A cat's tale: the impact of genetic restoration on Florida panther population dynamics and persistence

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Summary

1. Genetic restoration has been suggested as a management tool for mitigating detrimental effects of inbreeding depression in small, inbred populations, but the demographic mechanisms underlying population-level responses to genetic restoration remain poorly understood.

2. We studied the dynamics and persistence of the endangered Florida panther *Puma concolor coryi* population and evaluated the potential influence of genetic restoration on population growth and persistence parameters. As part of the genetic restoration programme, eight female Texas pumas *P. c. stanleyana* were released into Florida panther habitat in southern Florida in 1995.

3. The overall asymptotic population growth rate (λ) was 1·04 (5th and 95th percentiles: 0·95–1·14), suggesting an increase in the panther population of approximately 4% per year. Considering the effects of environmental and demographic stochasticities and density-dependence, the probability that the population will fall below 10 panthers within 100 years was 0·072 (0–0·606).

4. Our results suggest that the population would have declined at 5% per year (λ = 0·95; 0·83–1·08) in the absence of genetic restoration. Retrospective life table response experiment analysis revealed that the positive effect of genetic restoration on survival of kittens was primarily responsible for the substantial growth of the panther population that would otherwise have been declining.

5. For comparative purposes, we also estimated probability of quasi-extinction under two scenarios – implementation of genetic restoration and no genetic restoration initiative – using the estimated abundance of panthers in 1995, the year genetic restoration was initiated. Assuming no density-dependence, the probability that the panther population would fall below 10 panthers by 2010 was 0·098 (0·002–0·332) for the restoration scenario and 0·445 (0·032–0·944) for the no restoration scenario, providing further evidence that the panther population would have faced a substantially higher risk of extinction if the genetic restoration initiative had not been implemented.

6. Our results, along with those reporting increases in population size and improvements in biomedical correlates of inbreeding depression, provide strong evidence that genetic restoration substantially contributed to the observed increases in the Florida panther population.

Key-words: density-dependence, Florida panther, genetic introgression, genetic restoration, population viability analysis, probability of extinction, *Puma concolor coryi*, stochastic population growth rate, stochasticity, uncertainty

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Introduction

Loss of genetic variation and an increase in inbreeding are inevitable in small, isolated populations (Frankham, Briscoe, & Ballou 2002). Many such populations ultimately suffer from inbreeding depression due to reduced heterozygosity and/or increased expression of deleterious recessive alleles (Frankham, Briscoe, & Ballou 2002; Tallmon, Luikart, & Waples 2004; Charlesworth & Willis 2009). Indeed, many field studies have reported that inbreeding leads to reduction in survival (Keller *et al.* 1994; Liberg *et al.* 2005; Hostetler *et al.* 2010), reproduction (Ortego *et al.* 2007; Charpentier *et al.* 2008) and, ultimately, fitness (Slate *et al.* 2000; Höglund *et al.* 2002). At the population level, inbreeding depression can reduce population growth rate and increase extinction risk (Nieminen *et al.* 2001; Haag *et al.* 2002; Reed, Nicholas, & Stratton 2007; Aguirre & Marshall 2012). Low levels of immigration have been suggested to help alleviate detrimental effects of inbreeding depression (Ingvarsson 2001; Tallmon, Luikart, & Waples 2004). Improvements in fitness of inbred populations from natural or experimentally induced immigration of genetically divergent individuals (known variously as genetic rescue, genetic restoration and genetic introgression; Tallmon, Luikart, & Waples 2004) has been successful in enhancing demographic performance of several wildlife species (Westemeier *et al.* 1998; Vilà *et al.* 2003; Madsen, Ujvari, & Olsson 2004; Hogg *et al.* 2006).

The endangered Florida panther *Puma concolor coryi* exemplifies the challenges faced by small populations that are genetically and demographically isolated from other conspecific populations. Once widely distributed throughout the south-eastern US, panthers currently occur in a single, isolated population in south Florida (Onorato *et al.* 2010). After a remnant population of panthers was documented in 1972, a sustained research programme initiated in 1980 revealed that the population had declined to fewer than 20–25 adults (McBride *et al.* 2008). Furthermore, it became evident that the population was characterized by a low level of genetic variation and suffered from a plethora of morphological and biomedical abnormalities including atrial septal defects, cryptorchidism and low sperm count and quality (Roelke, Martenson, & O'Brien 1993; Barone *et al.* 1994; Culver *et al.* 2000). These abnormalities were thought to be indicative of inbreeding depression and ultimately led to the recommendation that genetic restoration might help alleviate these issues (Seal & Lacy 1994). This was a controversial recommendation (Maehr & Caddick 1995), but one that was implemented following vigorous debate and careful planning (Seal & Lacy 1994; Hedrick 1995; Beier *et al.* 2003). As part of this programme, eight female Texas pumas *P. c. stanleyana* were released into Florida panther habitat in south Florida in 1995, a process that would in essence mimic gene flow that historically occurred between the subspecies. Since 1995, panther abundance has increased substantially (McBride *et al.* 2008), genetic

variation has increased and the frequency of documented biomedical abnormalities has declined (Johnson *et al.* 2010). Recent studies suggest that age-specific survival rates were generally higher for admixed panthers than those for canonical (or pure) Florida panthers (Hostetler *et al.* 2010; Benson *et al.* 2011), but that admixed females did not have reproductive advantages (Hostetler *et al.* 2012b). Nonetheless, the role of the genetic restoration in observed demographic responses has been debated (Creel 2006; Maehr *et al.* 2006; Pimm, Bass, & Dollar 2006a; Pimm, Dollar, & Bass 2006b), and the demographic mechanisms underlying the observed population growth remain uncertain.

Concerns regarding persistence of the Florida panther population have led to several population viability analyses (PVA) (Maehr *et al.* 2002; Root 2004). After a thorough evaluation, a Scientific Review Team (SRT; commissioned by the Florida Fish and Wildlife Conservation Commission and U. S. Fish and Wildlife Service to review Florida panther science), noted that previous PVAs were based on unreliable estimates of demographic parameters, required too many arbitrary assumptions, performed insufficient sensitivity analyses, and used 'consensus' approaches to determine parameter values and model structure (Beier *et al.* 2003, 2006). The SRT recommended rigorous analyses of available data to estimate demographic parameters and to perform demographic analyses using those empirically estimated parameters. Now that statistically rigorous estimates of demographic parameters for the Florida panther are available (Hostetler *et al.* 2010, 2012b; Benson *et al.* 2011), the most appropriate next step is to use those parameters for a thorough assessment of the Florida panther population dynamics and persistence.

Our goal was to investigate whether and to what extent genetic restoration contributed to the observed population growth and to discern demographic mechanisms underlying the improvements in population growth and persistence parameters following the restoration. Specifically, we: (i) estimated deterministic and stochastic population growth rates for Florida panthers; (ii) performed prospective sensitivity analyses and quantified the elasticity of deterministic and stochastic population growth rates to vital demographic parameters; (iii) estimated probability of extinction and distribution of extinction times; (iv) estimated deterministic population growth rate for a hypothetical population of canonical panthers; (v) decomposed the observed difference in population growth rate between the overall population post-restoration and the canonical panther population, using a retrospective life-table response experiment analysis; and (vi) estimated probabilities of extinction between 1995 and 2010 for scenarios with and without genetic restoration. We explicitly address two sources of uncertainties that are frequently ignored (i.e. parametric and model selection uncertainty) and three factors that influence dynamics and persistence of small populations (i.e. environmental stochasticity, demographic stochasticity and density dependence).

Materials and methods

FIELD AND GENETIC METHODS

Since 1981, Florida panthers have been treed, captured, radio-collared and tracked by biologists from the Florida Fish and Wildlife Conservation Commission (FWC) and National Park Service (NPS) using methods described previously (Belden *et al.* 1988; Land *et al.* 2008; Onorato *et al.* 2011). Starting in 1995, Florida panther kittens from radio-collared females were counted, sexed and PIT tagged at the den; some kittens were subsequently recaptured alive or recovered dead as described previously (Land, Garman, & Holt 1998; Benson, Lotz, & Jansen 2008; Hostetler *et al.* 2010).

We divided female panthers into four age categories: kittens (age 0–1 year), sub-adults (age 1–2·5 years), prime adults (age 2·5–10 years), and older adults (age ≥ 10 years). Male age categories were the same except that sub-adult panthers became prime adult at age 3·5 years. Age of captured panthers was known for those captured and tagged as kittens; when exact age was not known, it was estimated based on tooth wear and pelage characteristics (Ashman & Greer 1976). The effect of potential error in estimates of age on parameter estimates was previously reported to be insubstantial (Benson *et al.* 2011).

Genetic samples for Florida panthers were analysed using 23 microsatellite loci to delineate genetic heritage of individuals (see Johnson *et al.* 2010 for analytical details). These data permitted us to categorize panthers as canonical (individuals representative of the population prior to genetic restoration) or as products of the genetic restoration process (e.g. F1 admixed, backcrosses).

POPULATION MODEL STRUCTURE AND DETERMINISTIC DEMOGRAPHIC ANALYSES

For deterministic and stochastic demographic analyses, we used age-structured matrix population models focusing on the female segment of the population (Caswell 2001). We assumed that female panthers can live until age 18·5 years, which corresponds to the estimated age of the oldest radio-collared female in this study (18·6 years). We also assumed female panthers can reproduce until age 18·5 years. However, the probability of successful reproduction by older adults was very low (Hostetler *et al.* 2010); this was because of the fact the oldest female panther in our data set that reproduced was age 11 years. Therefore, the population projection matrix had dimensions 19×19, and was of the form:

$$\mathbf{A}^t = \begin{bmatrix} F_1^t & F_2^t & \cdots & \cdots & F_{19}^t \\ P_1^t & 0 & \cdots & \cdots & 0 \\ 0 & P_2^t & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & P_{18}^t & 0 \end{bmatrix}$$

where F and P indicate age-specific fertility and survival rates for female panthers respectively; subscripts indicate age classes and t stands for time-specificity (superscripts do not apply to deterministic demographic analyses; Appendix S1, Supporting information). Because Florida panthers reproduce throughout the year, we used birth-flow methods for estimating P_i's and F_i's (Leslie 1945; Caughley 1977; Caswell 2001). Details on estimation of P_i's and F_i's and vital demographic parameters are presented in Appendix S1.

The asymptotic deterministic annual population growth rate (λ) and elasticity of λ to demographic parameters were estimated using methods described in Caswell (2001). We also estimated generation time (T) and life expectancy for female panthers starting in age class 1 following Caswell (2001).

INCORPORATING ENVIRONMENTAL STOCHASTICITY

Environmental stochasticity can substantially affect dynamics and persistence of populations (Tuljapurkar 1989; Caswell 2001; Boyce *et al.* 2006). Thus, we incorporated environmental stochasticity in demographic parameters for which there was evidence of temporal variation, as indicated by random effect of year-of-study (Hostetler *et al.* 2010, 2012b; Benson *et al.* 2011). Temporal variance of litter size was essentially zero, so we did not consider environmental stochasticity for this parameter (Hostetler *et al.* 2012b). However, there was evidence for environmental stochasticity in age-specific survival probabilities (kitten, sub-adult and adult survival), and age-specific probability of reproduction (Hostetler *et al.* 2010, 2012b; Benson *et al.* 2011). We assumed no temporal correlation among the three groups of parameters (survival of kittens, survival of sub-adult and adult panthers and reproduction probabilities); these parameters were estimated using different data sets and statistical methods, and we do not know of any reasonable methods to estimate temporal correlation among them.

ESTIMATING STOCHASTIC POPULATION GROWTH AND ELASTICITIES

We used a simulation approach (50 000 time steps) to estimate the stochastic population growth rate or $\log(\lambda_s)$ and elasticity of $\log(\lambda_s)$ to demographic parameters under the assumption of identically and independently distributed (iid) environments (Caswell 2001; Morris & Doak 2002; Haridas & Tuljapurkar 2005). In each time step, estimates of vital demographic parameters were obtained as described in Appendix S1 and a time-specific population projection matrix was compiled and stored. This sequence of 50 000 population projection matrices were then used to estimate $\log(\lambda_s)$ and elasticity of $\log(\lambda_s)$ to mean and standard deviation of demographic parameters as well as overall stochastic elasticities (Tuljapurkar, Horvitz, & Pascarella 2003; Caswell 2005; Haridas & Tuljapurkar 2005). We exponentiated $\log(\lambda_s)$ to obtain finite stochastic population growth rate (λ_s).

POPULATION VIABILITY ANALYSIS: ESTIMATING EXTINCTION PARAMETERS

We used a two-sex, age-structured matrix population model and a stochastic simulation approach to PVA, explicitly incorporating two sources of uncertainty (parametric and model selection uncertainty) and three factors that can strongly influence dynamics and persistence of small populations (environmental stochasticity, demographic stochasticity, and density-dependence). Simulation details are provided in Appendix S2, Supporting information.

The two-sex population projection matrix was of the form:

$$
\mathbf{A}_n^t =
\begin{bmatrix}
F_1^t(N) & F_2^t(N) & \cdots & \cdots & F_{19}^t(N) & 0 & \cdots & \cdots & 0 \\
P_1^t(N) & 0 & \cdots & \cdots & 0 & 0 & \cdots & \cdots & 0 \\
0 & P_2^t(N) & \ddots & \ddots & \vdots & \vdots & \ddots & \ddots & \vdots \\
\vdots & \ddots & \ddots & \ddots & \vdots & \vdots & \ddots & \ddots & \vdots \\
0 & 0 & \cdots & P_{18}^t(N) & 0 & 0 & \cdots & \cdots & 0 \\
M_1^t(N) & M_2^t(N) & \cdots & \cdots & M_{19}^t(N) & 0 & \cdots & \cdots & 0 \\
0 & 0 & \cdots & \cdots & 0 & Q_1^t(N) & \ddots & \ddots & \vdots \\
\vdots & \vdots & \ddots & \ddots & \vdots & 0 & \ddots & \ddots & \vdots \\
0 & 0 & \cdots & \cdots & 0 & 0 & \cdots & Q_{14}^t(N) & 0
\end{bmatrix}
$$

where $F_i^t(N)$ and $P_i^t(N)$ are age- and time-specific fertility and survival rates for female panthers as described previously, except that these rates can now be density-dependent [indicated by (N), where N is total population size at time t; see *Incorporating Density-dependence*, below]; M is the rate at which male kittens are produced by females of different age classes (assuming 50 : 50 sex ratio), and Q is age-specific survival of male panthers. We estimated survival probabilities of sub-adult, prime adult, and older adult males (Q_{sa}, Q_{pa} and Q_{oa}) using Cox proportional-hazard models as described in Benson *et al.* (2011, also see Appendix S1). We assumed that male panthers can live up to age 14·5 years, which corresponds to the estimated age of the oldest radio-collared male in our study (14·4 years). The population was then projected as: $\mathbf{n}(t + 1) = \mathbf{A}_n^t\, \mathbf{n}(t)$, where $\mathbf{n}(t)$ is the population vector at time t, and includes age-specific abundance of both male and female panthers. In this modelling framework, males contribute to the population dynamics only via survival.

Environmental stochasticity was incorporated using a simulation approach as described previously. A brief description of how we incorporated density-dependence and demographic stochasticity and estimated extinction parameters follows.

Incorporating density-dependence – There was strong evidence for negative density-dependence in kitten survival, moderate evidence for positive density-dependence in probability of successful reproduction, weak evidence for negative density-dependence in sub-adult and adult survival, and weak evidence for positive density-dependence in litter size (Hostetler *et al.* 2010, 2012b, Appendix S1). Because density-dependence in demographic parameters and functional form thereof can affect the estimated probability of extinction (Henle *et al.* 2004; Coulson *et al.* 2008), we included models that did and did not include the effect of panther abundance on demographic parameters in our simulations, and ran a separate PVA with no density-dependence in demographic parameters.

The annual index of abundance was the minimum count of older kittens (out of the den, but still dependent on their mothers), sub-adult and adult panthers of both sexes (McBride *et al.* 2008). Since age class 1 in our birth-flow model represents panthers of age 0·5–1·5 years, total population size at each time step was the sum of the population vector at time t.

Most, but not all models in our model sets included density-dependence on demographic parameters, and simulations with density-independent demographic parameters could grow exponentially, leading to very large population sizes. For biological plausibility and computational efficiency, we imposed a ceiling density limit of 1 000 panthers for all simulations. When the population size exceeded 1 000 panthers, we removed panthers proportionally from all age and sex classes to reach a total population size of 1 000.

Incorporating demographic stochasticity – At small population sizes, demographic stochasticity can substantially affect population dynamics and persistence (Caswell 2001; Morris & Doak 2002). We used an individual-based simulation approach (Caswell 2001) to incorporate the effect of demographic stochasticity by applying the time-specific demographic parameters (influenced by environmental stochasticity and/or density-dependence) to the fates of individual panthers. The number of females and males that survived a year in each age class was determined by drawing uniform random numbers between 0 and 1 and comparing them with the annual survival probabilities (P_i^t and Q_i^t respectively). We also used an individual-based simulation approach to incorporate the effect of demographic stochasticity on reproductive parameters; details are given in Appendix S1.

When the number of panthers in every sex and/or age class exceeded 20, we did not incorporate demographic stochasticity, but rounded entries of the projected population vector so they could be used with demographic stochasticity (which operates on individuals) the next year (Morris & Doak 2002). This was done to reduce computational burden and because the influence of demographic stochasticity is insubstantial in large populations (Morris & Doak 2002).

Estimating extinction parameters – We simulated populations for 200 years starting with the 2010 abundance index (100 total panthers), distributed according to stable age and sex distributions. If a simulated trajectory fell below a critical threshold (N_{crit}; set at 1, 10 and 30 panthers) it was considered extinct or quasi-extinct from that point forward. In addition to probabilities of extinction and quasi-extinction, we were interested in the timing of these events. Therefore, we also noted when each simulation first fell below each N_{crit} (extinction and quasi-extinction times; Morris & Doak 2002).

Sensitivity analysis of extinction probabilities – To examine the effects of model parameters on probabilities of extinction, we performed a life stage simulation analysis (LSS; Wisdom, Mills, & Doak 2000; Tinker, Doak, & Estes 2008; Bakker *et al.* 2009). For the top set of statistical models for demographic parameters (Appendix S1), we ran 1 000 parametric bootstraps of 1 000 simulations (Appendix S2, Supporting information, but without selecting different models), and regressed the intercept and slope parameters that determine the demographic parameter values (Appendix S1) against arcsine square-root transformed probabilities of extinction. We present squared semi-partial correlation coefficients (SSPCC) for each intercept and slope parameter, which quantify a combination of the influence of these parameter on extinction probability, and of the uncertainty in estimates of those parameters (Bakker *et al.* 2009). Because this approach cannot explicitly incorporate model selection uncertainty, we also performed this analysis using estimates of intercept and slope parameters obtained from the second-best supported statistical model for each group of demographic parameters (Appendix S1).

INCORPORATING PARAMETER AND MODEL SELECTION UNCERTAINTY

We incorporated parameter uncertainty into our demographic analyses and PVA via a parametric bootstrap method (Efron & Tibshirani 1993). We estimated a variance-covariance matrix for each set of parameters: (i) kitten survival (on logit scale); (ii) sub-

adult and adult survival (baseline survival converted to logit scale and log-hazard effect sizes); (iii) probability of reproduction (on complementary log-log scale); and (iv) number of kittens produced in the year per female panther that successfully reproduced (on cumulative logit scale). We assumed no overall covariance among the four categories of demographic variables, but used the estimated covariances within categories. We ran multiple parametric bootstraps (50 000 for deterministic and stochastic demographic analyses and 1 000 for PVAs), selecting slope and intercept parameter values from a multivariate normal distribution with vectors of the parameter means (on the appropriate scales) and variance-covariance matrices, and then converting the results to the real scale, for each bootstrap run. In this way, we sampled values of parameters from the empirically estimated distributions and parameter values.

Another source of statistical uncertainty in demographic analyses is model selection uncertainty – incertitude about whether the minimum AIC (or QAIC$_c$) model for a parameter is actually the best model in the model set (Burnham and Anderson 2002). We incorporated this form of uncertainty by randomly selecting statistical models for each set of parameters, weighted by their AIC (or QAIC$_c$) weights (Bakker *et al*. 2009). For deterministic and stochastic demographic analyses, we restricted model sets to density-independent models only (Appendix S1).

For each analysis for which model selection uncertainty was incorporated, parameter uncertainty was also incorporated by selecting parameter values from their distributions as discussed above for the model selected, and then estimating the output parameters (such as deterministic λ, sensitivities, and elasticities). This process was repeated multiple times (50 000 for deterministic and stochastic demographic analyses and 1 000 for PVAs).

For all output variables (except for generation time and life expectancy) we report mean and/or median and 5th and 90th percentiles of parametric and/or model selection bootstrap runs. We also report minimum and maximum extinction and quasi-extinction times.

WHAT IF THE RESTORATION NEVER HAPPENED?

An interesting question in the management of the Florida panther is: what might have happened to the panther population if genetic restoration had not occurred? To address this question, we estimated demographic parameters for canonical panthers only (Appendix S3, Supporting information). However, we used estimates of temporal variation in vital rates estimated for the overall population as described previously. We assumed that demographic parameter estimates based on canonical panthers after restoration were representative of a hypothetical post-1995 canonical panther population. Using these estimates, we parameterized a population projection matrix and estimated population growth rate for a canonical population ($\lambda^{(c)}$); we incorporated parametric and model selection uncertainty as described above. To determine which demographic parameters contributed the most to the observed difference between λ and $\lambda^{(c)}$, we performed a fixed effect, one way life-table response experiment analysis (LTRE; Caswell 2001).

In addition to population growth rate, we also investigated how genetic restoration affected the probability of extinction. We conducted PVAs for the overall population and canonical population with a starting population size estimated for 1995 (McBride *et al*. 2008). Of the 26 pumas believed to be present in

1995, 18 were Florida panthers (assumed to be at stable age and sex distribution for the canonical population) and 8 were female Texas pumas (two of age 3 years, and six of age 4 years; McBride *et al*. 2008; Onorato, unpublished data). To separate the potential benefits of 'genetic rescue' and 'demographic rescue' (Creel 2006), we conducted PVAs using a starting population size that did and did not include the eight Texas puma females. We ran 1000 runs of 1000 simulations, each for 15 years (1995–2010) using procedures described previously. We chose to run these simulations until 2010 only because we were examining 'what might have happened', rather than what could happen in the future. As the starting panther population sizes were <30, we only estimated probability of quasi-extinction and extinction times for critical thresholds of 1 and 10 panthers. Because the population size in 1995 was quite small and we had difficulty obtaining realistic estimates of the effects of abundance and genetic ancestry simultaneously, we assumed no density-dependent effects on demographic parameters. However, we imposed a ceiling density-dependence of 100, the minimum panther count for 2010.

Results

GROWTH RATE AND PERTURBATION ANALYSIS

The estimate of deterministic λ using demographic parameters obtained from the best supported set of statistical models for vital rates was 1·06 (5th and 95th percentiles: 0·96–1·16). When model selection uncertainty was incorporated, λ dropped to 1·04 (both mean and median; 0·95–1·14). Estimated generation time was 4·45 years. A female starting in age class 1 (average starting age of 6 months) was estimated to live on average 5·05 more years (SD = 4·85 years).

The estimate of λ_s obtained from the best supported set of statistical models for vital rates was 1·05 (0·96–1·13). With model selection uncertainty included, the estimate of λ_s was 1·03 (0·95–1·11). Overall stochastic elasticities were similar to deterministic ones (data not shown), with log (λ_s) being proportionately most sensitive to changes in prime adult survival (Fig. 1a). The log(λ_s) was proportionately most sensitive to the mean of prime adult survival, but was proportionately most sensitive to the standard deviation of kitten survival. Most of the overall stochastic elasticity was contributed by elasticity to the means of parameters; contribution of temporal standard deviations to overall stochastic elasticities was substantially smaller (Fig. 1b and c).

EXTINCTION PARAMETERS

The probability of extinction within the next 100 years was 0·057 (0–0·458; Fig. 2a). The probabilities of quasi-extinction within the next 100 years were 0·072 (0–0·606) and 0·128 (0–0·839) for critical threshold (N_{crit}) of 10 and 30 panthers respectively (Fig. 2b and c). At 200 years, 5th and 95th percentiles for probability of (quasi-)extinction ranged from zero to >0·8 at all critical thresholds. Conditional on extinction within 200 years, the median
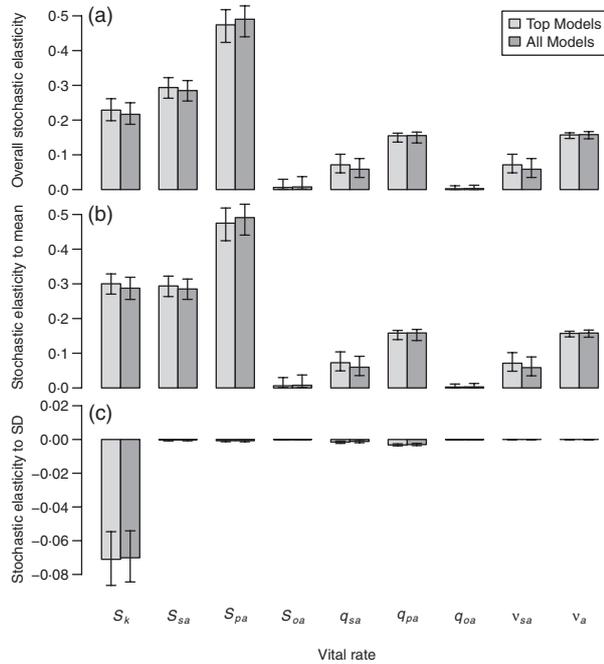
Fig. 1. Elasticity of stochastic population growth rate (λ_s) to demographic parameters for the set of top-ranked density-independent statistical models. (a) Overall stochastic elasticity; (b) Elasticity of λ_s to mean vital rates; and (c) Elasticity of λ_s to standard deviation (SD) of vital rates. Symbols are: S_k = survival probability for kittens (age 0–1 years); S_{sa} = survival probability for sub-adult females (age 1–2·5 years); S_{pa} = annual survival probability for prime adult females (age 2·5–10 years); S_{oa} = annual survival probability for older adult females (age 10 years and above); q_{sa} = annual probability of reproduction for sub-adult females; q_{pa} = annual probability of reproduction for prime adult females; q_{oa} = annual probability of reproduction for older adult females; v_{sa} = average number of kittens produced by an sub-adult female in a year, given that she reproduces; and v_a = average number of kittens produced by an adult female (ages 2·5 years and above) in a year, given that she reproduces. Error bars represent 5th and 95th percentiles of parametric bootstrap runs.

time to extinction was 80 years, and declined as N_{crit} increased (Table 1). Ignoring density-dependence in demographic parameters increased probabilities of extinction and quasi-extinction within the next 100 years at all thresholds (Appendix Fig. S1, Supporting information; N_{crit} = 1: 0·176, 0–0·878; N_{crit} = 10: 0·237, 0–0·978; N_{crit} = 30: 0·315, 0–0·997). Median time to (quasi-) extinction was not affected substantially by ignoring density-dependence in demographic parameters for N_{crit} = 1 and 10, but was lowered for N_{crit} = 30 (Appendix Table S1, Supporting information).

Probability of extinction was by far most sensitive to variation and uncertainty in kitten survival parameters (η_0 and η_{ab}; Fig. 3). The intercepts for probability of reproduction and adult survival were the next most influential parameters (Fig. 3). These results did not change when we repeated our analyses using the second-best sets of statistical models for demographic parameters (Fig. 3a and b), although total variation in probability of extinc-
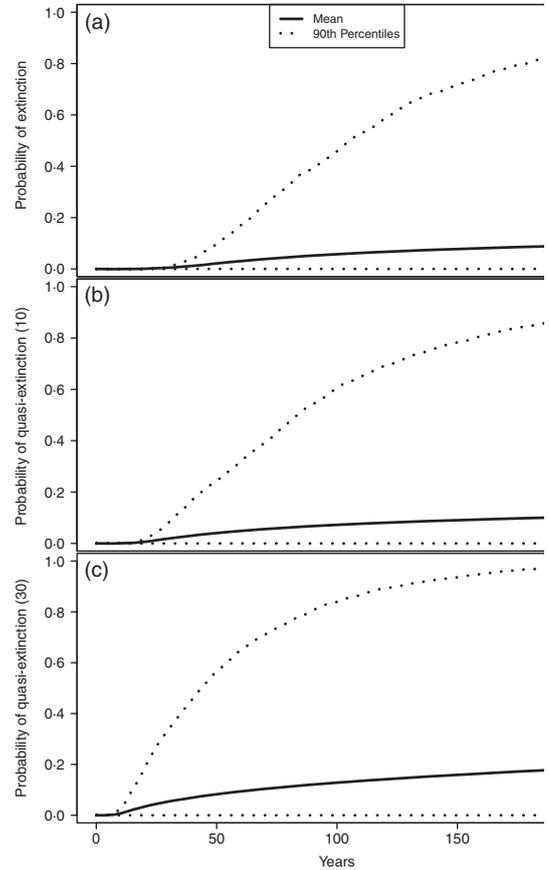
Fig. 2. Cumulative probability that the Florida panther population falls below (a) 1 panther, (b) 10 panthers and (c) 30 panthers over 200 years of simulation. The solid lines represent means and the dotted lines 5th and 95th simulation percentiles. Starting population size was 100 panthers.

Table 1. Summary statistics for extinction and quasi-extinction times (number of years until the population size falls below critical thresholds). The maximum (quasi-)extinction time was always 200, the number of years all simulations were run

Critical threshold	Minimum	Median	Mean	5th and 95th percentiles
1	14	80	90·7	31–181
10	6	65	78·3	19–179
30	1	58	73·7	11–180

tion explained by parametric uncertainty was greater for analyses based on demographic parameters estimated from the top set of models.

WHAT IF THE GENETIC RESTORATION NEVER HAPPENED?

The estimate of $\lambda^{(c)}$ for the hypothetical canonical population based on the top set of statistical models for demographic variables was 0·97 (mean and median = 0·96; 0·87–1·06). When model selection uncertainty was included, the mean and median of $\lambda^{(c)}$ were 0·95 (0·83–

1·08). Patterns of elasticities (data not shown) were similar to those for the overall population.

The difference between λ and $\lambda^{(c)}$ was 0·10; the largest contributor to this difference in growth rate was kitten survival probability (S_k; Fig. 4). This was due to both a substantially lower kitten survival for canonical panthers (−0·133; Fig. 4b) and a large sensitivity of population growth rate to S_k (Fig. 4a). Prime adult survival probability also contributed to the lower λ for the canonical population, while prime-adult probability of reproduction, sub-adult probability of reproduction and sub-adult survival probability had slightly compensatory effects on λ for the canonical population (Fig. 4). Although canonical panthers in general had lower survival than the overall panther population, the estimate for $S_{sa}^{(c)}$ based on the best supported model for this parameter was slightly higher for canonical panthers (Appendix S1 and S3, Supporting information); this was likely because of the fact that estimates of $S_{sa}^{(c)}$ came from a subset of the data for a period (1997–2006) when survival rates of sub-adult females were generally higher. The $\lambda^{(c)}$ predicted by the LTRE model (calculated as the sum of the contributions from all demographic parameters) was also 0·95, indicating that the LTRE approximation was reasonable.

With the starting population size of 26 panthers, the probability of extinction between 1995 and 2010 was 0·004 (0–0·015; Fig. 5a) for the overall population, and 0·069 (0·001–0·285; Fig. 5b) for the canonical population.

The probabilities of quasi-extinction (N_{crit} = 10 panthers) within the same period were 0·098 (0·002–0·332) and 0·445 (0·032–0·944) for the overall and canonical populations respectively (Figure 5c and d). Probabilities of extinction and quasi-extinction were higher for both populations with a starting population size of 18 panthers (Appendix S4, Supporting information). However, these probabilities were higher for the canonical population with the higher starting population size than those for the overall population with the lower starting population size (Fig. 5b and d and Appendix Fig. S2 A and C, Supporting information). These results suggest that improved demographic performance of the population was due primarily to improvement in demographic parameters attributable to genetic restoration; demographic benefits of an additional eight females were much smaller.

Discussion

Several laboratory and field studies have revealed that genetic restoration can help alleviate detrimental effects of inbreeding depression (Westemeier *et al.* 1998; Ebert *et al.* 2002; Vilà *et al.* 2003; Bijlsma *et al.* 2010). However, evidence to the contrary also exists, and one cannot rule out the possibility of potentially detrimental effects of such management actions, including outbreeding depression and genomic sweep (Tallmon, Luikart, & Waples 2004; Reed 2010; Adams *et al.* 2011). With many species being
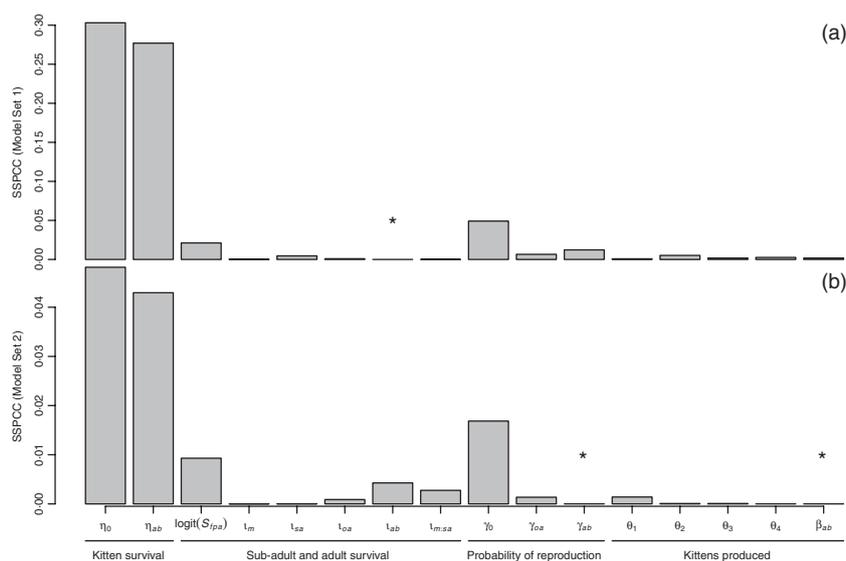
Fig. 3. Squared semi-partial correlation coefficients (SSPCC) for population viability analysis parameters, representing a sensitivity analysis of probability of extinction to model parameters. Panels depict SSPCC for (a) the top set of statistical models and (b) the set of 'next to top' statistical models for each group of parameters (see Appendix S1). Asterisks (*) represent parameters not included in that set of models. Symbols are: η_0 = intercept for kitten survival; η_{ab} = slope for abundance index on kitten survival (above threshold; see Appendix S1); $\text{logit}(S_{pa})$ = annual survival probability for prime adult females (ages 2·5–10 years) transformed to the logit scale; ι_m = log-hazard slope for being male on sub-adult and adult survival; ι_{sa} = log-hazard slope for being a sub-adult on survival; ι_{oa} = log-hazard slope for being an older adult on survival; ι_{ab} = log-hazard slope for abundance index on sub-adult and adult survival; $\iota_{m:sa}$ = log-hazard interaction term for male sub-adults survival; γ_0 = intercept for probability of female reproduction; γ_{oa} = slope for being an older adult on probability of female reproduction; γ_{ab} = slope for abundance index on probability of female reproduction; θ_s = intercept for annual number of offspring being at most s; and β_{ab} = slope for abundance index on kitten production.
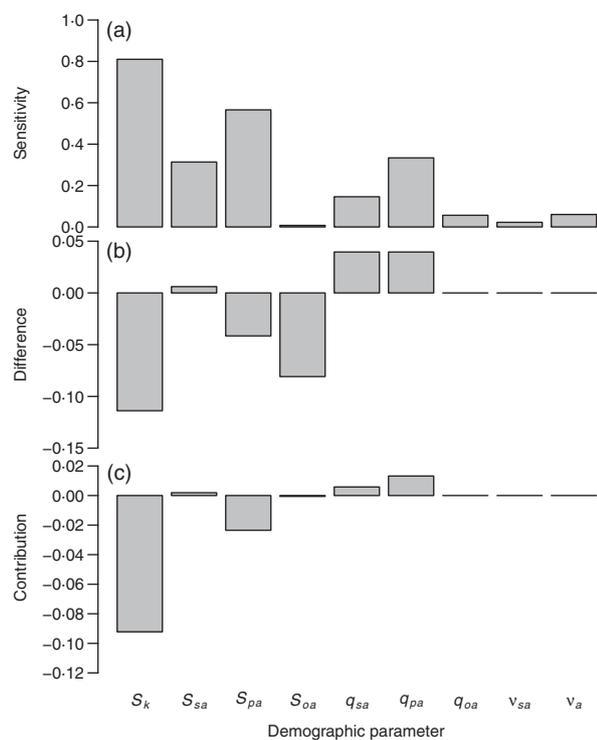
Fig. 4. Results of life-table response experiment (LTRE) analysis, decomposing observed difference in deterministic population growth rate between the overall population of the Florida panther (λ), and a hypothetical population of canonical Florida panthers ($\lambda^{(c)}$). Panels depict (a) sensitivity of deterministic population growth rate to demographic parameters (see Fig. 1), calculated at the mean of parameter values; (b) differences in demographic parameters between the canonical and overall populations; (c) contribution of demographic parameters to $\Delta\lambda = \lambda^{(c)} - \lambda$.

impacted by increased habitat fragmentation, the question of whether to restore gene flow between isolated populations or not is likely to arise often in the future. Developing guidelines and models that help predict the occurrence of inbreeding and outbreeding depression should be a critical part of this decision-making process (Keller & Waller 2002; Frankham *et al.* 2011). Detailed case studies on the demographic mechanisms of genetic restorations are key to improving such guidelines.

Our goal was to objectively evaluate the effect of genetic restoration on the dynamics and persistence of the Florida panther population and to discern demographic mechanisms underlying the population increase that occurred following the genetic restoration. Long-term demographic and genetic data allowed us to estimate and model demographic parameters and test for the effect of genetic ancestry (canonical Florida panthers, F1 admixed, and other admixed) on these parameters (Hostetler *et al.* 2010, 2012b; Benson *et al.* 2011). Thus, we were able to estimate demographic parameters by ancestry where the effect of ancestry was evident, and to project the consequences of overall or ancestry-specific demographic parameters.

The overall estimate of population growth rate (deterministic growth rate, $\lambda = 1 \cdot 04$; stochastic growth rate,

$\lambda_s = 1 \cdot 03$) suggested a *c.* 3–4% annual population growth. These results are generally consistent with the abundance index for the period 1995–2008 (McBride *et al.* 2008), the period over which most of the data were collected. Deterministic and overall stochastic elasticities were consistent with a well-established pattern documented for long-lived species (Heppell, Caswell, & Crowder 2000; Morris & Doak 2005; Stahl & Oli 2006) where λ and $\log(\lambda_s)$ were proportionately most sensitive to changes in survival of prime adults, followed by survival of sub-adults and kittens (Fig. 1). Although fewer studies have examined elasticity patterns for birth-flow populations, the same patterns seem to hold there (Fisher, Hoyle, & Blomberg 2000; Alberts & Altmann 2003; Federico & Canziani 2005). Interestingly, $\log(\lambda_s)$ was proportionately most sensitive to changes in mean survival of prime adult females, but it was proportionately most sensitive to changes in standard deviation of kitten survival.

Dynamics and persistence of biological populations are generally determined by complex interplays between intrinsically driven density-dependence and stochastic influences (Leirs *et al.* 1997; Coulson *et al.* 2001; Goswami *et al.* 2011; Hostetler *et al.* 2012a). Generally speaking, density-dependence tends to stabilize population dynamics and reduce probability of (quasi-) extinction, whereas stochastic forces tend to destabilize population dynamics and increase the probability of (quasi-) extinction. Considering all of these factors, our estimate of the probability of extinction within 100 years (baseline scenario) was 5·7%. The probabilities that the population would fall below 10 and 30 panthers (i.e. probability of quasi-extinction) were 7·2% and 12·8%, respectively, suggesting a moderate risk of (quasi-)extinction. The median and mean times to extinction were 80 and 90·7 years, respectively, suggesting that if the panther population does go extinct within the next 200 years it is most likely to happen within 100 years. Although our analyses were thorough and utilized rigorous estimates of demographic parameters based on long-term demographic data, we recommend caution in interpreting these results for at least three reasons. First, because we lacked actual estimates of population size, we used an index of abundance in our analysis of density-dependent effects on demographic variables. This may have led to an overestimation of the strength of density-dependence, especially on kitten survival, leading to an underestimation of the probability of (quasi-)extinction, although the reverse is also possible. Ignoring density-dependence in all demographic parameters, the probabilities that the population would decline below 1, 10 and 30 panthers within 100 years were 17·6%, 23·7% and 31·5% respectively. This difference in the probability of extinction between models with and without density-dependence is partly attributable to the higher estimates of temporal variance (particularly of kitten survival) from density-independent models. Second, we have not considered potential effects of factors such as catastrophic events, climate change, invasive species, and anthropogenic influences (e.g. habitat loss and
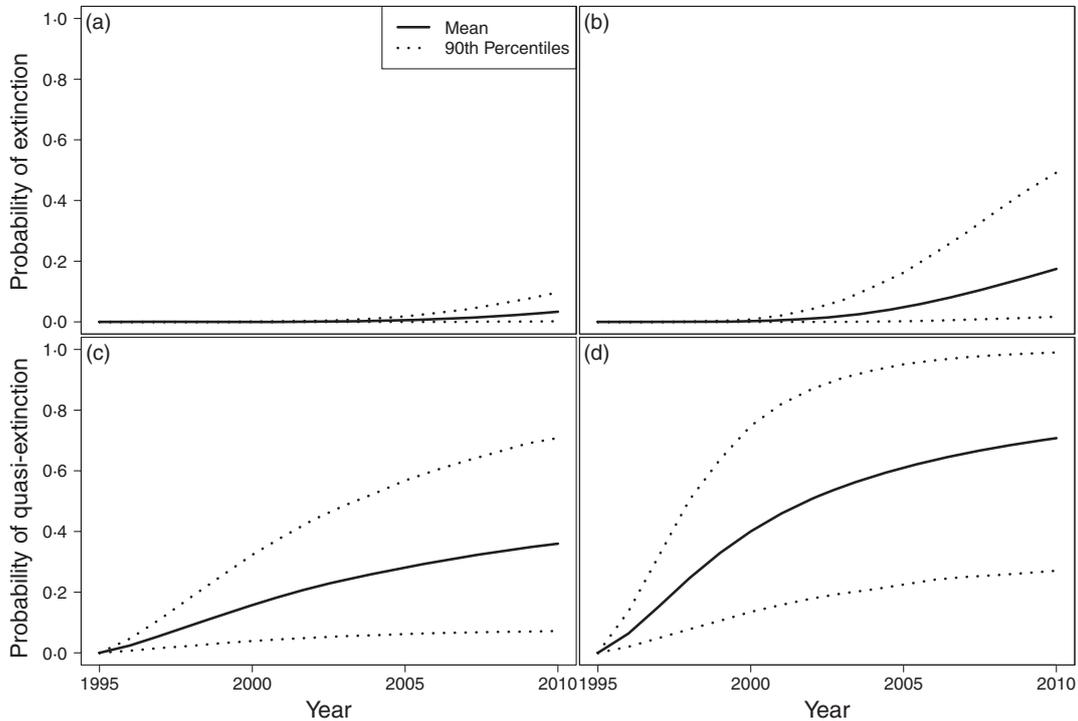
Fig. 5. Cumulative probability that the Florida panther population would have fallen below (a and b) 1 panther and (c and d) 10 panthers between 1995 and 2010. Panels a and c are for the whole population and b and d are for a hypothetical canonical population. Starting population size was at 1995 estimated population levels (total 26 panthers, including Texas females). The solid lines represent means and the dotted lines 5th and 95th percentiles.

fragmentation, increases in road-related mortalities). Finally, the percentile-based confidence intervals for the probability of (quasi-) extinction were wide, often ranging from zero to near one. Whereas such wide ranges are not surprising considering the many sources of variation and uncertainties considered in this study (Fieberg & Ellner 2001; Ellner & Fieberg 2003), they do nonetheless point to the fact that one must be careful in interpreting these results (Coulson *et al.* 2001).

The probability of extinction was by far most sensitive to kitten survival (Fig. 3). These results represent a combination of the effect of a parameter on extinction probability and uncertainty in the estimate of that parameter, and are most usefully interpreted as a guide to future research. Because kitten survival probabilities and the effect of panther abundance on them are very influential and their estimates somewhat imprecise, obtaining more precise estimates of these parameters (possibly through fitting young kittens with expandable radio-collars) should be a priority for future research.

Many of the earlier demographic analyses for the Florida panther suffered from lack of rigorous estimates of demographic variables and a failure to appropriately consider sources of variation and uncertainty, and also did not perform sensitivity analyses (Beier *et al.* 2003). Our study adequately addresses these concerns as per SRT recommendations and represents a significant improvement because: (i) our analyses are based on reliable estimates of demographic variables using long-term field data and robust statistical analyses; (ii) we have used a more realistic birth-flow approximation to matrix model parameter estimation (Caswell 2001); (iii) we have explicitly incorporated the three factors that substantially influence population dynamics and persistence: density-dependence, environmental stochasticity and demographic stochasticity; (iv) we have explicitly considered two important sources of uncertainties that are often ignored in PVAs: model selection uncertainty and parametric uncertainty (White 2000; Ellner & Fieberg 2003; Bakker *et al.* 2009; McGowan, Runge, & Larson 2011); and (v) we have performed sensitivity analyses at multiple levels: deterministic and stochastic elasticity analyses (Caswell 2001, 2005; Tuljapurkar, Horvitz, & Pascarella 2003; Haridas & Tuljapurkar 2005), sensitivity analysis of the probability of extinction (Bakker *et al.* 2009), and a retrospective perturbation analysis using life table response experiments (LTRE analysis; Caswell 1989).

It is not possible to address the question of what would have happened to the Florida panther population without genetic restoration directly, but we attempted to address this issue indirectly by conducting a series of analyses using estimates of demographic parameters for canonical Florida panthers. The estimated deterministic growth rate for a canonical population ($\lambda^{(c)} = 0.95$) suggested that the population would have declined at the rate of *c.* 5% per year if restoration had not taken place. Assuming

exponential growth and ignoring stochastic factors, the population would have declined to half its original size in about 14 years (from 18 canonical panthers in 1995 to *c.* 9 in 2009). Next, we asked: which demographic parameter(s) affected by genetic restoration contributed most to the observed post-restoration population growth? Retrospective LTRE analysis revealed that most of the observed increase in population growth rate was attributable to improvements in kitten survival in response to the genetic restoration, followed by prime adult survival. Thus, improved survival of kitten and prime adults in response to genetic restoration likely turned the declining panther population to an increasing one. Finally, we asked: would the panther population have gone extinct by now without genetic restoration? Without the eight Texas cougars released in the panther habitat, and without improvements in demographic parameters due to genetic restoration, we estimated that the probability that the population would have fallen below 10 panthers by 2010 was 70·8% (Appendix S4), as opposed to 9·8% with both benefits. Demographic parameters for canonical panthers were estimated using data collected for a period when canonical and admixed panthers coexisted; a purely canonical population might have experienced a higher growth rate and lower (quasi-) extinction probabilities in the absence of competition from admixed panthers. Conversely, because this hypothetical population would not have benefitted from the improved reproductive capacity of admixed males (Johnson *et al.* 2010), reproductive output might have been lower and this population's demographic performance might have been even worse than reported here. Given the evidence that a canonical population would have declined, possibly catastrophically (or at least continued to not increase), concerns of conservation and wildlife biologists and managers about the demographic and genetic health of the panther population in the early 1990s seem warranted (Fergus 1991; Roelke, Martenson, & O'Brien 1993; Seal & Lacy 1994).

Population projection models in general and PVAs in particular tend to be forward-looking. However, backwards-looking models can also be useful for model validation (Brook *et al.* 2000; Ball, Lindenmayer, & Possingham 2003; Lindenmayer *et al.* 2003) and for evaluating the impacts of past management actions (Schwartz 1999; Kauffman, Frick, & Linthicum 2003). Determining 'what would have happened' if management actions had been different can be at least as challenging as trying to predict the future. However, notwithstanding these challenges, both forward- and backward-looking demographic analyses can be useful; evaluating the effects of past management actions on population dynamics and persistence, and modelling potential future population changes and risks are critical tools for choosing appropriate management actions. Coulson *et al.* (2001) have persuasively argued that PVA results must be interpreted relative to the quantity and quality of data, future distribution of vital rates, and factors and processes affecting population dynamics and persistence. Fieberg & Ellner (2001) and Ellner *et al.* (2002) showed that long-term estimates of extinction risk can be highly imprecise, even with considerable quantities of data. Our results are consistent with these findings. Despite this, we disagree with the notion that the apparently inherent imprecision of PVA results makes them useless to science or management. PVAs are generally the best tools science has to offer for assessing extinction risk and it is generally preferable to make use of what science has to tell us (taking into account the uncertainty) than to ignore it in favour of subjective assessments (Brook *et al.* 2002). An estimate of probability of extinction, even with wide confidence intervals, still represents an objective and quantitative measure of risk faced by a population.

Although the imminent demise of the Florida panther population appears to have been averted for now, the population continues to face a multitude of threats. Panthers still exist in a single, isolated population, with no realistic possibility of natural gene flow (Onorato *et al.* 2010). Consequently, inbreeding remains a concern, and additional genetic management may be required to prevent future episodes of inbreeding depression. The population faces substantial risk due to demographic stochasticity, and catastrophic events such as disease outbreaks can potentially negatively impact the population (Onorato *et al.* 2010). Road-related mortalities, an important cause of panther mortality (Benson *et al.* 2011), have continued to increase, and given the projected human population growth and imminent increase in traffic volumes (Zwick & Carr 2006), this source of mortality is unlikely to subside. The south Florida ecosystem faces threats from many invasive species of plants and animals, which can alter prey availability; some invasive species (e.g. pythons) may directly compete with panthers for prey (Dorcas *et al.* 2012). Finally, models of climate change suggest that the sea level is likely to rise dramatically in south Florida, which (along with human population growth) can negatively affect habitat availability (Fei, Cox, & Whittle 2011). The long-term persistence of the Florida panther, a flagship species and one of the last remaining symbols of wilderness in Florida, will depend on the ability of wildlife managers to identify and address these potential threats in a timely fashion, a process that may be assisted via the findings of this and subsequent incarnations of population persistence models.

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Supporting Information

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

Appendix S1. Parameter estimation and methodological details.

Appendix S2. Pseudo-code used for estimating probability of extinction and quasi-extinction.

Appendix S3. Demographic parameters for canonical Florida panthers.

Appendix S4. Results of additional population viability analysis scenarios.