



# Inbreeding Depression Accumulation across Life-History Stages of the Endangered Takahe

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**Abstract:** *Studies evaluating the impact of inbreeding depression on population viability of threatened species tend to focus on the effects of inbreeding at a single life-history stage (e.g., juvenile survival). We examined the effects of inbreeding across the full life-history continuum, from survival up to adulthood, to subsequent reproductive success, and to the recruitment of second-generation offspring, in wild Takabe (*Porphyrio hochstetteri*) by analyzing pedigree and fitness data collected over 21 breeding seasons. Although the effect size of inbreeding at individual life-history stages was small, inbreeding depression accumulated across multiple life-history stages and ultimately reduced long-term fitness (i.e., successful recruitment of second-generation offspring). The estimated total lethal equivalents (2B) summed across all life-history stages were substantial (16.05, 95% CI 0.08–90.8) and equivalent to an 88% reduction in recruitment of second-generation offspring for closely related pairs (e.g., sib-sib pairings) relative to unrelated pairs (according to the pedigree). A history of small population size in the Takabe could have contributed to partial purging of the genetic load and the low level of inbreeding depression detected at each single life-history stage. Nevertheless, our results indicate that such “purged” populations can still exhibit substantial inbreeding depression, especially when small but negative fitness effects accumulate across the species’ life history. Because inbreeding depression can ultimately affect population viability of small, isolated populations, our results illustrate the importance of measuring the effects of inbreeding across the full life-history continuum.*

**Keywords:** lethal equivalents, model averaging, pedigree, population viability, Takahe

Acumulación de Depresión Endogámica en Etapas de la Historia de Vida de *Porphyrio hochstetteri* en Peligro

**Resumen:** *Los estudios que evalúan el impacto de la depresión endogámica sobre la viabilidad poblacional de especies amenazadas tienden a concentrarse en los efectos de la endogamia en una sola etapa de la historia de vida (e.g., supervivencia de juveniles). Examinamos los efectos de la endogamia a lo largo del continuo de la historia de vida, desde la supervivencia hasta la edad adulta, hasta el éxito reproductivo subsecuente y el reclutamiento de individuos de la segunda generación en *Porphyrio hochstetteri* mediante el análisis de datos del pedigrí y de la adaptabilidad recolectados a lo largo de 21 épocas reproductivas. Aunque el efecto de la endogamia en etapas individuales de la historia de vida fue pequeño, la depresión endogámica se acumuló en múltiples etapas de la historia de vida y a fin de cuentas redujo la adaptabilidad a largo plazo (i.e., reclutamiento exitosos de descendientes de segunda generación). Los equivalentes letales totales estimados (2B) a lo largo de todas las etapas de la historia de vida fueron sustanciales (16.05, 95% IC 0.08–90.8) y corresponden a una reducción de 88% en el reclutamiento de descendientes de segunda generación en el caso de parejas cercanamente relacionadas (e.g., apareamientos entre hermanos) en relación con parejas no relacionadas (según el pedigrí). Una historia de tamaño poblacional pequeño en *P. hochstetteri* pudo haber contribuido a la purga parcial de la carga genética y al bajo nivel de depresión endogámica detectado en cada etapa individual de la historia de vida. Sin embargo, nuestros resultados indican que tales poblaciones “purgadas” todavía pueden exhibir depresión endogámica sustancial, especialmente cuando los efectos de la adaptabilidad pequeña pero negativa se acumulan a lo largo de la historia de vida de la especie. Debido a que la depresión endogámica puede, en última instancia, afectar la viabilidad poblacional de poblaciones*

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*pequeñas y aisladas, nuestros resultados ilustran la importancia de medir los efectos de la endogamia a lo largo del continuo de toda la historia de vida.*

**Palabras Clave:** equivalentes letales, pedigrí, *Porphyrio hochstetteri*, promedio de modelos, viabilidad poblacional

## Introduction

Inbreeding depression can substantially affect population growth and viability (Amos & Balmford 2001; O'Grady et al. 2006; Boakes et al. 2007). The effects of inbreeding at early life-history stages, such as embryo and nestling survival in birds or juvenile survival in mammals (Keller & Waller 2002), have been well studied, but few researchers have investigated inbreeding depression beyond these early life stages. Evaluation of inbreeding depression taken from single life-history stages tends to underestimate its true impact in population viability models (O'Grady et al. 2006), but they can also overestimate its effects. For example, birds may compensate for a decrease in hatching rate due to inbreeding depression by increasing the number of breeding attempts; thus, reduction in individual fitness is avoided (e.g., van de Casteele et al. 2003). Conversely, close relatives that breed may exhibit mean levels of reproductive output, but the offspring they produce have low fecundity (e.g., Keller 1998; Jamieson et al. 2003). Measuring inbreeding depression across all life-history stages can indicate which stages are most sensitive to inbreeding, and provide a better estimate of the overall effect of inbreeding on fitness (e.g., Szulkin et al. 2007).

Few studies of wild populations have investigated the effect of inbreeding across generations. In one notable example, Szulkin et al. (2007) found independent effects of inbreeding at different life-history stages in the Great Tit (*Parus major*): inbred offspring showed reduced fledging success and recruitment, and those that survived to breed eventually had fewer hatchlings. Examining the effects of inbreeding depression at all life-history stages can be difficult because long-term monitoring and reliable pedigree data are needed. Large sample sizes are difficult to obtain for wild populations of threatened species, particularly those with long generation times, and identifying populations with large variances in inbreeding levels (necessary for statistical power) can also be difficult (Bierne et al. 2000; Kruuk et al. 2002). Consequently, most knowledge of the effects of inbreeding in wild populations comes from long-term studies of nonthreatened species (e.g., Keller 1998; Kruuk et al. 2002; Szulkin et al. 2007). Despite the increasing interest in molecular measures of inbreeding (e.g., reductions in heterozygosity), pedigree-based methods remain the most powerful means of evaluating inbreeding depression (Pemberton 2004, 2008). Nevertheless, studies that exclusively use results derived from pedigree analyses to inform manage-

ment of threatened populations are rare (but see Ralls & Ballou 2004; Ewing et al. 2008). Because of these difficulties in collecting pedigree information, the actual effects of inbreeding on the mean fitness of threatened wild populations are likely to go unreported.

We evaluated the effects of inbreeding over the complete life history of a closed but free-ranging population of a highly endangered flightless rail, the Takahe (*Porphyrio hochstetteri*). Our Takahe dataset provided an opportunity to evaluate the long-term fitness consequences of inbreeding because there is a high rate of inbreeding in the Takahe pedigree (Grueber & Jamieson 2008). In addition, the Takahe population we examined has undergone a prolonged bottleneck (Lee & Jamieson 2001), which raises issues about possible genetic purging of deleterious alleles, which is not likely to occur in populations in which inbreeding has always been rare.

Results of previous research on Takahe, based on 14 years of pedigree data showed that whereas a pair's relatedness had no effect on their reproductive fitness, female (but not male) offspring of related pairs fledged significantly fewer young than outbred birds (Jamieson et al. 2003). We built on this earlier work by including seven additional breeding seasons, which allowed us to assess the ability of related pairs to produce offspring and the reproductive ability of those inbred offspring. We used an information-theoretic approach, including model averaging, to determine the predictors of each fitness response. This approach allowed us to estimate effect sizes of inbreeding on fitness, even when sample sizes were small, and to incorporate model-selection uncertainty (Burnham & Anderson 2002).

## Methods

### Study Species

The Takahe (*Porphyrio hochstetteri*), once widespread throughout New Zealand, was thought to be extinct by the end of the 1800s. In 1948 a remnant population was discovered in the remote Fiordland region of the South Island of New Zealand (Lee & Jamieson 2001). During the 1980s and 1990s, the New Zealand Department of Conservation translocated a total of 25 Takahe (mostly juveniles) from the Fiordland population to four offshore islands from which introduced predators had been eradicated. (For location maps and details of translocations, see Jamieson et al. [2003] and Grueber and Jamieson

[2008]). Eighteen of the 25 founders bred successfully, although a small proportion of founder genomes are disproportionately represented among the descendents (Grueber & Jamieson 2008). As of 2007 there were 72 adult Takahe on the four islands (15 on Kapiti Island, 35 on Mana Island, 12 on Maud Island, and 10 on Tiritiri Matangi Island) (Wickes et al. 2007). These birds are managed as a single metapopulation and thought to be at carrying capacity (C.E.G. & I.G.J., unpublished data). Island Takahe are free ranging and select their own territories and mates. They all have unique color bands, which allow Department of Conservation staff to monitor the population and collect breeding data.

### Pedigree and Reproductive Data

Methods of pedigree development and of analysis of the pedigree structure for the island Takahe population as a whole are described in Jamieson et al. (2003) and Grueber and Jamieson (2008). Here we examined 21 years (1986–2007) of annual breeding data from the Takahe populations of Kapiti, Mana, Maud, and Tiritiri Matangi islands (hereafter referred to as the “island population”). We used data collected by the Department of Conservation on egg fertility, hatching rate, fledging rate, and rate of survival to 2 years (breeding age) and to recruitment (defined as successful pairing and laying at least one egg). Animals that hatched fewer than 2 years prior to the end of the study were not included in our analysis of survival to 2 years or of survival to recruitment.

Inbreeding first occurs when a pair of related individuals breed for the first time; their kinship coefficient is equivalent to the inbreeding coefficient of their offspring. In this first generation, inbreeding depression can negatively affect the survival or “vital” stages of the pair’s offspring. Assuming the offspring survive and are recruited into the breeding population, they enter the “reproductive” phase, and their reproductive fitness is measured by the survival rates of second-generation offspring (i.e., the grand-offspring of the original related pair). Our analysis of the latter, reproductive phase, included only data from seasons in which birds bred with unrelated ( $f = 0$ ) or distantly related ( $f < 0.125$ ) partners. There were too few data to include only unrelated pairings. Limiting the data in this way could introduce a bias if inbred individuals are themselves more likely to breed with close relatives. Using logistic regression, we determined that  $f$  did not explain the likelihood of subsequent close inbreeding ( $f \geq 0.125$ ) (logistic regression, model AIC<sub>c</sub> [Akaike information criterion corrected for small sample sizes] was not improved by the addition of coancestry).

All paired females laid eggs, so we could only assess the effect of inbreeding on fertility rates in males (i.e., the ability of inbred males to provide viable sperm and produce fertile eggs). To maximize sample size, we assessed male fertility rates for all paired individuals, including those

that had related partners. We analyzed the effects of inbreeding depression on male fertility separate from the analysis of all other stages.

Individual inbreeding coefficients depend on the depth of pedigree data (Keller 1998); therefore, it is standard practice to restrict analysis to offspring for which all four grandparents are known. Observational and genetic data indicate that Takahe are typically monogamous (Lettink et al. 2002; C.E.G. & I.G.J., unpublished data). In our vital-stage analysis, to examine the effect of parental relatedness on offspring survival, we calculated inbreeding coefficients for breeding pairs. Eighteen percent ( $n = 104$ ) of putative breeding “pairs,” however, actually consisted of groups of two adult males and/or two adult females that were unrelated (i.e., nonsibling groups) and shared a nesting site. Reproductive outputs, such as the number of eggs, could not be assigned reliably to individual parents within such groups because DNA sampling of offspring did not occur until banding. In these cases we assigned pairwise kinship coefficients as the mean of all possible male–female pairwise combinations of the individuals sharing the nest site (following Grueber & Jamieson 2008). In the reproductive-stage analysis, we examined the effect of individual inbreeding coefficients on breeding success. Thus, the sampling unit we used was the individual. We included annual breeding data only from individuals that were monogamous in a given year so that reproductive outputs (e.g., number of eggs) could be assigned unambiguously to individuals. Only 7 of the 69 birds included in the reproductive analysis were the offspring of polygamous groups. We assigned parents to three of these seven through analysis of DNA from tissue samples taken at banding; thus, their inbreeding coefficients could be calculated reliably (C.E.G. & I.G.J. unpublished data, see also Grueber et al. 2008). We assigned the remaining four (6%) birds inbreeding coefficients that were the mean of all possible pairwise combinations from their parents (Grueber & Jamieson 2008).

### Modeling

We modeled the effect of inbreeding on each life-history stage for vital and reproductive stages with generalized linear mixed models (GLMMs) with model selection and model averaging based on AIC<sub>c</sub> (Burnham & Anderson 2002).

### MODEL FITTING

We fitted GLMMs with a binomial error structure and logit link (Armstrong & Cassey 2007) with functions implemented in the lme4 library (Bates & Maechler 2009) of R (R Core Development Team 2009). To model survival at each life-history stage the binomial numerator (event) was the number of successes (e.g., number of offspring

that fledged) and the denominator (trials) was the number of successes in the previous stage (e.g., number of offspring that hatched).

Because 62% of pairs bred in multiple years, GLMMs allowed us to incorporate a random factor, identity of the pair, to control for pair effects in the vital-stage analysis. The analysis of the reproductive stage also required that a random factor, individual identity, be included to control for breeding in multiple years (81% of birds) with different partners. Although the inclusion of random factors increased the number of parameters to be estimated, the random factors improved the  $AIC_c$  of models relative to equivalent generalized linear models without random factors (data not shown).

Takahe breed annually from 2 years old up to approximately 20 years old (Lee & Jamieson 2001), so we included age (of the breeder) and its polynomial ( $age^2$ ) as continuous covariates. We also wanted to control for possible effects of increasing population density over time. Including each year of the study as a continuous covariate inhibited model convergence. Therefore, we grouped years as an ordinal covariate with three levels categorized on the basis of time since founding: early (prior to 1995), mid (1995–2002), and late (2003–2006). We also included breeding site (island) as a categorical factor. Differences between male and female responses to inbreeding have been observed in Takahe (Jamieson et al. 2003) and other species (e.g., Keller 1998), so we included sex in the reproductive-stage analysis. We also investigated second-order interactions between inbreeding and age and inbreeding and sex.

To directly compare effect sizes of fixed effects on different measurement scales and to allow comparison of main effects when interactions are present (Gelman 2008), we standardized input variables with the function available in the R library arm (Gelman et al. 2009).

#### MODEL AVERAGING

We used model averaging (Burnham & Anderson 2002), with the functions available in the R library MuMIn (Bartoń 2009), to determine the most important explanatory variables in the GLMM. For each life-history stage, we generated a global model that incorporated all relevant explanatory variables and plausible interactions and then derived a set of models with all possible combinations of variables. Our set of top models was defined as those that fell within two  $AIC_c$  of the best model in the set. We used the natural-average method to conduct model averaging (Burnham & Anderson 2002; Nakagawa & Freckleton 2010). There are two outputs of this analysis: standardized parameter estimates (and their unconditional standard errors, which incorporate model-selection uncertainty) and relative importance of each parameter for explaining the variance in the response variable.

#### INBREEDING DEPRESSION AND LETHAL EQUIVALENTS

All final models included inbreeding, so we generated conditional survival estimates ( $S$ ) for  $f = 0$  ( $S_0$ ) and  $f = 0.25$  ( $S_f$ ), at the population mean for all other parameters in the models. Because the GLMMs were constructed with a logit link, we back transformed these survival estimates with

$$p = 1/(1 + 1/e^x), \quad (1)$$

where  $p$  is the predicted probability of survival of outbred or inbred individuals and  $x$  is the probability of survival on the logit scale.

To identify the long-term effect of fitness reductions at individual stages when  $f = 0$  and 0.25, we scaled survival of both groups to an initial fertile egg. Subsequent survival rates were the product of the probability of survival at a given stage and the proportion of survivors from the previous stage. To illustrate the fitness losses across the full life-history continuum and to provide the starting point for the reproductive phase, we multiplied the proportion of recruits from the vital stage by the mean number of fertile eggs laid in a season for  $f = 0$  and 0.25 birds. We interpreted these proportions as the number of survivors (vital phase) or the number of subsequent offspring produced (reproductive phase) per initial fertile egg per season.

We derived lethal equivalents for each life-history stage from Morton et al.'s (1956) equation

$$B = -\ln(S_f/S_0)/f, \quad (2)$$

where  $B$  is the number of lethal equivalents per gamete and  $f$  is set to 0.25 by convention. Calculating lethal equivalents in this way generates a value for each life-history stage that controls for other factors in the model (Laws 2009). The model-averaging analysis also provided the upper and lower bounds of the 95% CI of the parameter estimate for inbreeding ( $\beta_f$ ). These were substituted as the upper and lower bounds of the effect size of inbreeding to derive arithmetic confidence intervals for lethal equivalents (Nakagawa & Cuthill 2007) through use of the model to generate survival probabilities at  $f = 0$  and 0.25.

Sample sizes differed for each life-history-stage response variable, so we used parametric bootstrapping in R (Faraway 2006) to estimate the 95% CI for lifetime lethal equivalents. In our analysis, GLMMs were applied with a logit link, so we assumed the effect sizes for inbreeding followed normal distributions, where the mean is  $\beta_f$  and the standard deviation is the unconditional standard error of  $\beta_f$ . For each life-history stage, we re-sampled random  $\beta_f$  values and used these to calculate survival probabilities for  $f = 0$ ,  $f = 0.25$ , and lethal equivalents (Eq. 2). We summed single-stage lethal equivalents across the eight life-history stages and 100,000 bootstrap iterations yielded a distribution of simulated total lethal

equivalents values, from which the median and 95% CI could be obtained.

## Results

We derived offspring vital rates from 104 breeding pairs (66 males and 55 females) (mean kinship coefficient = 0.0794,  $s^2 = 0.0090$ ). Subsequent reproductive rates of the offspring that survived to recruitment were derived from 69 individuals (32 males and 37 females) that bred with unrelated ( $f < 0.125$ ) partners (mean individual  $f = 0.0473$ ,  $s^2 = 0.0054$ ). Our fertility analysis included data from 45 males (mean  $f = 0.0604$ ,  $s^2 = 0.0087$ ). Overall, the mean coancestry of all pairs in the data set (0.0794) was relatively high, as was variance in  $f$ .

Prior to model averaging at least one model containing  $f$  was within two AIC<sub>c</sub> of the best model for all life-history stages so that the effect of inbreeding could be estimated. The parameter estimate of inbreeding was negative, indicating that fitness decreased as inbreeding coefficient increased, in six of eight life-history stages (Table 1). The unconditional standard errors (which incorporated model-selection uncertainty) of the effect sizes of  $f$  were very large relative to the effect sizes for all life-history stages (for seven of the eight stages SEs were larger than that of the effect size; Table 1). The importance of  $f$ , relative to other model parameters (e.g., year, age, island) ranged from 0.13 for vital-stage hatching rate to 0.77 for reproductive-stage fledging rate (Table 1). The 95% CI ranges for lethal equivalents, whether derived arithmetically or through simulations, included zero for all life-history stages and therefore were not significant at  $\alpha = 0.05$  (Table 1).

Fledging during the reproductive stage appeared to be the most sensitive to inbreeding, although the standard error of the parameter estimate was high (standardized effect size of inbreeding =  $-0.569$  [SE 0.328]). Fledging in the reproductive stages was also associated with the greatest reduction in survival relative to outbred individuals (Fig. 1). Lethal equivalents per gamete ( $B$ ) for inbred adults at the fledging stage were high (3.34), although the CI<sub>95%</sub> included zero, and therefore was not significant at  $\alpha = 0.05$ . Although fertility of female Takahe could not be assessed, we observed that the effect of inbreeding on male fertility tended to be negative (Table 2), although again the CI included zero.

Despite the uncertainty of the estimates at each life-history stage (standard errors were high relative to the effect sizes), the negative effects of inbreeding accumulated over time (Fig. 1). As additional life-history stages were taken into account, the difference in fitness between outbred and inbred Takahe increased to the point where lethal equivalents per haploid genome ( $B$ ), across all life-history stages, was 8.03 (Table 1; 95% CI

0.039–45.4), which is significantly greater than zero. The final relative fitness estimate, measured in terms of recruitment of second-generation offspring, was reduced by 88.0% in closely related pairs ( $f = 0.25$ ) relative to unrelated pairs ( $f = 0$ ) (Fig. 1).

## Discussion

Our analysis of the Takahe pedigree indicates that the detection of inbreeding depression at single life-history stages is generally imprecise due to the high standard errors around the effect sizes for inbreeding at each stage (Table 1) and that the effect of inbreeding is sometimes even in the unexpected direction (inbreeding has a positive effect on fitness) (Table 1). Nevertheless, the weak effect of inbreeding on fitness at all life-history stages accumulates over all stages to result in a large reduction in fitness for inbred ( $f = 0.25$ ) pairs relative to outbred pairs (Fig. 1). The summed lethal equivalent for a haploid organism ( $B$ ) from the embryo stage through to the production of second-generation offspring was 8.03 (95% CI 0.039–45.4). When we included the effect of inbreeding on male fertility (Table 2), assuming that inbreeding does not affect female Takahe fertility and that the sex ratio on the islands is equal, then cumulative lethal equivalents ( $B$ ) for the island Takahe population as a whole could be as high as 8.80. Although the CI for total lethal equivalents was wide, and it was thus difficult to quantify with high certainty the magnitude of the effect of inbreeding on fitness, we concluded that inbreeding depression was occurring because the CIs did not include zero and had the potential to be very high.

When we converted the summed lethal equivalents to  $2B$  (for diploid organisms), the summed LE ( $2B = 16.03$ ) was closer to the 12 lethal equivalents O'Grady et al. (2006) found than it was to the widely cited value of 3.1 lethal equivalents calculated by Ralls et al. (1988). O'Grady et al. (2006) conducted a meta-analysis of the effects of inbreeding depression across various species' life-history stages up to survival to sexual maturity, and Ralls et al. (1988) derived their value from a single life-history stage. An accumulation of the effect of inbreeding depression across life-history stages is also consistent with the work of Szulkin et al. (2007), who found similar evidence of this accumulation in the Great Tit, a widespread passerine species. Furthermore, our results are similar to those of Jamieson et al. (2003), who detected a significant effect of inbreeding on the ability of female (but not male) Takahe offspring to fledge chicks. Differences between the latter study and our results are likely due to differences in sample size (larger in this study) and statistical methods. In addition, the data we used were collected over a longer period, which allowed us to

Table 1. Standardized predictors of fitness in vital life-history stages and reproductive life-history stages of island Takahē (*Porphyrio hochstetteri*).<sup>a</sup>

Predictor <sup>b</sup>	Hatching rate		Fledging rate		2-year survival		Offspring recruitment	
	$\beta$ (SE)	RI	$\beta$ (SE)	RI	$\beta$ (SE)	RI	$\beta$ (SE)	RI
Vital stage								
intercept	0.599 (0.155)		0.073 (0.183)		1.520 (0.240)		2.130 (0.431)	
<i>f</i>	-0.086 (0.233)	0.13	-0.203 (0.266)	0.14	-0.240 (0.457)	0.18	0.316 (0.732)	0.18
year ID	0.426 (0.235)	0.78	-0.386 (0.255)	0.52	-0.150 (0.506)	0.17	-3.760 (0.732)	1.00
maternal age <sup>2</sup>	-0.644 (0.333)	0.87	0.552 (0.469)	0.30	-	-	0.702 (1.270)	0.19
maternal age	-0.309 (0.291)	0.29	0.302 (0.294)	0.27	-0.396 (0.558)	0.21	0.474 (0.771)	0.19
island	-		-0.557 (0.330)	0.06	-		-	
Lethal equivalents <sup>b</sup>	0.177 (-0.566, 1.462)		0.762 (-0.474, 3.581)		0.270 (-0.379, 2.357)		-0.179 (-0.338, 2.022)	
Simulated lethal equivalents	0.177 (-0.699, 1.232)		0.762 (-1.120, 2.876)		0.270 (-0.570, 1.723)		0.179 (-0.737, 1.072)	
Reproductive stage								
intercept	0.847 (0.257)		0.071 (0.252)		2.810 (0.767)		1.770 (0.439)	
<i>f</i>	0.238 (0.306)	0.19	-0.569 (0.328)	0.77	-0.281 (0.879)	0.14	-0.644 (1.250)	0.17
year ID	0.641 (0.238)	1.00	-0.120 (0.296)	0.09	0.387 (0.668)	0.16	-4.540 (1.070)	1.00
sex	0.182 (0.206)	0.20	-0.133 (0.254)	0.09	-0.234 (0.534)	0.15	-0.266 (0.776)	0.16
age <sup>2</sup>	-0.492 (0.319)	0.50	0.538 (0.337)	0.09	-0.346 (0.753)	0.15	0.708 (0.886)	0.21
age	0.411 (0.286)	0.47	0.447 (0.295)	0.91	-		-	
age <sup>2</sup> <i>f</i>	-		-1.280 (0.786)	0.40	-		-	
island	-0.381 (0.283)	1.00	-0.531 (0.323)	0.21	-1.422 (0.793)	1.00	-	
Lethal equivalents <sup>c</sup>	-0.691 (-0.838, 1.997)		3.339 (-0.201, 9.092)		0.952 (-0.063, 22.212)		3.383 (-0.376, 40.179)	
Simulated lethal equivalents	-0.691 (-1.886, 1.337)		3.338 (-0.369, 7.839)		0.949 (-1.337, 16.035)		3.391 (-0.862, 36.540)	

<sup>a</sup>Abbreviations:  $\beta$ , standardized coefficient for model predictors; SE, unconditional standard error; RI, relative importance of each parameter to the other parameters in the final model; *f*, inbreeding coefficient. Results shown are model predictors derived after averaging submodels within 2AIC<sub>c</sub> of the best model (see Methods); submodel sets that were used to generate these averages are provided in Supporting Information.

<sup>b</sup>All vital-stage models included the random factor "pair identity"; reproductive-stage models included the random factor "individual identity." All predictors were standardized to a mean of 0 (SD 0.5). Abbreviations for vital stage: maternal age, age of the female at breeding. Abbreviations for the reproductive stage: age and age<sup>2</sup>, age of the individual at breeding; year ID, ordinal variable for period since founding (early, mid, or late). For island, Kapiti was the reference category and the effect size is a weighted mean based on the number of pairs in the vital-stage analyses or the number of individuals from each island in the reproductive-stage analyses.

<sup>c</sup>Haploid lethal equivalents (B) are based on Eq. 2 and conditional on the mean of all other parameters in each model; numbers in parentheses are upper and lower limits of the 95% CI, respectively, calculated arithmetically with the CIs of the parameter estimates. A positive value indicates decrease in fitness as inbreeding coefficient increases. Simulated lethal equivalents are calculated with parametric bootstrapping; numbers in parentheses are upper and lower limits of the 95% quantiles, respectively.

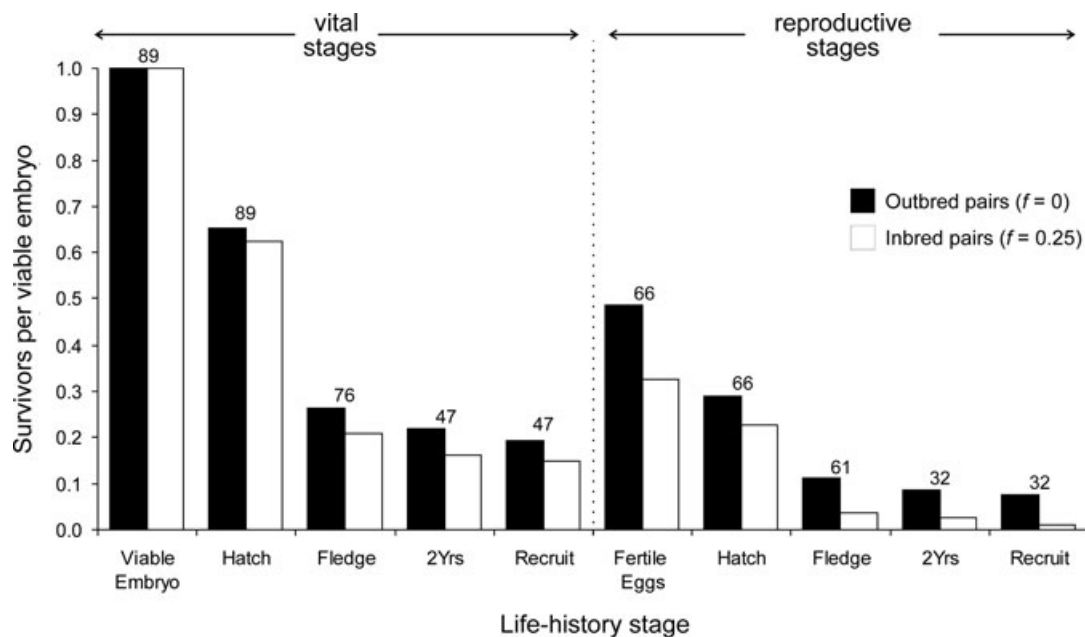


Figure 1. Cumulative predicted effects of inbreeding over all life-history stages for outbred ( $f = 0.00$ , black bars) and inbred ( $f = 0.25$ , white bars) Takaha. The sample size (above bars) is the number of levels of the random factor (pairs for “vital stages”; individuals for “reproductive stages”; as per Table 1). The proportion of successful transitions to the next life-history stage (x-axis) is the proportion of successes from the previous stage multiplied by the probability of successful transition. Proportions are scaled to one fertile egg (viable embryo) at the beginning of the vital stage; thus, subsequent stages can be interpreted as proportions surviving per embryo. The beginning of the reproductive stage is the proportion of recruits from the vital stage multiplied by the mean number of fertile eggs produced per season by outbred (2.52,  $n = 36$ ) and maximally inbred (2.19,  $n = 5$ ) individuals. The proportion of recruits in the final stage is the annual probability that an egg laid by an initial pair (outbred or inbred) will give rise to a second-generation offspring recruit.

analyze inbreeding depression over all life-history stages of Takaha.

Our results support the assertion that lethal equivalent values derived from studies of single life-history stages are likely to underestimate the effect of inbreeding in population viability models (O’Grady et al. 2006; Szulkin et al. 2007), even if the population has experienced ancestral inbreeding. Studies of single life-history stages may show

that inbreeding has little effect relative to other predictors of survival, as observed here (Table 1). In our case, however, this conclusion would be erroneous because the summed consequence of each of these small effects was greater than zero (Fig. 2).

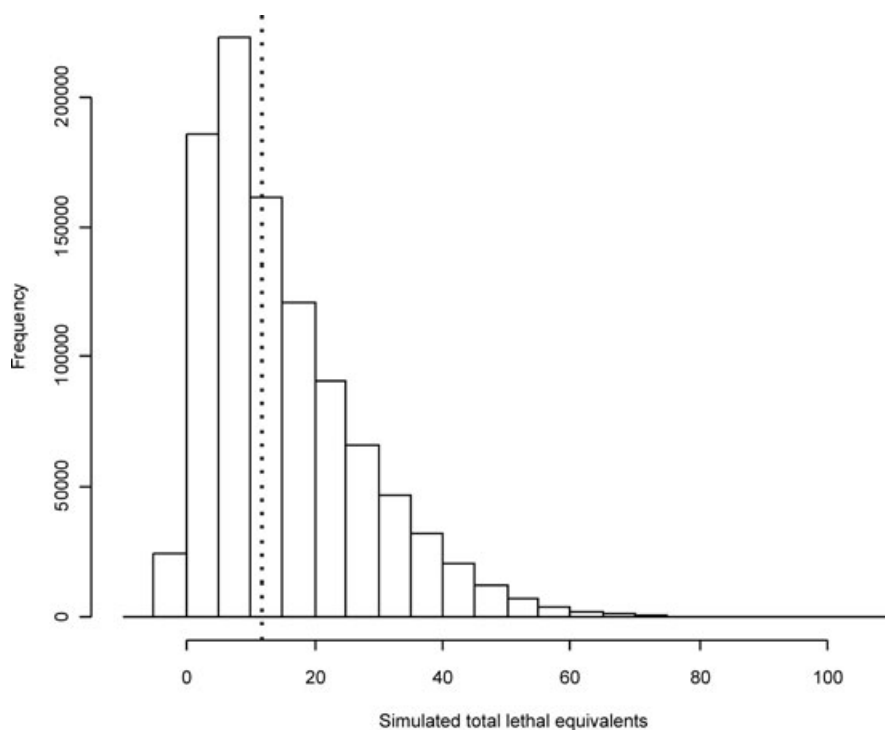
In addition to (and probably a result of) the prolonged bottleneck experienced by Takaha historically (Lee & Jamieson 2001), the species has low genetic diversity relative to other endangered species (Jamieson et al. 2006; C.E.G. & I.G.J., unpublished data). This bottleneck suggests that inbreeding coefficients derived from the Takaha pedigree may be underestimates of the true level of inbreeding. Nevertheless, our finding that inbreeding is negatively affecting overall fitness of Takaha indicates that even species subjected to ancestral inbreeding can lose fitness as a result of recent inbreeding (see also Boakes et al. 2007).

If Takaha have experienced ancestral inbreeding, then why have they not purged their genetic load? For example, severe inbreeding depression ( $LE [2B] = 14.94$ ) was detected in a large population of Collared Flycatchers (*Ficedula albicollis*), a species with the lowest frequency of inbreeding of any inbred bird (Kruuk et al. 2002). This severe inbreeding depression indicates that few

Table 2. Standardized predictors of male fertility of island Takaha (*Porphyrio hochstetteri*)<sup>a</sup>.

Predictor	Male fertility	
	$\beta$ (SE)	RI
Intercept	2.270 (0.559)	
$f$	-0.670 (0.494)	0.42
Year ID	0.057 (0.407)	0.25
Age	0.406 (0.285)	0.45
Island	-1.301 (0.592)	1.00
Lethal equivalents	1.570 (-0.069, 8.698)	
Simulated lethal equivalents	1.571 (-0.510, 4.970)	

<sup>a</sup>See Table 1 for definitions of abbreviations and how model predictors and lethal equivalents were derived.



*Figure 2. Distribution of simulated total lethal equivalents (B) for Takahē generated by resampling the effect size of inbreeding (based on point estimate and unconditional standard error) and recalculating lethal equivalents at each life-history stage. Lethal equivalents were summed across all eight life-history stages to generate the distribution of 100,000 lethal-equivalent values (dashed line, median of the distribution).*

deleterious alleles have been purged from the large population (Kruuk et al. 2002). By contrast, in Takahē, which has a long history of inbreeding (and thus opportunity for purging), we could not detect inbreeding depression at any individual life-history stage (Table 1). Although some genetic purging may have occurred in Takahē, the species is not fully resilient to inbreeding, especially when inbreeding is considered across all life-history stages. This observation is consistent with evidence that genetic purging may not effectively reduce inbreeding depression over all fitness traits (Boakes et al. 2007).

It seems likely that the cumulative inbreeding depression we detected in Takahē will affect the viability of the population if the frequency of inbreeding increases. It is inevitable that the mean inbreeding coefficient of a closed population at carrying capacity will increase (Frankham et al. 2002). Based on our results and results of a related study documenting loss of genetic variation in Takahē (Grueber & Jamieson 2008), the New Zealand Department of Conservation plans to remove individuals with high mean kinship values (Lacy 1995) from the island population and translocate them to the larger source population in Fiordland. At the same time they will introduce unrelated birds from Fiordland to the island population. These activities should reduce the rate at which local inbreeding is increasing.

More generally, we found it is important to evaluate inbreeding depression over all the life-history stages of a species because subtle effects at each life-history stage can be difficult to detect, but may accumulate to markedly reduce overall fitness and negatively affect population vi-

ability. In addition, our results contribute to the ongoing debate (Crnokrak & Barrett 2002; Keller & Waller 2002; Boakes et al. 2007) about the relative effectiveness of purging of deleterious genes in wild populations that have undergone prolonged bottlenecks. Our study highlights the importance of collecting and maintaining pedigrees, where possible, in wild populations of threatened species because unrestrained inbreeding has the potential to seriously hinder their recovery.

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### Supporting Information

Characteristics of each submodel in the top model sets that were averaged for each fitness response (Appendices



S1–S9) are available as part of the online article. The authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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