameter estimates from the population models of *Litoria raniformis* under various fire frequencies and immigration rates.

Appendix S1. OpenBUGS code for stochastic population models

Appendix S2. Population genetic data results as calculated by Genodive, using each pond each year as a population within the system**.**

Table 1. Count and acoustic survey results over the seven-year study period. Numbers reflect only males, since surveying was done using acoustic recordings of male advertisement calls and frogs were located for capture using calls. Acoustic results are pooled for both species (*Litoria ewingii*, LE and *Litoria paraewingi*, LP) due to the similarity of calls between species.

	Sample sizes (actual count estimates)				**Acoustic count estimates**
	LE	LP	Hyb	Total	Total
2007	42	31	8	81	70
2008	52	18	12	82	70
2009	75	17	13	105	89
2010	49	26	6	81	63
2011	61	27	16	104	83
2012	81	7	17	105	54
2013	62	9	16	87	84
TOTAL	422	135	88	645	513

Table 2. Mean number of alleles, corrected heterozygosity (H_s), inbreeding values (G_{is}), effective population size (N_e), number of migrants (N_m) and Fst values between ponds for *Litoria* populations during pre-fire (2007-2008), post-fire (2009-2011) and recovery years (2012-2013). 95% Confidence Intervals are given for all means except for N_m (data unattainable).

	2007	2008	2009	2010	2011	2012	2013
Litoria ewingii							
Alleles	9.625±2.72	8.750±2.42	7.750±2.35	9.286±1.83	12.000±2.07	6.714±1.98	12.286±1.22
H_s	0.746±0.056	0.747±0.039	0.749±0.019	0.785±0.022	0.774±0.032	0.802±0.041	0.726±0.037
G_{is}	0.191±0.086	0.140±0.095	0.136±0.034	0.317±0.060	0.249±0.038	0.208±0.043	0.139±0.065
N_e	11.5±8.3	73±41.4	12.5±0.2	27.5±0.5	14.5±1.5	26.2±6.3	10.9±2.2
N_m	2.133	3.515	9.127	4.075	7.249	6.858	3.813
Fst	0.030±0.025	0.029±0.027	0.018±0.011	0.00±0.00	0.014±0.013	0.008±0.005	0.065±0.027
Litoria paraewingi							
Alleles	13.000±1.61	13.625±1.49	14.250±2.24	12.143±2.06	12.857±2.72	14.571±0.70	14.571±0.07
H_s	0.826±0.021	0.794±0.037	0.801±0.056	0.779±0.12	0.768±0.040	0.757±0.058	0.743±0.037
G_{is}	0.070±0.03	0.089±0.13	0.088±0.08	0.337±0.29	0.270±0.08	0.172±0.22	0.085±0.07
N_e	59.6±41.6	104.2±39.0	26.7±0.0	*too low	6.1±2.9	7.3±4.4	206.2±82.6
N_m	1.538	0.942	1.888	0.631	2.561	0.412	1.050
Fst	0.006±0.003	0.00±0.00	0.00±0.00	0.017±0.035	0.027±0.018	0.004±0.006	0.009±0.002

		Pre-Fire	**Post-Fire effect**			**Recovery Period effect**		
		mean ± sd	mean	2.5%CI	97.5%CI	mean	2.5%CI	97.5%CI
Litoria ewingii	G_{is}	0.198 ± 0.07	0.042	-0.049	0.133	0.016	-0.021	0.053
	AR	9.177±	-0.66	-6.98	5.71	1.16	-4.65	6.93

Table 3. The mean (± sd) immediate effect of the fires (2009-2010) and subsequent recovery period (2011-2012) on the inbreeding level (G_{is}), allelic richness (AR) and effective population size (N_e) of the *Litoria ewingii* and *Litoria paraewingi* metapopulations, as determined by Bayesian analysis. Table includes 95% credible intervals of the effects.

		2.30						
	N_e	42.1 ± 23.2	-30.81	-95.31	34.07	-24.93	-83.91	33.91
Litoria paraewingi	G_{is}	0.08 ± 0.005	0.115	-0.028	0.26	-0.028	-0.089	0.033
	AR	13.31 ± 1.05	-0.11	-2.98	2.78	0.69	-1.94	3.31
	N_e	80.58 ± 79.38	-66.78	-287.3	158.2	-7.419	-210.0	196.4

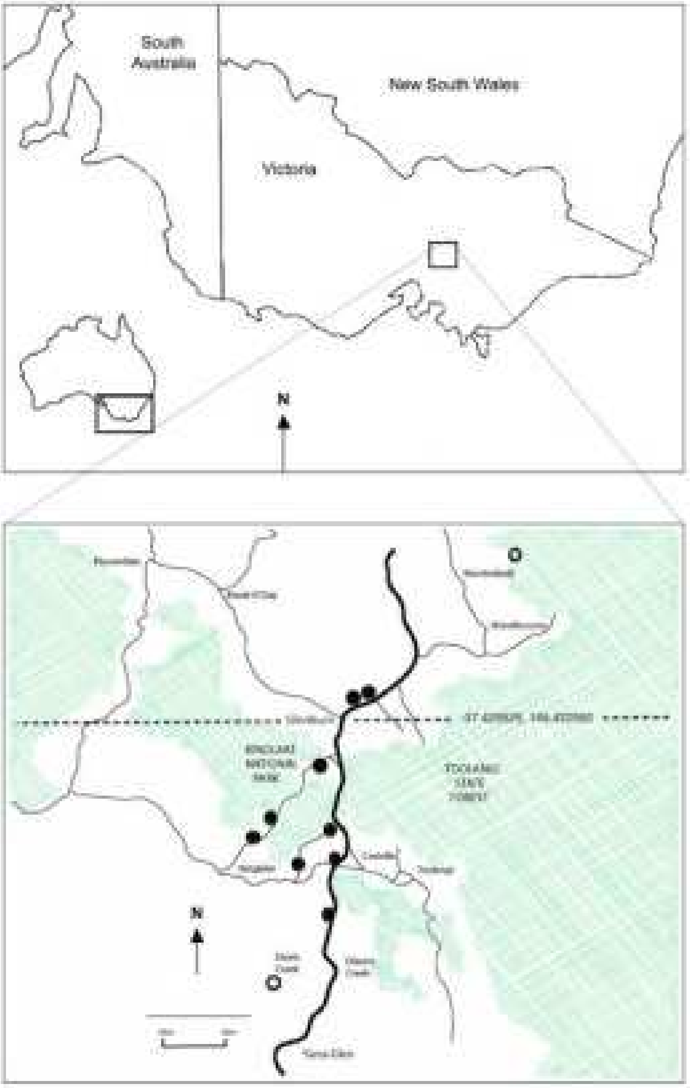
Figure 1. Map of the Glenburn transect, including the locations of each of the eleven sites sampled 2007-2013. Filled circles indicate sites burned in the 2009 wildfires, open circles indicate sites that remained unburned. Shaded areas indicate protected forested regions versus cleared land (unshaded).

Figure 2. Inbreeding levels (G_{is}) and effective population size (Ne) over the study period for *L. ewingii* (Le) and *L. paraewingi* (Lp). Both species show increased G_{is} immediately post-fire, resulting in low effective population sizes. Genetic diversity has not completely recovered after five years. Values are not provided for LP in 2012 and 2013 because, although the total number of frogs caught did not drop, the proportion of LP was too low to provide accurate estimates (Table S1).

Figure 3. Population genetic structure for *L. ewingii* and *L. paraewingi* pre-fire, post-fire and during the recovery period. Graphs presented are STRUCTURE plots (*22*), where individuals are assigned probabilistically to populations, or jointly to two or more populations if their genotypes indicate they are admixed, with each color representing a separate population. The x-axis represents the North-South transect from left-right (Fig. 1). Our results indicate that immediately post-fire, allele and genotype frequencies are significantly affected, with a return to

pre-fire conditions in *LP* 2011-13, while in *LE* the fire appears to drive some spatial structure in 2011-2013.

Figure 4. Expected minimum population size (EMP) and the probability of extinction of populations of *Litoria ewingii*, *L. paraewingi* and *L. raniformis* over 100 years under four fire regimes. EMP is expressed as a proportion of initial population size, which was set at the equilibrium effective population size (Ne). Error bars show the 95% credible intervals around the estimated values.



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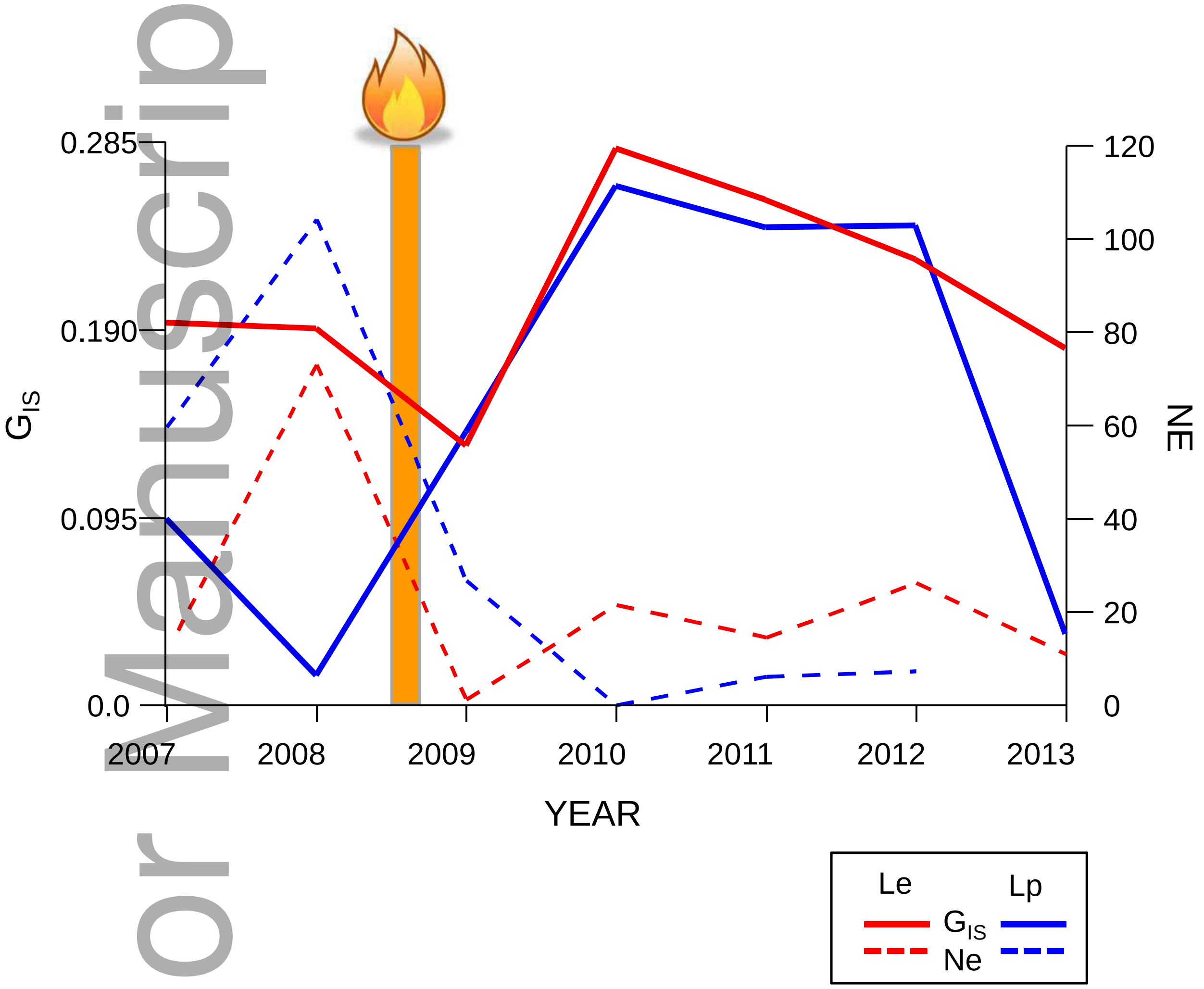
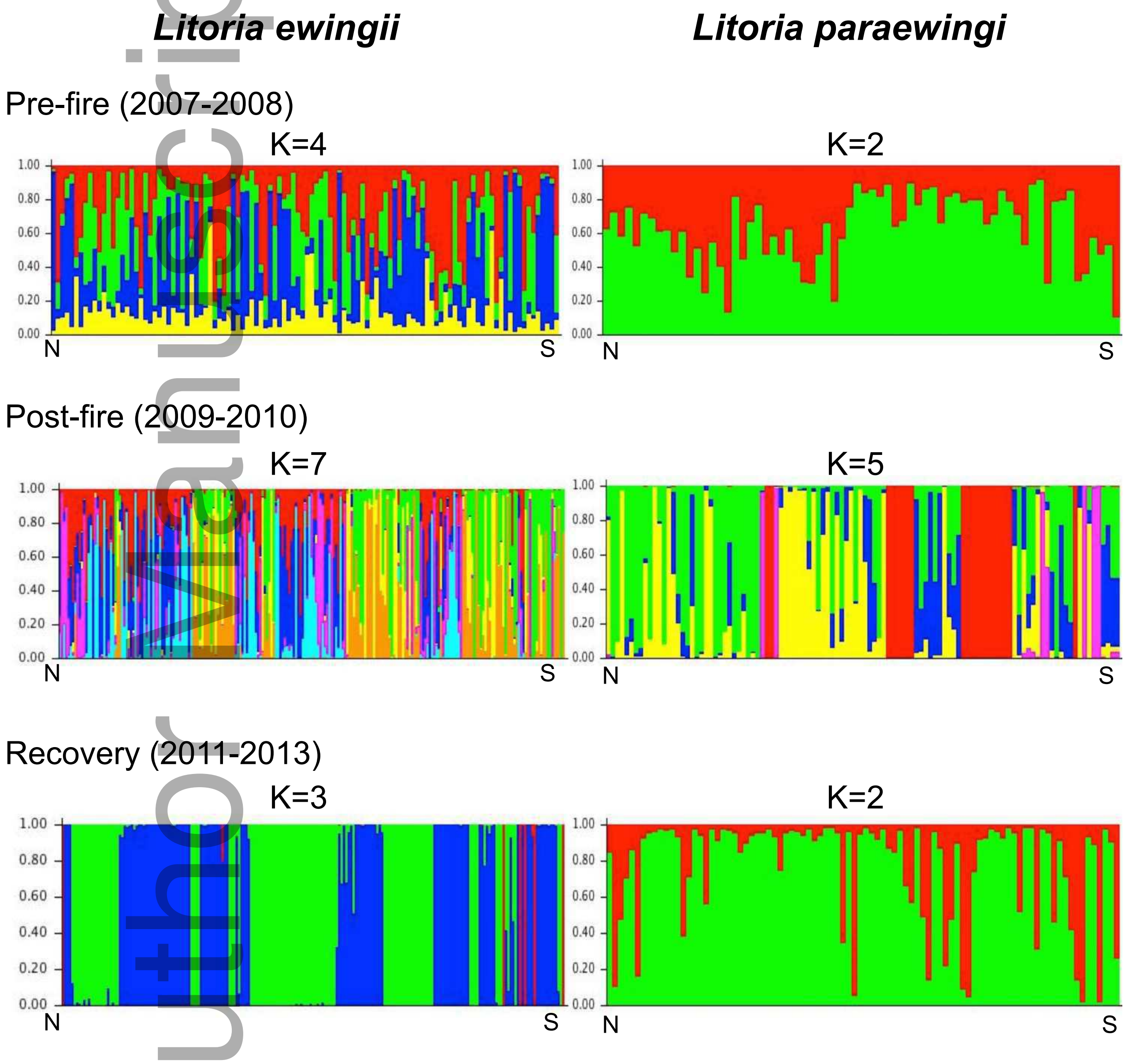
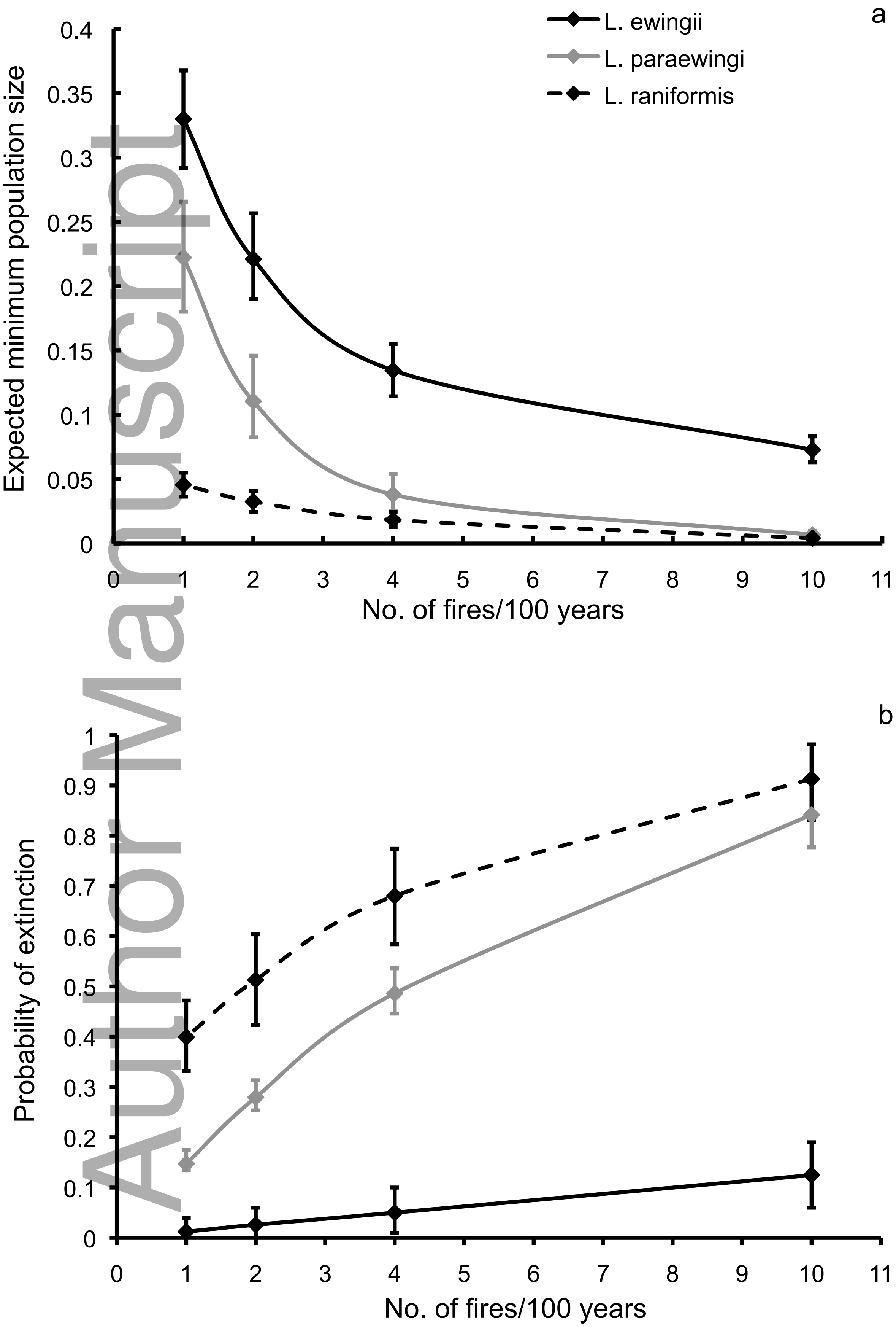

0.285
0.190
0.095
0.0
G_{IS}
120
100
80
60
40
20
0
NE
2007
2008
2009
2010
2011
2012
2013
YEAR
Le
Lp
G_{IS}
Ne


Litoria ewingii
Litoria paraewingi
Pre-fire (2007-2008)
K=4
K=2
Post-fire (2009-2010)
K=7
K=5
Recovery (2011-2013)
K=3
K=2
1.00
0.80
0.60
0.40
0.20
0.00
N
S


a
L. ewingii
L. paraewingi
L. raniformis
Expected minimum population size
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0
1
2
3
4
5
6
7
8
9
10
11
No. of fires/100 years
b
Probability of extinction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5
6
7
8
9
10
11
No. of fires/100 years