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MODELING *PLASMODIUM* PARASITE ARRIVAL IN THE GALAPAGOS PENGUIN (*SPHENISCUS MENDICULUS*)

ROBERT J. MEILE,[1,6] ROBERT C. LACY,[2] F. HERNÁN VARGAS,[3,4] AND PATRICIA G. PARKER[1,4,5]

[1]*Department of Biology, University of Missouri–St. Louis, St. Louis, Missouri 63121, USA;*
[2]*Chicago Zoological Society, Brookfield, Illinois 60513, USA;*
[3]*The Peregrine Fund, 5668 West Flying Hawk Lane, Boise, Idaho 83709, USA;*
[4]*Charles Darwin Foundation, Puerto Ayora, Santa Cruz, Galápagos, Ecuador; and*
[5]*WildCare Institute, Saint Louis Zoo, St. Louis, Missouri 63110, USA*

ABSTRACT.—The recent discovery of a *Plasmodium* parasite in the endangered Galapagos Penguin (*Spheniscus mendiculus*) poses a threat to the long-term persistence of this endangered species and, potentially, much of the endemic avifauna of the Galapagos Islands. However, little information is available on the transmission dynamics or pathogenicity of *Plasmodium* in the Galapagos. We added a simple model of infection to the population model of the Galapagos Penguin devised by Vargas et al. (2007). Two variables (the probability of an individual becoming infected each year, and the increase in annual mortality caused by infection) define the dynamics of the disease component of the model; the stress from El Niño events could affect parasitized individuals in different ways, so three forms of stress-induced relapse are also explored. All models show a high impact due to mortality from infection, and there are large parts of parameter space that have a 0% probability of persistence for the next 100 years. To estimate the mortality that might be associated with *Plasmodium* infection, a comparison was made between census data from 1998–2009 and model predictions based on the same years. A range of plausible mortality values was determined from the best-fitting models, ranging from 0–5% to 0–10% depending on the type of relapse modeled. Even at these relatively low levels of impact, *Plasmodium* infection has the potential to drastically reduce the probability of persistence of the Galapagos Penguin population over the next 100 years. *Received 9 August 2012, accepted 18 April 2013.*

Key words: El Niño, Galapagos Penguin, malaria, population viability analysis, *Spheniscus mendiculus*.

Modelamiento de la Llegada del parásito *Plasmodium* a los Pingüinos de Galápagos (*Spheniscus mendiculus*)

RESUMEN.—El descubrimiento reciente del parásito *Plasmodium* el pingüino amenazado *Spheniscus mendiculus* representa una amenaza para la persistencia a largo plazo de esta especie y, potencialmente, para mucha de la avifauna de las islas Galápagos. Sin embargo, hay poca información disponible sobre la dinámica de transmisión o la patogenicidad de *Plasmodium* en las Galápagos. Añadimos un modelo simple de infección al modelo poblacional de *S. mendiculus* propuesto por Vargas et al. (2007). Dos variables (la probabilidad de que un individuo sea infectado por año, y el incremento en la mortalidad anual causada por la infección) definen la dinámica del componente del modelo relacionado con la enfermedad. El estrés causado por los eventos de El Niño podría afectar a los individuos infectados en diferentes maneras, por lo que también se exploraron tres formas de reincidencia inducida por el estrés. Todos los modelos mostraron un alto impacto de la mortalidad debida a la infección. Además, grandes áreas del espacio paramétrico presentaron una probabilidad del 0% de persistencia de la especie en los próximos 100 años. Para estimar la mortalidad que podría estar asociada con la infección de *Plasmodium*, comparamos datos de censos obtenidos entre 1998 y 2009, y las predicciones del modelo basadas en los mismos años. Se determinó un rango de posibles valores de mortalidad a partir de los modelos con mejor ajuste, que varian entre 0-5% a 0-10% dependiendo del tipo de reincidencia modelado. Incluso a estos niveles relativamente bajos de impacto, la infección por *Plasmodium* tiene el potencial de reducir drásticamente la probabilidad de persistencia de la población de *S. mendiculus* en los próximos 100 años.

[6]E-mail: rjm8g8@umsl.edu

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There has been serious concern that *Plasmodium*, a blood parasite capable of causing avian malaria, would find its way to the Galapagos Islands (Wikelski et al. 2004). The introduction of *Plasmodium* and a suitable vector have been implicated in the extinction of several endemic bird species in Hawaii over the past century (Warner 1968, Atkinson et al. 1995). Like Hawaii, the Galapagos Islands are home to many small populations of endemic birds, long isolated from the mainland (Harris 1973). These factors make disease-induced extinctions more likely (De Castro and Bolker 2005). Therefore, the discovery by Levin et al. (2009) of a *Plasmodium* lineage in the endangered Galapagos Penguin (*Spheniscus mendiculus*) represents a serious threat, not just to this particular species, but to the entire avifauna of the Galapagos Islands.

The Galapagos Penguin is endemic to the archipelago, with a small population of ~1,800 individuals (F. H. Vargas pers. comm.), down from an initial estimate of 4,000 individuals in the 1970s (Vargas et al. 2005). Although the population has experienced positive growth over the short term, the species has experienced a long-term decline over the past 40 years (Vargas et al. 2005). This fluctuation is driven by the periodic occurrence of intense El Niño events, which are associated with >50% reductions in the population, believed to be caused by reduced food availability (Vargas et al. 2006). In addition to reducing the size of an already small population, a stressful event such as El Niño could also worsen the effects of parasite infection (Atkinson and van Riper 1991, Valkiūnas 2005), which were not known to be present during Vargas et al.'s (2006) study. Another cause for concern is that related species of penguins in the genus *Spheniscus* have shown high susceptibility to and mortality from *Plasmodium* infection in captivity (Stoskopf and Beier 1979, Fix et al. 1988, Cranfield et al. 1990, Graczyk et al. 1994). This vulnerability may be exacerbated in the Galapagos Penguin population because of its low overall genetic diversity (Nims et al. 2008), including significantly low variation in major histocompatibility complex (MHC), which may indicate a poor immune response to infection (Bollmer et al. 2007). The Galapagos Penguins' small population, their potential vulnerability to *Plasmodium* infection, and the periodic occurrence of devastating El Niño events all suggest that this species could be severely threatened by the presence of *Plasmodium*.

Unfortunately, little is known about *Plasmodium* in the Galapagos or how the penguins respond to it (LaPointe et al. 2012). *Plasmodium* parasites were initially discovered in Galapagos Penguins by using polymerase chain reaction (PCR) to amplify parasite DNA in the hosts' blood. Using this technique, PCR-positive Galapagos Penguins were found on the islands of Isabela, Fernandina, and Santiago, with a total prevalence of 5% (n = 362 unique penguins; Levin et al. 2009). However, an enzyme-linked immunosorbent assay (ELISA), which is used to detect previous exposure to *Plasmodium*, was conducted on a subset of the same birds and found that ~95% of the sampled individuals had been exposed (Palmer et al. 2013). Unanswered questions regarding *Plasmodium* in Galapagos Penguins include estimates of its pathogenicity and the identities of the mosquito vector and vertebrate reservoir. These parameters will be important in understanding how critical the situation in the Galapagos Islands is, and in determining management actions to minimize the risk of avian extinctions, and all are under investigation.

Investigating these factors will take time, and meaningful conservation decisions need to be made in the meantime to manage this vulnerable species. A useful tool in making these decisions is population viability analysis (PVA), a technique used to predict the probability of extinction for a population by utilizing a stochastically driven computer simulation of future population growth (Possingham et al. 1993). It has been successfully used in predicting viability for some species (Brook et al. 2000), but PVA is more robust to limited data when it is used to make relative comparisons, among threats or the effects of management actions on future trajectories, rather than absolute predictions (Coulson et al. 2001, Ellner et al. 2002). Population viability analysis can also be valuable for exploring the effects of uncertainty about parameter values on our predictions and, when long-term field data exist against which to compare model projections, for narrowing the plausible range of parameters. A previous PVA for the Galapagos Penguin was conducted by Vargas et al. (2007) using the simulation program VORTEX (Lacy et al. 2010). The presence of *Plasmodium* in the population was unknown at that time, so their model focused on how El Niño events might influence the probability of persistence of the Galapagos Penguin population. Under the current frequency of El Niño events, they predicted a 70% probability of persistence for the next 100 years. They found that the less frequent but more damaging strong El Niño events have a greater effect on the Galapagos Penguin population than the more frequent but weak El Niño events. Another important factor is the adult mortality rate, with rates >5% being especially damaging. The presence of *Plasmodium* was not accounted for in the previous model, so it is unknown if these results are still applicable.

The present work extends the model of Vargas et al. (2007) to include a disease component. We explore possible consequences of *Plasmodium*'s introduction on the Galapagos Penguin's long-term probability of persistence and use the modeling framework to estimate a range of plausible parameter values for mortality from *Plasmodium* infection.

Methods

The model is a stochastic, individual-based model developed in VORTEX, version 9.99b (Chicago Zoological Society, Brookfield, Illinois), a program used for PVA (Lacy 2000, Miller and Lacy 2005, Lacy et al. 2010). This type of modeling includes the effects of variable demography, environmental conditions, and rare catastrophes, instead of deterministic factors alone (see Table 1 for terminology). The inclusion of these semirandom events means that a single run of the model gives only one possible outcome for the population, so each scenario (combination of parameter values) is run 1,000 times to create a distribution of outcomes. Each run simulates the Galapagos Penguin population 100 years into the future in 1-year increments, and the probability of population persistence can be calculated as the proportion of the 1,000 runs that predict an intact population after 100 years, with an intact population being defined as having at least one individual of each sex still alive. All of our modeled scenarios have three main components: the demographic parameters of the Galapagos Penguin population, the occurrence of El Niño events, and the dynamics of *Plasmodium* infection.

Table 1. Terminology used in the present study.

Term	Definition
Scenario	A single scenario refers to the specific combination of parameters used for a particular simulation.
Year	One increment of time in the model; the current population size, births, deaths, catastrophes, etc. are used to calculate the new population size after that year. Each scenario runs for 100 years.
Run	An independent, 100-year calculation of the model; the probability of persistence is calculated from 1,000 runs of the model.
Stochastic events	Events that occur randomly with set probabilities; they do not have the exact same value in each run of the model.
Probability of persistence	The proportion of runs that end with an intact population (defined as one or more individuals of each sex remaining alive).
Probability of infection	The probability of a susceptible individual becoming infected each year.
Susceptible	An individual in the model that is not infected with *Plasmodium*.
Acute infection	An individual in the model that is infected with *Plasmodium* and is currently experiencing symptoms of disease (increased mortality).
Chronic infection	An individual in the model that is infected with *Plasmodium,* but is otherwise not affected.
Pathogenicity	The probability of an individual dying in a year when acutely infected or suffering a relapse, in addition to (added to) their mortality rate without disease.
Relapse	A reoccurrence of symptoms (i.e., pathogenicity) in a chronically infected individual, triggered in the model by El Niño events.
Strong El Niño	A 2-year event that reduces the penguins' survival and reproductive success.
Weak El Niño	A 1-year event that reduces only the penguins' reproductive success.

Galapagos Penguin demographics.—The Galapagos Penguin demographic parameters are the same as used in Vargas et al.'s (2007) Current El Niño (CEN) model (parameters given in Table 2), with two exceptions. First, the initial population size is now set at 1,800 individuals, in accordance with the Galapagos Penguin population census estimate from 2009 (H. Vargas pers. comm.; for details on the census estimation technique, see Vargas et al. 2005). The sizes of the four island subpopulations are taken to be proportional to the population sizes reported in table 3 of Vargas et al. (2007). Second, the mortality rates for each age class are now functions that take the infection status of an individual into account (see below; details are provided in the online Appendix [see Acknowledgments]).

El Niño events.—As in Vargas et al. (2007), there are two types of El Niño events included in the model, strong and weak (Vargas et al. 2006), and in VORTEX they are treated as "catastrophes" that occur randomly with a set probability. The frequency of occurrence for El Niño events is the same as in the previous model (Table 2), but their duration and severity have been slightly altered. An oversight in Vargas et al.'s (2007) model implementation allowed both types of El Niño to occur in the same year; this has been fixed. Also, strong El Niño events are now modeled as 2-year events, with differing severity for each year, to more closely match the dynamics of observed El Niño events. Details on these changes are provided in the online Appendix.

Disease states.—Each individual Galapagos Penguin in the model can be in one of three disease states at any time: susceptible, acutely infected, or chronically infected. Susceptible individuals are not infected with *Plasmodium* and, thus, experience normal rates of mortality. Individuals become acutely infected for the first year after contracting malaria, and they experience increased mortality due to their infection. If an individual survives the first year of infection, they then become chronically infected; infection with *Plasmodium* can lead to persistent, long-term infections (Valkiūnas 2005). Chronic infections are considered to be under control, so infected individuals in the model do not experience any increased mortality as a result of their infection, except under certain circumstances (see below).

Variables of infection.—There are two variables that control the spread and severity of infection by *Plasmodium* in the model. The probability of infection gives the probability each year that a susceptible individual will become infected. The pathogenicity variable is the increase in the probability of mortality that an individual experiences as a result of infection.

Relapse scenarios.—It is believed that individuals in high-stress situations can become immunocompromised, leading to a worsening of symptoms from an existing infection (Atkinson and van Riper 1991, Valkiūnas 2005). El Niño events can be stressful periods for Galapagos Penguins, which are believed to become food limited because of changes in the Cromwell Current system leading to reduced fish numbers (Vargas et al. 2006). However, it is unknown how infected Galapagos Penguins will respond to different El Niño conditions; they may suffer a recurrence of their symptoms (hereafter "relapse") during some events or be unaffected. Thus, three separate modeling scenarios of relapse were considered: a scenario in which no relapses occur, one in which relapses occur during strong El Niño events, and one in which relapses occur during all El Niño events, weak and strong. In the two scenarios in which relapses are included, whenever a relapse-triggering event occurs, chronically infected individuals experience increased mortality because of their infection, according to the pathogenicity for the current model (see below). The model does not take into account the possibility of pathogenicity changing between the acute infection phase and subsequent relapses, because of either increased resistance in the host or increased susceptibility from the stressful El Niño conditions.

Exploration of parameter space.—In order to assess the possible effect of malaria on the Galapagos Penguin population, the two variables that define the malaria dynamics (the individual probability of infection and pathogenicity) were varied over the full parameter space. Looking at the full range of possible values allows us to assess how each parameter affects the model and how the parameters interact with each other. Each variable could take on values from 5% to 100%, in 5% intervals, along with a baseline model that did not include any disease component; each unique combination of parameter values is a separate model of 1,000 runs. In addition, this entire parameter space was separately analyzed for each of the three relapse scenarios described above. This resulted in the analysis of 1,201 separate models (400 for each

TABLE 2. Parameters of the model. Adapted from Vargas et al. (2007).

Parameter	Parameter value in basic model
Number of iterations	1,000
Number of years	100
Extinction definition	One sex remains
Number of populations	4
Inbreeding depression	No
Correlation of demographic rates among subpopulations	0.9
Concordance of variation in reproduction and survival	Yes
Breeding system	Long-term monogamy
Number of types of catastrophes	2
Dispersing age range (youngest–oldest)	(1–1)
Dispersing sex(es)	Both
Percent survival of dispersers	80
Dispersal rates	[a]
Age of first offspring for females	3
Age of first offspring for males	3
Maximum age of reproduction	20
Maximum number of broods per year	1
Sex ratio at birth (% male)	50%
Annual reproductive rates	
Percentage of adult females breeding	56.7
Annual variation in percentage breeding	SD = 13
Percentage of females producing 1 progeny	33.5
Percentage of females producing 2 progeny	46.4
Percentage of females producing 3 progeny	12.4
Percentage of females producing 4 progeny	7.7
Mortality rates (same for both sexes)	
Percentage mortality between ages 0 and 1	67
Annual variation in % 0–1 mortality	SD = 10
Percentage mortality between ages 1 and 2	25
Annual variation in % 1–2 mortality	SD = 5
Percentage mortality between ages 2 and 3	5
Annual variation in % 2–3 mortality	SD = 3
Percentage mortality after age 3	5
Annual variation in % 3+ mortality	SD = 3
Catastrophe 1: Strong El Niño	
Frequency	5%
Multiplicative effects on reproduction, survival	[b]
Catastrophe 2: Weak El Niño	20% [c]
Frequency	
Multiplicative impacts on reproduction, survival	0.8 [d], 1.0
Percentage of males in breeding pool	100%
Initially at stable age distribution?	Yes
Initial population size	1,800 [e]
Carrying capacity (*K*)	4,200
SD in *K* due to environmental variation (EV)	420
Harvest	No
Supplementation	No

[a] See Vargas et al. (2007).
[b] See Appendices A and B.
[c] A weak El Niño will not occur in the same year as a strong El Niño; see Appendices A and B.
[d] The multiplicative impact on reproduction was incorrectly reported as 0.2 in Vargas et al. (2007) (R. C. Lacy pers. obs.).
[e] The population size of each subpopulation uses the same "mean percent of population" reported in Table 3 of Vargas et al. (2007).

relapse scenario, plus the baseline), for a total of 1,201,000 runs. For each model, the probability of population persistence for the next 100 years was recorded.

Model assumptions.—Some assumptions of the disease component of the model include the following. (1) The individual probability of infection and pathogenicity are the same for every individual, in every year, and on every island (within a set of 1,000 runs for any model). Although these probabilities are likely to vary spatially and temporally, the distribution of such variation is wholly unknown. (2) The pathogenicity of infection is the same for

the initial and subsequent episodes. (3) Relapses occur only during El Niño years, if at all. This ignores other sources of stress that could lead to relapses, such as molting or reproduction (Richner et al. 1995). (4) The dynamics of the vector(s) or possible reservoirs are not taken into account.

Effect of increased breeding success on probability of persistence.—In their previous modeling work in this system, Vargas et al. (2007) made several suggestions for management actions to increase the probability of persistence for the Galapagos Penguin. One suggestion was to increase the percentage of females that successfully breed in a year; on the basis of the results from their modeling, they recommended an increase of 10%. To determine how effective this strategy might be in the face of *Plasmodium*'s presence in the population, the analysis of 1,201 models discussed above was repeated, but with the adult female breeding success increased by 10%, from 56.7% to 66.7%.

Estimation of the probability of infection.—In order to assess the current threat posed by *Plasmodium*, as opposed to the range of possible outcomes, it is necessary to estimate values for the two malaria variables, the probability of infection and the pathogenicity. The ELISA performed by Palmer et al. (2013) found around 95% exposure of sampled Galapagos Penguins to *Plasmodium*. The 95% exposure rate from the ELISA is therefore taken as an estimate of the probability of infection in the analysis on pathogenicity below.

Estimation of pathogenicity.—Although the effects of *Plasmodium* infection on Galapagos Penguin mortality have not been directly observed, the pathogenicity of infection will have population-level effects that may be detectable. To estimate the pathogenicity experienced by Galapagos Penguins, multiple scenarios were run that differed only in the value of the pathogenicity parameter. Their results were compared with the Galapagos Penguin population census data from 1998–2009 (Vargas et al. 2005; F. H. Vargas pers. comm.), specifically the growth rate, r, from one year to the next, calculated as

$$r = \frac{N_{t+1}}{N_t}$$

where N_t and N_{t+1} are the population sizes during years t and $t + 1$. Some values of the pathogenicity parameter will provide a better fit to the census data than others.

The scenarios were run for 11 years, instead of 100 years as in the main model. These 11 years simulate those following the last strong El Niño event, unlike the previous modeling that projected future population growth. Choosing these years avoids complicating the analysis with the possible interactions among infection, pathogenicity, and strong El Niño events, while still allowing enough data points for comparisons to be made. The years 2006, 2008, and 2009 were weak El Niño years, as determined by sea-surface temperature data from the Charles Darwin Foundation Climate Database (see Acknowledgments) and the definition of "weak El Niño" given in Vargas (2006) and were modeled accordingly. The demographic parameters of the model were kept the same, except that the starting population size was set to 780, the population estimate from 1998 (Vargas et al. 2005).

Six scenarios included in this analysis had pathogenicity values from 5% to 30% in 5% intervals, along with a scenario that did not include any disease component. The decision to stop at 30% was arbitrary. The probability of persistence was set at 95% for each value of pathogenicity (additional scenarios were run with probabilities of infection from 80% to 100% in 5% intervals, but the results were consistent with the 95% scenarios and so are not reported here). The fit of each scenario was defined as follows: the difference between the population growth rate of the model, averaged over all 1,000 runs, and the growth rate observed in the census data was found for each year, and the differences were then averaged across all years. This whole analysis was repeated for the scenario without relapses and the scenario with relapses during all El Niño events.

Although only one parameter value will give the best-fitting model to the data for each relapse scenario, other values may not be significantly worse fits to the data. Using normal statistical tests, such as two-sample t-tests, to determine which models were significantly different would not be appropriate; the standard error in the fit of the models could be arbitrarily increased or decreased by changing the number of model runs. Instead, we took the 1,000 runs of the best-fitting model and calculated the fit of each individual run to the census data. These 1,000 values can be ordered into a distribution, which gives the range of typical values found when assuming the best-fitting model to be true. The average fit of the other scenarios can then be compared to this statistical distribution; if the proportion, P, of the distribution that falls outside of the average fit of a scenario is less than the 0.05 alpha level, that scenario is a significantly worse fit to the data compared to the best-fitting model (for using simulations for hypothesis testing, see Whitlock and Schluter 2009).

Results

The probability of persistence for 100 years depends on the disease parameter values and the type of El Niño relapse (Fig. 1). In all three scenarios, increasing pathogenicity leads to a steep decline in the probability of persistence, whereas increasing the individual probability of infection causes a less severe decline. Increasing both parameters causes a rapid decrease. There are large portions of the parameter space that show a 0% probability of persistence after 100 years. The scenarios without relapses and with relapses during strong El Niño events have similar shapes across parameter space, whereas the scenario with relapses for all El Niño events predicts a smaller probability of persistence for all parameter values. Increasing the proportion of adult females successfully breeding by 10% leads to an increase in the probability of persistence for parts of parameter space, and in a similar fashion for all three relapse scenarios (Fig. 2). When the value of at least one of the disease parameters is kept low, there is a modest increase in the probability of persistence. When both parameters increase, though, the benefits of increasing the breeding success quickly decline, and there are still large areas of parameter space that have a 0% probability of persistence.

The scenario that best fit the census growth rates was the scenario that did not include any effect of *Plasmodium* infection (Table 3 and Fig. 3A). The no-disease model underestimates the average trend in the actual growth rate from 1999 to 2005, and slightly overestimates it from 2006 to 2009. Most of the

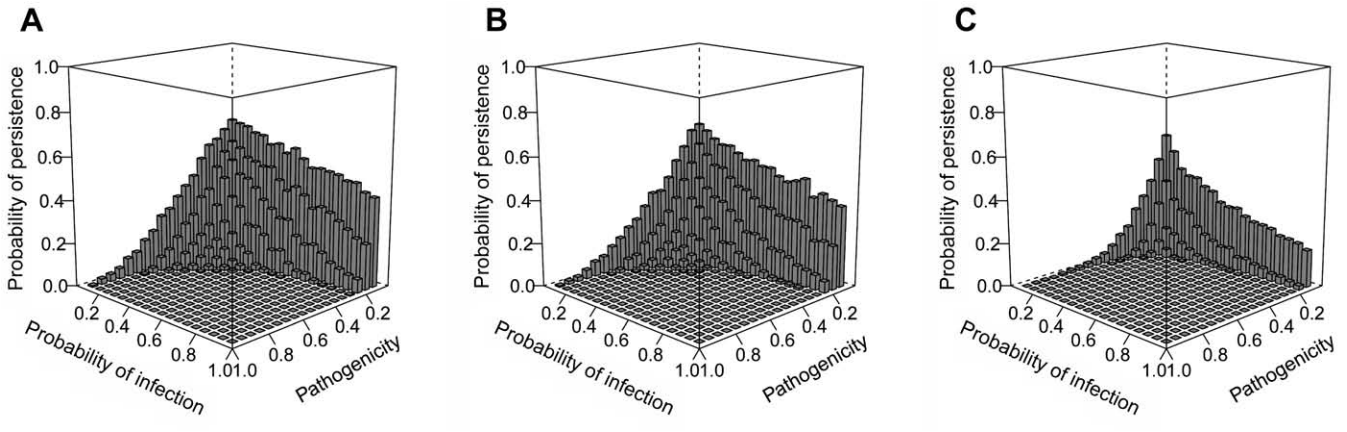

FIG. 1. Mean probability of persistence of the Galapagos Penguin after 100 years under (A) the scenario without relapses, (B) the scenario with relapses during strong El Niño events, and (C) the scenario with relapses during all El Niño events. Each graph shows the probability of persistence for each combination of parameter values (probability of infection and pathogenicity), starting at 5% and increasing in 5% intervals. Pathogenicity is the increased mortality due to infection. The baseline model used by Vargas et al. (2007) gave a probability of persistence of 70%.

disparity comes from the strong recovery of the population in 1999, immediately after the previous strong El Niño; this can be seen by showing the same data while omitting the results from 1999 (Fig. 3B). For the years 1999 to 2009, only 15 of 1,000 runs of the no-disease model generated mean growth rates greater than that observed in the census data. Without the year 1999, 446 of 1,000 runs generated mean growth rates greater than the census, reflecting the close fit of the no-disease model to those census data. When the scenarios with malaria are compared to the distribution of the best-fit model, significantly worse fits were obtained for the models with no relapses when pathenogenicity was ≥15%, and for models with relapses when pathenogenicity was ≥10% (Table 3). The probability of persistence is high for the next 25 years, regardless of the relapse scenario or level of pathogenicity, but is significantly reduced for the next 50 to 100 years (Fig. 4).

TABLE 3. Model fit for the estimation of pathogenicity. "Fit" is the average difference between the growth rate each year in that model and the growth rate in the census. *P* is the proportion of runs of the best-fit (no disease) model that deviated more from the census data than did the mean for the model in that row. A model is a significantly worse fit to the census data when $P < 0.05$.

Pathogenicity	Probability of infection	No relapses fit	*P*	Relapses fit	*P*
0	0	−0.0367			
5	95	−0.0516	0.231	−0.0641	0.083
10	95	−0.0646	0.083	−0.0932	0.002
15	95	−0.0797	0.015	−0.1252	0
20	95	−0.0997	0.001	−0.1607	0
25	95	−0.1201	0	−0.1987	0
30	95	−0.1458	0	−0.2467	0

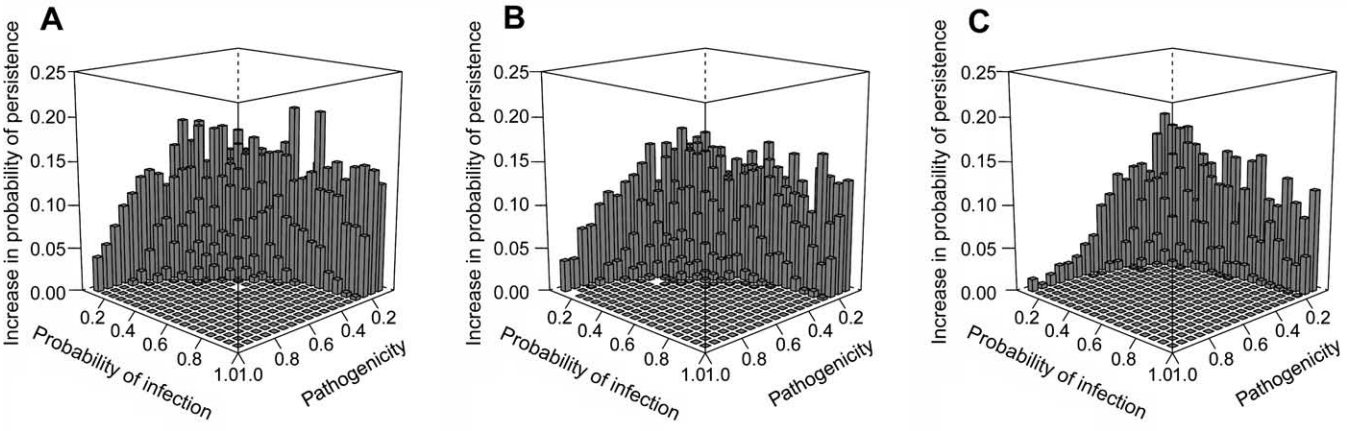

FIG. 2. The improvement over the base model due to increasing the proportion of adult females successfully breeding by 10%, for (A) the scenario without relapses, (B) the scenario with relapses during strong El Niño events, and (C) the scenario with relapses during all El Niño events. The surfaces are not smooth because of the random variation between the stochastic simulations of the different models.

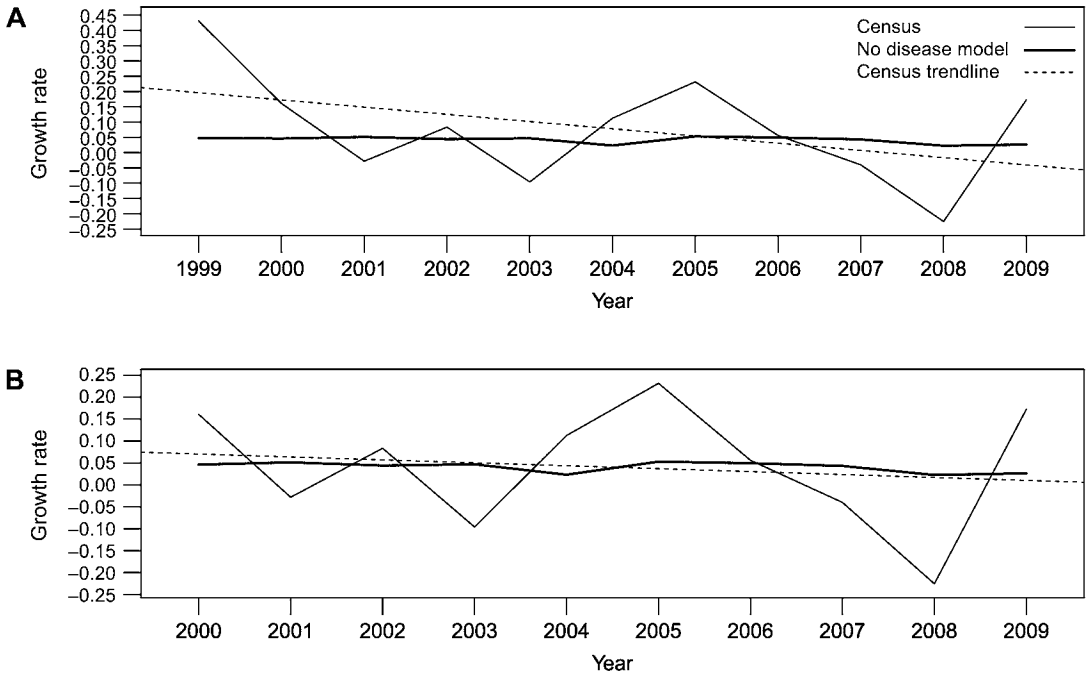

FIG. 3. Comparison of Galapagos Penguin population growth trends with the predicted growth from the best-fitting model. (A) Yearly growth rates (thin line) and a linear regression (dashed line) for the census data, and the average predicted growth rates over 1,000 runs for the best-fitting model, with no disease component (thick line). (B) The predictions of the no-disease model are similar to the linear trend of the census data, but only if the high growth in 1999 is not included.

DISCUSSION

The recently documented arrival of *Plasmodium* could have devastating consequences for the long-isolated avifauna of the Galapagos. Our modeling work provides the first estimate for the pathogenicity of *Plasmodium* infection in an endemic Galapagos species. Under both the relapse scenarios considered, the estimated levels of mortality associated with infection were relatively low. This is consistent with what information is available on *Plasmodium*'s presence in the Galapagos. The Galapagos Penguin population has continued to grow since 2003 (Vargas et al. 2005, F. H. Vargas pers. comm.), despite near ubiquitous exposure of Galapagos Penguins to *Plasmodium* (Palmer et al. 2013). Over this period, 10 individuals have been found and recaptured

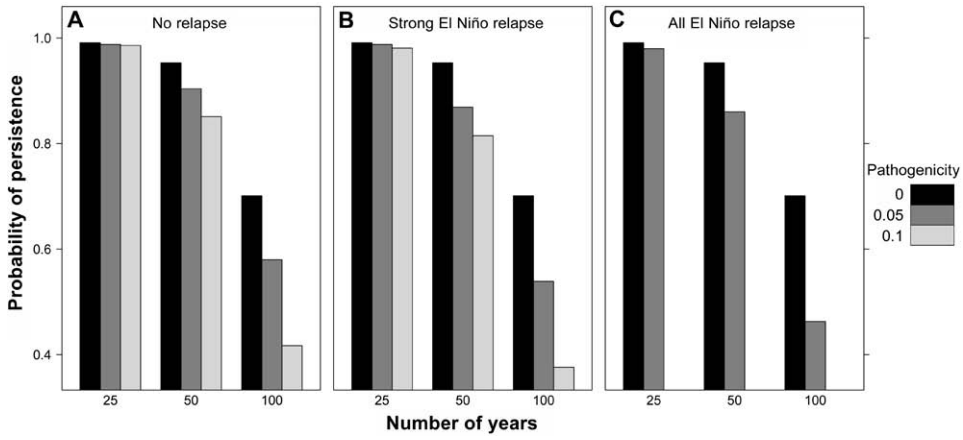

FIG. 4. Probability of persistence of the Galapagos Penguin for the next 25, 50, and 100 years for the range of plausible parameter values for pathogenicity for the scenarios (A) without relapses (0–15% pathogenicity), (B) with relapses during strong El Niño events (0–15%), and (C) with relapses during all El Niño events (0–10%). For B, plausible mortality values given are the same as for A, as they have the same relapse schedule for the years tested here. All scenarios with malaria included had the probability of infection set at 95%. The data for 100 years are the same as reported in Figure 1.

that were PCR-positive for *Plasmodium* at both times (Levin et al. 2009, Palmer et al. 2013). Five of these individuals have survived for ≥3 years. This all suggests that at least some Galapagos Penguins are suffering only minimal effects from *Plasmodium* infections. However, the recaptures and estimates of *Plasmodium* exposure started in 2003, several years after the latest strong El Niño event. It is possible that infection is relatively benign under most conditions (as our results suggest) but that pathogenicity is high during strong El Niño events. Our models do not investigate this, because they assume that the pathogenicity of infection is the same for the initial, acute infection and for subsequent relapses.

It is possible that *Plasmodium* has played a role in previous Galapagos Penguin population crashes. A reanalysis of Miller et al.'s (2001) Galapagos Penguin blood samples, taken in 1996, found one infected bird out of 109 retested (P. G. Parker pers. comm.), showing that *Plasmodium* was present in the Galapagos Penguin population during the 1997–1998 strong El Niño. We do not know when *Plasmodium* arrived in the Galapagos Islands, leaving open the possibility that it was also present during the 1982–1983 strong El Niño, but undetected. The heavy mortality observed during strong El Niño events may, in part, be due to the presence of infected Galapagos Penguins that cannot cope with the stressful conditions. The lack of archived samples from this period makes drawing any conclusions difficult; the Galapagos National Park and the Charles Darwin Foundation could provide a valuable resource by maintaining such materials for retrospective study. In the meantime, observational studies of penguins during the next strong El Niño event, undertaken alongside continued disease monitoring, will help shed light on this issue.

It appears that the type of relapse that may occur during El Niño events, and even the pathogenicity of infection, does not have an appreciable effect on the probability of persistence over the short term (Fig. 4). It is only when considering the population's persistence for 50 or 100 years that differences in these parameters lead to different predictions for the population's fate. All of the models that include the effects of malaria experience a precipitous drop in the probability of persistence after 25 years. According to the previous modeling of Vargas et al. (2007), the most significant factor determining the probability of persistence for the Galapagos Penguin is the level of adult mortality. An increase of even 5% can have a severe effect. All of the most plausible disease models increase mortality by ≥5% for ≥80% of the population, which leads to lower probabilities of persistence over relatively short periods. The presence of *Plasmodium* in the Galapagos Penguin population represents a serious, long-term threat.

Vargas et al. (2007) recommended that a controlled breeding program be considered when the predicted probability of persistence falls below 90% for the next 50 years. This occurs over a large part of the parameter space, especially when the probability of infection is high (Fig. 4; R. J. Meile et al. unpubl. data), even at the current population size of 1,800 individuals. We reiterate their suggestion to have a management plan in place, in case of a precipitous population decline, and to continue censusing the population to detect such a decline. Their conservation recommendations to improve the demographic success of the population are still relevant as well.

Looking at the whole of parameter space, and not just at the range of most plausible values, allows us to consider the effect of reducing one variable or the other on the 100-year probability of persistence. For instance, would conservation effort be more effectively spent on reducing disease transmission or pathogenicity? Somewhat counter-intuitively, given the near ubiquity of exposure to *Plasmodium*, each percent decrease in pathogenicity has a greater effect than a similar decrease in the individual probability of infection. However, the effectiveness of an intervention also depends on how cost effective it is; the expense and logistical difficulty of protecting already infected Galapagos Penguins from the effects of their disease, if even possible, may be prohibitive compared with a campaign to greatly reduce or eliminate transmission to susceptible individuals. Alternatively, the arrival of *Plasmodium* in the Galapagos may have added a mortality factor that our models suggest could push the Galapagos Penguin toward extinction. Effective conservation may therefore require reducing other sources of mortality so that the cumulative mortality is sustainable.

Despite the gaps in our knowledge about the effects of *Plasmodium* on Galapagos birds, our work highlights the flexible and modular nature of population-modeling research. A PVA will be useful only if it contains reliable information on all of the relevant threats to a population's persistence; this will be possible only for the most well-studied systems, if at all. However, models can be updated as new threats are discovered, reparameterized as new data are gathered, and rerun when novel analyses are devised. There are benefits to modeling, despite its limitations and biases. In order to maximize these benefits, we recommend that modelers design their PVAs to facilitate future modification. How the model is programmed and implemented will have an effect on this, and extensive documentation of what the model is doing, and why, is essential. This allows other researchers to replicate or expand upon models already developed.

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Supplementary Online Material for

MODELING *PLASMODIUM* PARASITE ARRIVAL IN THE GALAPAGOS PENGUIN (*SPHENISCUS MENDICULUS*)

ROBERT J. MEILE,[1,6] ROBERT C. LACY,[2] F. HERNÁN VARGAS,[3,4] AND PATRICIA G. PARKER[1,4,5]

[1]*Department of Biology, University of Missouri–St. Louis, St. Louis, Missouri 63121, USA;*
[2]*Chicago Zoological Society, Brookfield, Illinois 60513, USA;*
[3]*The Peregrine Fund, 5668 West Flying Hawk Lane, Boise, Idaho 83709, USA;*
[4]*Charles Darwin Foundation, Puerto Ayora, Santa Cruz, Galápagos, Ecuador; and*
[5]*WildCare Institute, Saint Louis Zoo, St. Louis, Missouri 63110, USA*

Appendix A: Duration and Severity of Weak and Strong El Niño Events

In VORTEX, catastrophes such as El Niño events are handled as rare, 1-year events that alter the survival probability and/or reproduction of individuals in the population. Although Vargas et al. (2007) used constants for these values, our model treats them as functions to achieve two ends: to prevent overlapping of El Niño events (equation 15 in Appendix B) and to alter the duration and severity of strong El Niños to more closely match observations (equations 3, 4, 6, and 12–14 in Appendix B). VORTEX treats each catastrophe as an independent event, and so, using the default settings, a weak and a strong El Niño event could occur simultaneously in the model with a probability of 0.01 (0.05 probability of strong El Niño * 0.2 probability of weak El Niño) per year. Having both types of events occurring together could affect the frequency of relapse events in the different model scenarios.

Two strong El Niño events have been observed over the past 47 years, in 1982–1983 and 1997–1998, which lasted for 18 and 17 months, respectively (Vargas 2006). The intensity of these events followed a bell-shaped curve—each started off as a weak El Niño, intensified into a strong El Niño for ~1 year, then tapered off into another weak El Niño. This contrasts with the representation of strong El Niño events in Vargas et al.'s (2007) model, as 1-year events with a single level of severity. Our model treats strong El Niños as 2-year events (equations 6 and 12). Each time a strong El Niño event occurs, one year is randomly chosen (using equations 3 and 4) as the year with higher severity, whereas the other year has weaker El Niño activity (equations 13 and 14). The stronger year has the same effect on the population as the strong El Niño event used in Vargas et al. (2007)—reproduction is reduced to 1% of its normal rate, and survival is reduced to 30% of its normal rate. The weaker year has no effect on survival and reduces reproduction to 90% its normal rate. A full weak El Niño event, unassociated with a strong El Niño, reduces reproduction in the model to 80%; the weak activity before and after a strong El Niño will likely overlap with only part of the breeding season, and so the effect in our model has been halved.

Appendix B: Equations Used in VORTEX to Define the Model

These equations are presented in the same form that they were input into VORTEX. In version 9.99b, there are three types of user-created variables: global state (GS) variables, population state (PS) variables, and individual state (IS) parameters. Each of these types of variables requires different inputs or functions to determine their behavior. GS variables require a function that specifies its value for the first year of the simulation (the initial function), and how the value of the variable changes from one year to the next (the transition function). GS variables operate at the level of the metapopulation. PS variables use only a transition function, and they operate independently for each subpopulation. IS variables are assigned to every individual in the population; in addition to the initial and transition functions, IS variables also have a function that determines their value for new-born individuals. For the mortality rates and catastrophe frequency–severity, these equations are functions of the original variables of the model.

Global State Variables

(B.1) GS1: Probability of infection—Initial function is a proportion between 0 and 1.0. Transition: =GS1.

This variable represents the probability of an individual becoming infected in a single year. This variable is included for ease of data entry; the variable PS1 (equation 5) is what is actually used for transmission in the equations.

(B.2) GS2: Pathogenicity—Initial function is a whole number between 0 and 100. Transition: =GS2.

[6]E-mail: rjm8g8@umsl.edu
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In the model, an infected individual has an increased probability of dying in some years. This variable gives the amount of that increase; for example, a mature individual normally has a 5% probability of dying in a year, but an infected individual has a (5+GS2)% probability of dying. The total probability of an individual dying is capped at 99% (see the Mortality Rates section, equations 9–11).

GS variables 3–5 are used for bookkeeping but not as part of the model itself.

(B.3) GS6—Initial and Transition: =SRAND((R*100)+Y)

(B.4) GS7—Initial and Transition: =SRAND((R*100)+(Y–1))

A strong El Niño event in this model has two strengths, one for each of its years—a strong effect (the same as the strong El Niño event in the Vargas et al. 2007 model) and a weak effect (corresponding to the build-up and settle-down time surrounding a strong El Niño event; see Appendix A). These functions are used by the Catastrophe functions (equations 13 and 14) to determine the order in which these two effects occur. Note that GS7 returns the same result as GS6 from the previous year.

Population State Variables

(B.5) PS1: Probability of infection: =GS1

This variable represents the probability of an individual becoming infected in a single year. It is the same for each subpopulation and equals the value given in equation 1.

(B.6) PS2: =(CAT(1)<1)*(PS2<1)

This function is what causes a strong El Niño event to take 2 years instead of 1 (see equation 12). During the first year of the strong El Niño, PS2=1, then it reverts to PS2=0 after the second year.

PS variables 3–5 are used for bookkeeping, similar to the GS variables 3–5.

Individual State Parameters

(B.7) IS1: Chronic Infections—Initial: =(RAND<0.90); Birth: =0; Transition: =IS2

In this model, an individual is assumed to retain its infection for life. The initial, acute infection period causes increased mortality, whereas the chronic infection is considered to be under control (except when relapses are allowed during El Niño events; see equations 9–11 below). The acute stage lasts for only the first year of an individual's infection in this model. At the beginning of each run (at year 0), 90% of the population is chronically infected, in accordance with the *Plasmodium* exposure found by Palmer et al. (2013). Also see equation 8 below.

(B.8) IS2: Acute Infections — Initial: =((RAND<0.50)OR(IS1=0)); Birth: =(RAND<PS1); Transition: =IS2+((IS2<1)*(RAND<PS1))

At the beginning of each run of the simulation, 5% of the population is given to be acutely infected (arbitrarily set as the PCR-detected level of infection found in Levin et al. 2009). This, along with equation 7 above, gives 95% of the population as being exposed at the start of each run. Because 90% of the population has already been assigned to be chronically infected, half of the remaining 10% of the population is set as chronically infected. In every year following the first, PS1% (equation 5) of the population becomes infected. The name of this variable is a misnomer, though, because it stays non-zero after the acute period is over. The actual effect of infection on the model is handled by the Mortality functions.

An individual's infection status is determined by their values for IS1 and IS2 together (equations 7 and 8). If (IS1=0) and (IS2=0), then they are uninfected. If (IS1=0) and (IS2=1), they are acutely infected. If (IS1=1) and (IS2=1), then they are chronically infected.

Mortality Rates

Base mortality for each age class:

- 0–1 years old: 67%
- 1–2 years old: 25%
- 2–3 years old: 5%
- 3+ years old: 5%

Mortality functions for the no-relapse model:

(B.9) = 67+((MIN(GS2:32))*((IS1=0)*(IS2=1)))
= 25+((MIN(GS2:74))*((IS1=0)*(IS2=1)))
= 5+((MIN(GS2:94))*((IS1=0)*(IS2=1)))

When an individual is uninfected, it experiences an X% chance of mortality each year, according to its age class (with X = 67, 25, or 5). For an individual to experience increased mortality (GS2, capped at 99%), it must be infected (IS2=1; equation 8). Additionally, the infection must not yet have become chronic (IS1=0; equation 7). This means that, under this model, individuals experience increased mortality from disease only in the year in which they become infected, and not at any time afterward.

Mortality functions for the all El Niño relapse model:

(B.10)=67+((MIN(GS2:32))*(IS2=1)*((IS1=0)OR((CAT(1)=0)
OR(CAT(2)=0)))
=25+((MIN(GS2:74))*(IS2=1)*((IS1=0)OR((CAT(1)=0)
OR(CAT(2)=0)))
=5+((MIN(GS2:94))*(IS2=1)*((IS1=0)OR((CAT(1)=0)
OR(CAT(2)=0)))

As in equation 9, individuals will experience heightened mortality when (IS1=0; equation 7) and (IS2=1; equation 8); that is, they are acutely infected. However, in this model only (IS2=1) is strictly necessary; mortality for these individuals will also be increased during a strong El Niño year (CAT(1)=0) or a weak El Niño year (CAT(2)=0). This translates into chronically infected individuals experiencing increased mortality during all El Niño events.

Mortality functions for the strong El Niño relapse model:

(B.11)=67+((MIN(GS2:32))*(IS2=1)*((IS1=0)
OR(((PS2=1)*(GS6<0.5))
OR((CAT(1)=0)*(PS2=0)*(1–(GS7<0.5))))))
=25+((MIN(GS2:74))*(IS2=1)*((IS1=0)
OR(((PS2=1)*(GS6<0.5))
OR((CAT(1)=0)*(PS2=0)*(1–(GS7<0.5))))))
=5+((MIN(GS2:94))*(IS2=1)*((IS1=0)OR(((PS2=1)*(GS6<0.5))
OR((CAT(1)=0)*(PS2=0)*(1–(GS7<0.5))))))

This model includes relapses, but only during the stronger year of a strong El Niño event. Which of the 2 years is stronger is randomly chosen, determined in part by the variables GS6 (equation 3) and GS7 (equation 4). If (GS6<0.5), then the first year of the strong El Niño (as given by PS2=1, equation 6) will be the stronger year. If (GS6>0.5) (or equivalently, [1–(GS7<0.5)]), then the second year of the strong El Niño (given by [CAT(1)=0]*[PS2=0]) will be the stronger year. Again, this is used to determine when a relapse will occur for chronically infected individuals.

Catastrophe Functions

(B.12) Strong El Niño Frequency: =5+(100*(PS2!=0))

Strong El Niño events begin with a probability of 5% each year. At the beginning of the second year, PS2 (equation 6) equals 1, causing the strong El Niño event to continue for that second year, at which point PS2 returns to 0.

(B.13) Strong El Niño Reproduction Severity:=((PS2=1)*(((GS6<0.5)*0.01)+((1–(GS6<0.5))*0.9))) + ((PS2=0)*(((1–(GS7<0.5))*0.01)+((GS7<0.5)*0.9)))

During the first year of a strong El Niño (PS2=1, equation 6), the El Niño severity will be strong (if GS6<0.5, equation 3) or weak (if GS6>0.5). In the second year (PS2=0), the opposite effect will occur (because GS7, equation 4, returns the same number as last year's GS6). The value of 0.9, from the terms ((1–GS6<0.5)*0.9) and ((GS7<0.5)*0.9), represents the effect of the weaker year during a strong El Niño event (see Appendix A).

(B.14) Strong El Niño Survival Severity:=((PS2=1)*(((GS 6 <0.5)*0.3)+((1–(GS6<0.5))*1.0)))+((PS2=0)*(((1–(GS7<0.5))*0.3)+((GS7<0.5)*1.0)))

The effects of this function are similar to the above, but affecting survival instead of reproduction.

(B.15) Weak El Niño Frequency: = 20–(100*(CAT(1)=0))

Weak El Niños occur with 20% probability each year, except in years when a strong El Niño is already occurring.

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