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EXAMINING THE RISK OF INBREEDING DEPRESSION IN A NATURALLY RARE CETACEAN, THE VAQUITA (*PHOCOENA SINUS*)[1]

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ABSTRACT

Uncertainty about the magnitude of various risks facing endangered species can paralyze conservation action. The vaquita is a naturally rare porpoise that has declined to the low hundreds of individuals because of gillnet mortality over the past 57 years. No variability in mitochondrial DNA (mtDNA) was found in vaquitas (n = 43). Because reducing gillnet mortality will require strong conservation action, the question was raised whether vaquitas are doomed because of inbreeding depression and whether, therefore, mitigation efforts would be futile. We use simulations to investigate the "doom hypothesis" by first asking whether the current level of genetic variability results from the recent decline or from historical factors. If fixation was historical then deleterious alleles could have been selected out of vaquitas over thousands of years, reducing concerns about inbreeding depression. Simulations

[1] Ken Norris was for us not only important for his contribution to the recognition of the vaquita as a species but also as an inspired thinker. He was a role model for the hybrid vigor contributed to many fields by the creative thinker who will boldly cross from one area to the next pollinating our imaginations. With Ken in the field of marine mammal science there was never any danger of inbreeding depression. We therefore dedicate to Ken this manuscript, which we hope uses Ken's style of bringing all possible tools to bear on a problem by blending lessons from the genetics of captive animals, the population dynamics of wild marine mammals, and computer simulations of evolution to reject the hypothesis of certain doom of the species he brought to the attention of science.

showed that fixation most likely resulted from historical rather than recent loss. Of 1,000 simulations done at plausible abundances and mutation rates, 247 (84.3%) fixed before and 46 (15.7%) fixed during the recent decline. Fixation correlates with historical abundance, making it more likely that because vaquitas are fixed, they are also a naturally rare species. However, because studies on purging deleterious alleles have not shown purging to be universally beneficial we also examine the doom hypothesis using data on the response to inbreeding of a wide variety of captive animals. Responses are so variable that the doom hypothesis cannot be affirmed. We further explore whether more data from vaquitas would lead to conclusive results and found that the data required, such as the adult survival rate, will be impossible to obtain. We conclude that because the doom hypothesis cannot be affirmed this risk factor should not delay conservation actions.

Key words: genetic diversity, inbreeding depression, mitochondrial DNA, *Phocoena sinus,* rare species, risk assessment, risk of extinction, vaquita.

The vaquita (*Phocoena sinus*) is listed as one of only two critically endangered cetaceans (IUCN). Such a high-risk species requires strong conservation action, yet economic concerns demand that such action have some likelihood of success. The question has been raised whether vaquitas are doomed to extinction because of inbreeding depression and whether, therefore, any potential attempts at recovering the species would be predestined to fail. Unlike the case of any other cetacean examined so far, no variability was found in the hypervariable portion of the control region of mitochondrial DNA (mtDNA; Rosel and Rojas-Bracho, this issue). Further, other data suggest that vaquitas might be inbred: several carcasses displayed polydactyly (Ortega-Ortiz 1993), some had deformed vertebrae (Torre-Cosio 1995), and many adult females had odd calcifications in their ovaries (Hohn *et al.* 1996). However, inbreeding in itself does not necessarily lead to extinction, as evidenced by many apparently healthy wild populations that are known to be inbred (for examples see Rosel and Rojas-Bracho, this issue). In this paper, we address the question of whether vaquita population dynamics and demography lead us to conclude that vaquitas are doomed given their current abundance and level of genetic variability. We assume that the evidence supporting doom must be very strong if accepting the hypothesis results in no conservation actions, which in turn would result in extinction. To address the likelihood of the doom hypothesis we first review what is known both about inbreeding depression and the history of vaquitas. We then use this basic information to address the following questions: was the loss of variability in mtDNA historic or recent, do data on captive animals affirm the doom hypothesis, and is it feasible to obtain conclusive results supporting the doom hypothesis by gathering more data from vaquitas?

As populations become small two forces lead to reduced genetic variability and increased homozygosity. First, as the number of possible parents becomes small, over time the population becomes inbred and the chances of inheriting alleles identical by descent increases. Second, the random effects by which genes are passed on to the next generation lead to fluctuations in gene fre-

quencies, the loss of some genes and the fixation of others, which is called genetic drift. Thus, small populations have two genetic signatures that go together with inbreeding: an excess of homozygotes and a loss of alleles, both resulting in lower allelic diversity.

Inbreeding depression is a reduction in fitness caused by inbreeding, which in turn reduces population growth rate. Its mechanisms are incompletely understood. The most common explanation is the expression of double recessive deleterious alleles. Because mtDNA is maternally inherited and individuals have a single copy, it is not possible to inherit double recessive deleterious alleles that would reduce fitness, and for that reason, there cannot be inbreeding depression caused by a deleterious mitochondrial genome. However, both loss of mtDNA haplotypic diversity and inbreeding result from genetic drift. Thus, even though we cannot estimate homozygosity because research has not been done on the nuclear genome, we can examine the general level of genetic diversity using mtDNA data. We use diversity of mtDNA as indirect data on the general level of diversity, which correlates to inbreeding.

Individuals born with double recessive lethal alleles by definition do not survive to reproduce, so those alleles are purged from the population. Similarly, deleterious (but not lethal) alleles can be purged through time by selecting against less fit individuals. This purging hypothesis led to the conclusion that populations that have survived at low abundance for long periods of time, and thus have a historic loss of genetic diversity, are more resistant to the effects of further inbreeding (Nei *et al.* 1975, Lande 1988, Lande and Barrowclough 1987).

Various studies have examined the validity of the purging hypothesis. Ehiobu *et al.* (1989) bred two populations of *Drosophila* to the same level of inbreeding but at different rates. They showed that the slower rate of inbreeding led to lower inbreeding depression. Lacy and Ballou (1998) examined whether deleterious alleles were purged in experiments with three wild populations of mice brought into captivity. They did not find that inbred populations consistently showed the benefits of purging and gave other examples where purging has not been demonstrated. Instead, they found the effects of selection on inbreeding depression varied among populations and speculated that variability was caused by different histories of inbreeding and selection. Thus, understanding the history of how vaquitas lost mtDNA variability helps us evaluate the effect of future inbreeding. Will we expect to see strong inbreeding depression through the exposure of lethal recessive alleles or a more benign response because some genetic load has likely already been purged from the species?

Small populations become inbred through time through the process of genetic drift. If vaquitas have always been rare, then the lack of variability could have occurred over evolutionary time. And there is good reason to believe that this is the case for vaquitas. Despite many early accounts of other marine mammals in the Gulf of California, the vaquita was first described in 1958 (Norris and McFarland 1958). The vaquita is also endemic to the Gulf of California, and, at least now, is restricted to the extreme northern portion of

the Gulf (Brownell 1986, Gerrodette *et al.* 1995). Although the scarcity of reported sightings lends credence to the rarity of vaquitas in recent times, no data exist to evaluate historical abundance.

The most proximate occurrence of the closest living relative to the vaquita, Burmeister's porpoise (*Phocoena spinipinnis*), is in central Peruvian waters (Norris and McFarland 1958, Rosel *et al.* 1995). It has been suggested that vaquitas are a relict population from an ancestral species of both that crossed the equator from the Southern Hemisphere during a period of cooler water conditions sometime in the Pleistocene. The migrants were then later trapped as water temperatures rose (Norris and McFarland 1958, Barnes 1985). The current population could, therefore, result from either a colonizing event by a few founding individuals or from a range expansion followed by fragmentation.

It is also very likely that whatever the size of the original population, it has decreased since the 1940s because of continuing mortality in gillnets (Villa-Ramirez 1976, Barlow 1986). The current abundance estimate is 597 animals, with a 95% confidence interval from 177 to 1,073 (Jaramillo-Legorreta *et al.*, this issue). Further work is being done to estimate trends in abundance using these new data. However, a previous study estimated a decline of 17.7%/yr (95% CI = −43.2%, 19.3%, Barlow *et al.* 1997). D'Agrosa (1995) carried out the only specific study on incidental mortality of vaquitas and estimated between 39 and 84 vaquitas were killed each year in gillnets set by only one of three fishing villages within the vaquita range. Using this range of mortality estimates together with the current best estimate of abundance results in mortality rate estimates of 6.8%/yr and 14.5%/yr.

We already know that vaquitas lack variation in mtDNA. We do not know the original abundance of vaquitas and so we use simulations to recreate genetic drift for populations of different abundances. We then assess the effects of the decline over the past 57 yr by comparing the number of haplotypes and haplotypic diversity between a scenario where the population continues at the same abundance for 57 yr to a scenario where the population declines to 100 effective females. We compare how often fixation originates from historic to recent events.

Because the effects of purging remain unknown, we examine the doom hypothesis from the perspective of the best data available to quantify the effects of inbreeding depression: changes in survival rates from studies of captive animals. To accept the doom hypothesis, the effects of inbreeding would have to be sufficient to result in long-term negative population growth. Again, we emphasize that the evidence for the doom hypothesis must be very strong, because lack of conservation actions based on accepting the doom hypothesis will almost certainly lead to extinction. We then address the question: should difficult and costly conservation actions be delayed with the expectation that further research can determine whether the vaquita is already doomed because of inbreeding depression? Although we will never get direct data on survival rates of inbred *versus* non-inbred vaquitas, some may argue that we could gather sufficient demographic data to better assess the vulnerability of the vaquita to extinction as a result of inbreeding depression. We assess what data

would be required through a demographic sensitivity analysis using what few data are available for the vaquita (an analysis of 56 animals; Hohn *et al.* 1996) together with the survival rates of other marine mammals. We later discuss the feasibility of gathering sufficient data to confirm the doom hypothesis.

Methods

Interpreting Lack of mtDNA Variation

Haplotypic diversity (h) is measured as:

$$h = 1 - \sum_{i=1}^{j} p_i^2 \tag{1}$$

where j = the number of alleles and p = frequency of alleles. Note that this is the same formula as that for heterozygosity (Avise 1994). The expected loss of haplotypic diversity is:

$$h_t = h_0\left(1 - \frac{1}{N}\right)^t \tag{2}$$

where t = time measured in generations, and N is the number of breeding females. Note that this differs from the formula for loss of heterozygosity because each individual carries only one mtDNA haplotype instead of two nuclear alleles. Thus, the $2N$ in the standard formula for heterozygosity is replaced simply by N. Although this formula generally describes the decline in haplotypic diversity with time, it has some peculiarities that limit its use for our purposes. For example, note that fixation ($h = 0$) can occur only when $N = 1$, otherwise haplotypic diversity approaches zero as N becomes small and/or t becomes large. This simple model assumes that the effect of mutation is so negligible that it can be ignored. Because mutation rates are relatively high for the parts of the genome we are considering, this omission could lead to some incorrect conclusions when a population reaches an equilibrial state between drift and mutation and h remains essentially stable over long periods. If $h_t \approx h_0$ even when t is large then we could incorrectly infer that N must be very large. A greater problem for the questions addressed in this manuscript is that the formula yields only the expected value. At any given point in time, h is an average of a distribution of possible states of h because inheritance is random. As h approaches zero, this distribution will be highly non-Normal with most populations fixed (at $h = 0$) and a small proportion with some remaining diversity.

We are interested in whether the fixation observed in vaquitas is more likely a result of historical or recent loss. To answer this question, we cannot use the analytical formula, which gives only the expected loss. What we need to know is a conditional question: of the populations that are fixed, how many became fixed during the last 57 yr? The expected value (eq. 2) yields no insight here. Therefore, we simulate vaquita population dynamics.

Because little is known of age-specific birth and death rates of vaquitas (see

the demographic model below) we decided a simple birth and death model would suffice to capture the dynamics of the loss of haplotypes. Further, because we are concerned only about mtDNA, we model only females. A birth and death model does not have different probabilities for different ages. To find the appropriate birth and death rate for a genetic model, we iteratively found the birth and death rate that yielded a plausible generation time for vaquita. The average generation time for vaquitas was 10 yr (see demographic model below). To obtain a generation time of 10 yr required that the probability of birth equaled the probability of death = 0.07.

The model starts by randomly assigning each individual a haplotype using a frequency distribution for harbor porpoises (*Phocoena phocoena*) in Pacific waters.[2] This distribution consists of 57 different haplotypes ($h = 0.88$) of which a few are common and most are rare. Using a one-year time step, the birth-and-death model then randomly decides whether each individual will survive and/or reproduce. The birth rate is allowed to be density dependent so that a given maximum rate is achieved as abundance approaches zero (eq. 3).

$$b_N = b_{\max} - (b_{\max} - d)\frac{N_e}{K_e} \tag{3}$$

where b_N = the birth rate at an effective female size of N (N_e), b_{max} = maximum birth rate, d = death rate, and K_e = effective abundance at carrying capacity. Population growth rate (r) is $b - d$, so for a growth rate of 1%/yr $r = 0.01$) and a constant death rate 0.07, $b_{max} = 0.08$. The effective population size of an actual population is the number of individuals in a theoretically ideal population that would drift at the same rate as the actual population. This ideal population has a number of restrictive assumptions, including the ability of every individual to reproduce with an equal chance of breeding with every other individual and no population fluctuations through time. Such assumptions are never met by actual populations, which usually results in the effective population being much less than the actual abundance. Because mtDNA is maternally inherited, the effective population size can crudely be thought of as approximately the number of adult females (or about ⅓ or less of the total abundance) (Nunney 1991, 1993). Some data indicate that the ratio of effective population size to total estimated population size may be as low as 1/10 (Frankham 1995).

Conclusions about the origin of fixation will be affected by our estimate of the current number of effective females. The factors that would affect N_e relative to the estimated total abundance for mtDNA would be abundance, variance in reproductive contributions between females, and variance in abundance. The best estimate for current abundance exceeds 500 vaquitas. Approximately half of those individuals are females, and of those approximately half are breeding adults, for a rough calculation of 0.25*500 = 125 effective females. Variance in reproductive contributions is relatively even for cetaceans,

[2] Unpublished data from S. Chivers, Southwest Fisheries Science Center, P. O. Box 271, La Jolla, CA 92038-0271, May 1999.

which are constrained to a maximum of one birth/year. Variance in abundance is also likely to be low relative to that in many mammals. No die-offs have been reported for vaquitas and there are no known causes for large fluctuations in habitat quality in the Northern Gulf of California. Because we knew that these factors will cause some reduction in N_e we chose the current $N_e = 100$. If the current number of effective females is greater, then the ratio of historical to recent fixation would increase, because it would be even less likely that uncommon alleles would be lost. However, if the current number of effective females is less than 100 then it would be more likely that rare alleles were lost because the population was reduced to very small numbers, and, therefore, the probability of historical fixation would decrease relative to the probability of recent fixation. Franklin (1980) showed that immediate loss of diversity was of concern when N_e was less than 50. We feel it unlikely that the current effective number of females is much less than our chosen value of 100 and very unlikely that N_e is currently less than 50, which would substantially increase the probability of recent rather than historical fixation.

The first phase of each simulation allows random inheritance at constant abundance over a long enough period to reach a state near the mutation drift equilibrium (see description of mutation rates below). We assume that environmental conditions have remained more-or-less unchanged for the vaquita since the most recent cold period, which was approximately 10,000 yr ago (Pielou 1991). Although vaquitas no doubt existed in this area many thousands of years before this time, using a 10,000-yr time horizon is conservative in terms of maximizing haplotypic diversity and therefore provides some remaining diversity to be lost between 1940 and 1997. Assuming a longer time period simply allows more time for genetic drift to occur, resulting in a smaller number of extant haplotypes. As will be seen from the simulations, most loss of haplotypes occurs rapidly followed by long periods of little change. Thus, the choice of time scale will effect our results little as long as we are past the initial period of relaxation from our initial state of high allelic diversity. Thus, we examine the questions: "If vaquitas have only been rare for the last 10,000 yr, what level of haplotypic diversity should we expect just prior to the commencement of gillnetting and how does this diversity change as a result of the recent decline?"

The second phase simulates dynamics between 1940 and 1997 (57 yr). Because heterozygosity fluctuates with time, we compare the state of a population that remained stable to 1997 to one that declined to low effective population size ($N_e = 100$). We calculated the number of haplotypes and haplotypic diversity (eq. 1) before and after the decline (1940 and 1997).

Not surprisingly, we have insufficient data to estimate mutation rates for vaquitas. For this reason, to test the sensitivity of our conclusions to this unknown parameter, we examine a range of mutation probabilities (m): 0.00001, 0.0001, and 0.001. For each birth, if a randomly chosen number was $<m$, the newborn was given a new haplotype, *i.e.,* the mutation probabilities are per birth. Mutation rates are often given as rates per gene per generation or for mtDNA per site per generation. The hypervariable zone of

mtDNA D-loop that was sequenced for harbor porpoise was 402 base pairs and for a stable population ($r = 0$) there would be one birth/generation (of females to females), so our highest mutation rate (0.001) would translate to approximately (0.001/402)*(10-yr generation time) = 0.000025/site/generation/female. Thus, our highest mutation rate is about 5,000 times higher than the value (5×10^{-9}) estimated by Hoelzel *et al.* (1991) for the full cetacean D-loop (900 base pairs). Therefore, it is more likely that the actual mutation rate falls between $m = 0.00001$ and $m = 0.0001$, and we present results primarily for that range. Although the high mutation rate ($m = 0.001$) is not realistic for mtDNA it may better represent the dynamics of the most highly variable parts of the genome, which would include microsatellites and the genes responsible for immune system reactions to disease challenges. We will therefore discuss the results of the dynamics of the high mutation rate ($m = 0.001$) with respect to potential changes in the nuclear genome that would correlate with finding no diversity in the mtDNA genome.

The amount of variability in 1940 would also depend on how the vaquita arose as a population/species. We chose two possible scenarios to bracket population dynamics that would lead to higher and lower diversity. The first scenario (the "fragmentation hypothesis") assumes that the current population arose from an event that fragmented the current population from a larger distribution. Abundance, therefore, remained more or less constant through to 1940. Thus, the fragmentation hypothesis assumes that vaquitas have never experienced a bottleneck where the species numbered less than one hundred effective females. The second scenario (the "founder event hypothesis") assumes that the current population was founded by a small number of individuals and grew to its assumed maximum historical abundance (*i.e.,* that which existed in 1940). The length of time populations spent at very low abundance varied between different simulation scenarios that examined different sizes of the bottleneck, maximum population growth rates and effective carrying capacities (K_e). In addition to this short time period when the population was rare, we make two more assumptions that would result in a greater rather than lesser level of haplotypic diversity: the environment was sufficiently constant through time that the abundance remained relatively constant, and founding members were chosen randomly from the potential pool of haplotypes. Thus, if our results indicate, for example, that there is an 80% chance of fixation when the effective female population size (N_e) is 500, it is likely that the actual fixation probability is higher, because our assumptions are probably violated in a real population and the resulting bias would indicate a lessor rate of fixation.

The dynamics of random inheritance of mtDNA was simulated for both the fragmentation and founder-effect hypotheses for a range of plausible historical abundances. For the fragmentation scenario we consider effective population sizes between 500 and 8,000 (or total abundance between approximately 1,500 and 24,000 using $N_e = (⅓)N$ (Nunney 1991, 1993) or between 5,000 and 80,000 using $N_e = (1/10)N$ (Frankham 1995). This covers the range of interesting genetic behavior, *i.e.,* the range where the probability of

a haplotype becoming fixed changes from nearly one to nearly zero. Thus, examining either smaller or larger populations will not contribute any further insight.

The fragmentation hypothesis assumes that abundance remained constant throughout the first phase (10,000 yr). Because the simulations are stochastic, abundance does fluctuate around K_e but never falls to very low levels. In contrast, the founder-effect hypothesis starts the population at a very low abundance. We examine a range of founding numbers between 10 and 360 for K_e = 500, K_e = 1,000, and K_e = 2,000. We examine only these carrying capacities for the founder hypothesis because it is sufficient to address our question of whether fixation was recent or historical. Populations grow from the initial founding number to K_e. Those simulations that result in the population going to zero are eliminated because, obviously, vaquitas did not go extinct. Because the loss of haplotypes depends on the amount of time populations are in low abundance, we also examine a low population growth rate (1%/yr) and a relatively high rate (4%/yr) for an odontocete (see review of growth rates in Rojas-Bracho and Taylor, this issue). We ran 100 replicates for each type of simulation (for example, the fragmentation hypothesis with N_e = 500 and m = 0.001), where a replicate is one simulation of 10,000 years plus the 57-yr decline period.

Assessing the Risk of Inbreeding

We first assess the risk of inbreeding by reviewing data on changes in survival rates in captive mammals. If some captive mammals display no decrease in survival when comparing inbred to non-inbred individuals, then we will conclude that the hypothesis of certain doom of the vaquita is rejected. We then create a demographic model to see whether further research is warranted to obtain a better case-specific estimate of the likelihood of the doom hypothesis for the vaquita. To see what data would be required to refine our understanding of the possible effects of inbreeding depression, we explore plausible ranges for birth rates, survival rates, oldest age, and age at first reproduction (*AFR*). Because we will never have inbreeding depression data directly from vaquitas, we use the reduction due to inbreeding in first-year survival of captive mammals. Although inbreeding depression has been demonstrated in other demographic rates, examination of juvenile survival will be sufficient to address whether further research into required demographic parameters is warranted. These demographic parameters are used in a Leslie matrix (Caswell 1989) to calculate the population growth rate (r). Assuming exponential growth (eq. 4), a population will decline to extinction if $r < 0$.

$$N_t = N_0 e^{rt} \tag{4}$$

where N = abundance, t = time in years, and r = population growth rate. We systematically examined all combinations of the demographic parameters. The exercise reveals which parameters have the greatest influence on popula-

tion growth rate and hence would be the most important to resolve to improve our ability to assess the risk to vaquitas posed by inbreeding depression.

An analysis of a sample of 56 vaquitas revealed that an interbirth interval of two years was most likely (Hohn *et al.* 1996). No animals in the sample were between the ages of 3–6, which complicated the estimate of age of first reproduction. It turned out that all the <3-yr-old animals were immature and all the >6-yr-old animals were mature. We therefore had to examine a range of possible ages of first reproduction (*AFR*) between 3 and 6 yr. The oldest vaquita examined was 21 yr. Because the vaquita population has probably been subject to high mortality in gillnets for several decades, it is unlikely that this represents the true oldest age. We therefore examined a range for oldest age between 20 and 30 yr.

No survival rate data are available for vaquitas. The only survival estimates for both juvenile and adult odontocetes are for killer whales (*Orcinus orca*: adult = 0.99, first year = 0.96, ratio first year to adult = 0.96; Brault and Caswell 1993) and bottlenose dolphins (*Tursiops truncatus*: adult = 0.96, first year = 0.80, ratio first year to adult = 0.83; Wells and Scott 1999). Both of these species are longer-lived and have longer interbirth intervals than the vaquita. We found it unlikely, given the lower life expectancy and shorter interbirth interval of vaquitas, that vaquitas would have higher survival rates than bottlenose dolphins. Thus, our highest survival rates are close to the bottlenose dolphin rates. Pinnipeds give birth annually, which correlates with a relatively high juvenile mortality rate. Using the pinniped life history strategy for a cetacean would therefore represent the lowest expected survival rates for vaquitas (northern fur seals, *Callorhinus ursinus*: adult = 0.90, juvenile = 0.67, ratio first year to adult = 0.75; Barlow and Boveng 1991). Given the oldest age and interbirth interval, the adult survival is likely to be closer to the bottlenose dolphin than the fur seal, and the ratio of first-year survival to adult survival is likely to be between these two. We therefore examine the following ranges: adult survival rates from 0.90 to 0.96, ratio of first-year to adult from 0.75 to 0.85.

We also calculated generation time (Caswell 1989) for the above combinations of demographic parameters. Mean values were around 10 yr with a standard deviation of about 1 yr. We used this mean value to calculate the average birth and death rate used in the simulations to investigate changes in mtDNA variation.

Ralls and Ballou (1983) summarized changes in first-year mortality for 16 species of ungulates and 16 species of primates in captivity (Table 1). They defined inbreeding as any probability that alleles could be identical by decsent according to the pedigrees of each species. Thus, individuals were either categorized as inbred or not inbred; no attempt was made to correlate survival with a given level of inbreeding. Most increases in juvenile mortality ranged between 50% and 150%, though cases of no effect and complete reproductive failure were also observed. A later paper (Ralls *et al.* 1988) treated more taxa and determined the relationship between the level of inbreeding and juvenile survival. It presented results for a level of inbreeding representing the average

Table 1. Mean first-year mortality rates for captive bred animals. First-year survival is (1 − first-year mortality).

Taxa	Mean non-inbred mortality	Mean inbred mortality	Percent increase
Primates	0.295	0.498	69%
Ungulates	0.216	0.543	151%
Pooled	0.256	0.521	103%

individual being related approximately at a level of sibling-sibling or parent-offspring matings ($F = 0.25$). The range of increases in juvenile mortality were about the same, so we examined increases of 50%, 100% and 150% in juvenile mortality.

Results

Interpreting Lack of mtDNA Variation

Both haplotypic diversity and the number of haplotypes were lost rapidly in the simulations (Fig. 1a, 1b) as the initial high number of haplotypes from the harbor porpoise relaxed to the lower number supportable by the smaller hypothesized vaquita abundances. The figures show the results for $N_e = 500$, with the results of a sample of ten of the stochastic simulations plus the average of all 100 simulations (in bold). Note that even though heterozygosity is generally decreasing through time, within-simulation heterozygosity often increases over short periods. Thus, using the analytical equation for any particular simulated population may yield nonsensical results. For example, the only way to get an increase in heterozygosity over time is if N is negative (eq. 2). The equation predicts how heterozygosity will change on average but does not pertain to changes in any particular population. Our interest lies in the known fixation of a particular population. For that reason, our analysis seeks to find the probability of fixation and assess the relative likelihood of fixation under different plausible scenarios. We also show results using the analytical equation (eq. 2, Fig. 1a, bold smooth line). The analytical equation consistently overestimates h relative to the simulations. Perhaps this is caused by the analytical equation assuming non-overlapping generations whereas the simulation used annual increments.

The probability of fixation without the decline (eq. 5) decreases rapidly with increasing abundance (Fig. 2, 1940 symbols).

$$p_{\text{fixed}} = p_{1940} + p_{1997_s} \tag{5}$$

where p_{fixed} = the total probability of fixation without the decline, p_{1940} = probability fixed in 1940, p_{1997_s} = probability fixed between 1940 and 1997 if the population remained stable. Results for the two plausible mutation rates were indistinguishable and were therefore averaged. For these rates, high probabilities of fixation occurred at or below $N_e = 2{,}000$. The probability of

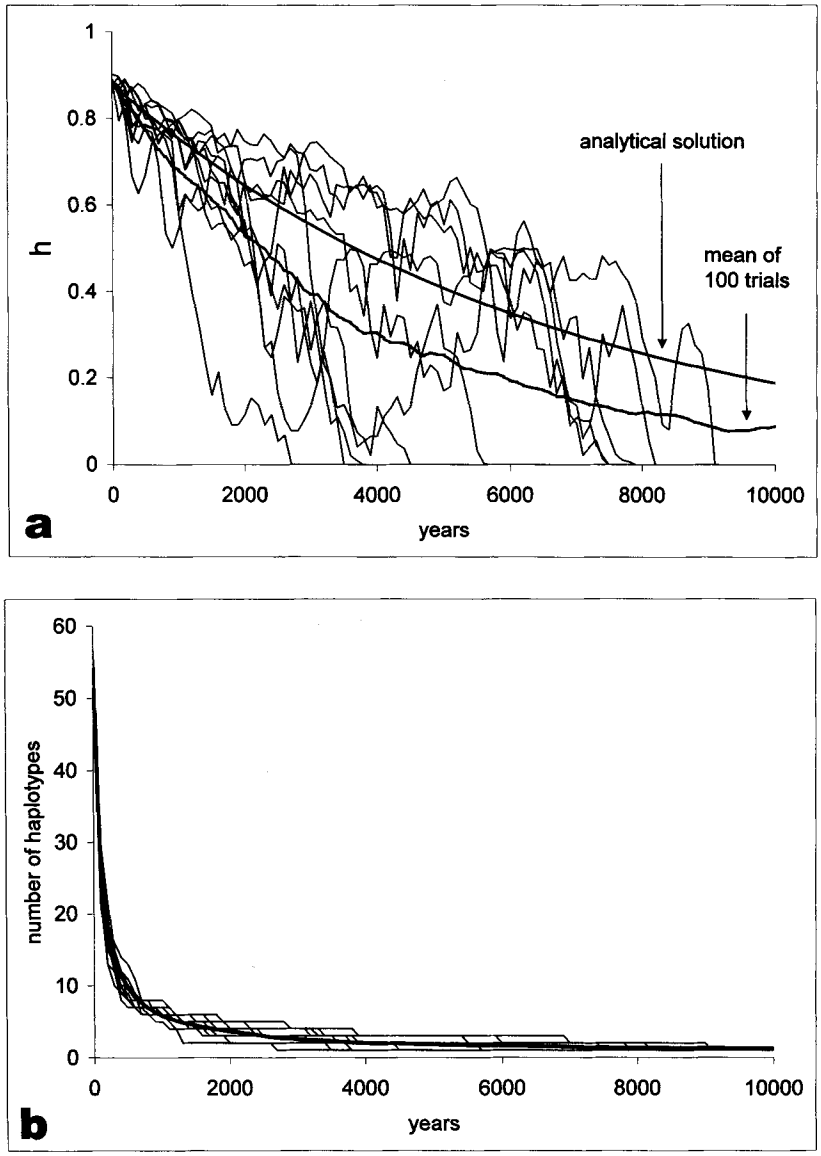

Figure 1. Changes in haplotypic diversity (h) and number of haplotypes through time in years (1a and 1b respectively) when N_e = 500. Thin lines show a sample of ten simulations. Bold lines in both figures show mean h for 100 simulations. Upper smooth curve in 1a shows the analytical solution to eq. 2. Mean haplotypic diversity (solid symbols) and loss in haplotypic diversity (diversity in 1940 − diversity in 1997, shaded symbols) for different mutation probabilities and for different N_e for r = 0.04.

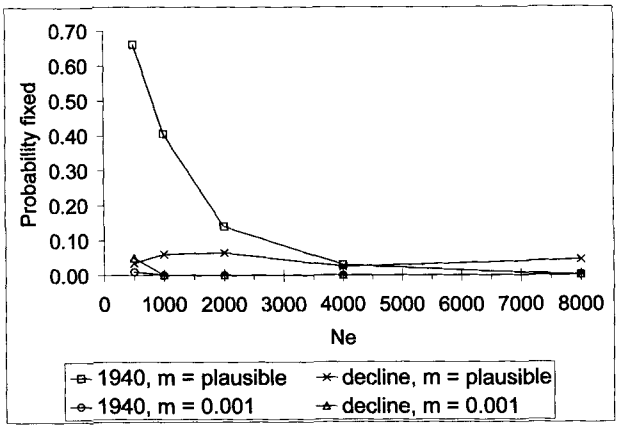

Figure 2. Probability of fixation for all stable simulations (symbolized as 1940) and for declining simulations for different effective female population sizes (N_e). "Plausible" refers to average of results for $m = 0.0001$ and $m = 0.00001$, because the results for these two different rates were indistinguishable.

fixation because of the decline for these mutation rates remains relatively low at about 0.05 for all abundances. When $m = 0.001$ (a very high mutation rate) fixation only rarely occurs and only when $N_e = 500$. In these few cases, most became fixed during the decline.

When N_e is very low (<1,000), populations either become fixed (and thus

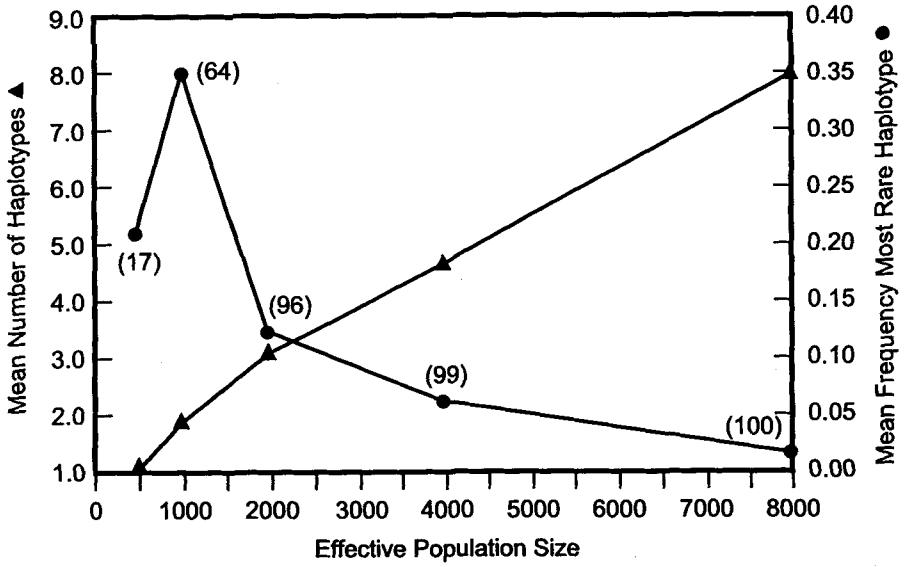

Figure 3. Mean number of haplotypes (triangle) and mean frequency of most rare haplotype (circle) for different N_e when $r = 0.04$. Rare haplotype statistic only computed when population not fixed, so sample sizes given in parentheses.

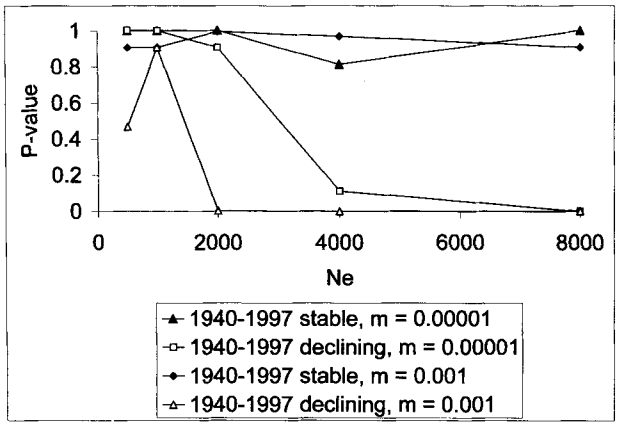

Figure 4. *P*-values for Kolmogorov Smirnov tests for goodness of fit for different N_e comparing distribution of haplotypic diversity (h) in 1940 to (1) population remaining stable to 1997 (filled symbols), and (2) population declining between 1940 and 1997 (open symbols). Only $m = 0.00001$ is shown for plausible mutation rates because it was indistinguishable from $m = 0.0001$.

have no diversity to lose) or have a few common haplotypes (Fig. 3). The mean number of haplotypes increases dramatically as abundance increases (Fig. 3, triangles). For those populations that did not become fixed for one haplotype, we show the mean frequency of the least frequent haplotype (Fig. 3, circles). Consider the example when $N_e = 500$. For the 100 simulation runs, 83 became fixed and had one haplotype while the remaining 17 had two haplotypes. These two haplotypes existed in high frequencies. The most rare haplotype had a mean frequency of 0.21. Thus, when the decline occurs between 1940 and 1997 it is very unlikely that small populations will lose alleles, because all alleles are relatively common. Contrast this to the case of $N_e = 8{,}000$. The

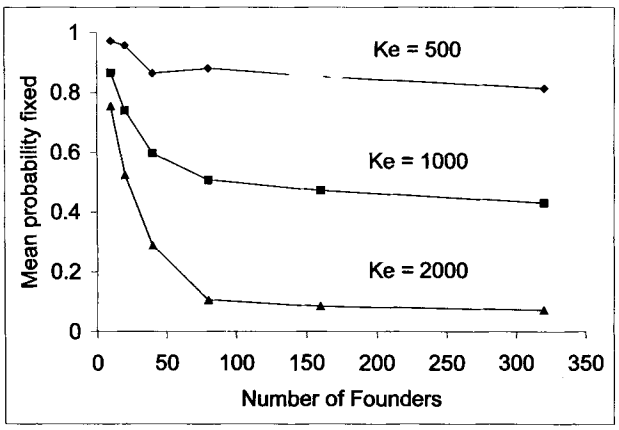

Figure 5. Probability of fixation for different numbers of founding females and different effective carrying capacities for $r = 0.01$.

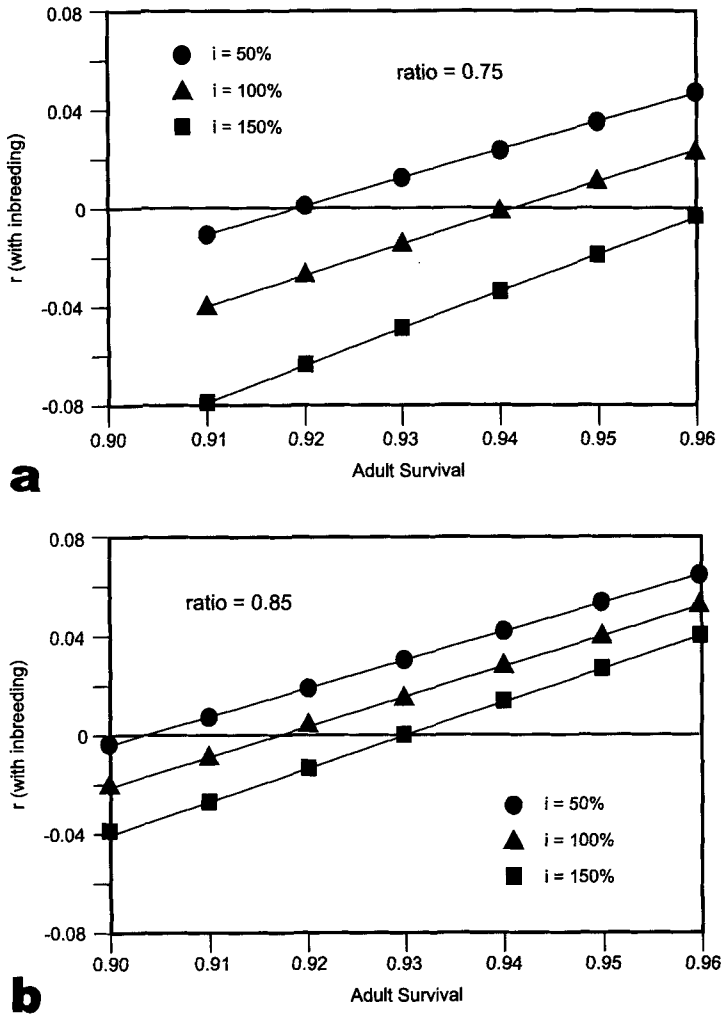

Figure 6. Growth rate assuming constant *AFR* (5) and oldest age (25) for extremes of different ratios of juvenile to adult survival: 6a–ratio = 0.75 and 6b–ratio = 0.85.

mean number of haplotypes was nearly 8 and consequently the mean frequency of the most rare haplotype was less than 0.02. Thus, abundant populations maintain rare alleles that can be easily lost in a rapid decline. However, this loss is unlikely to result in fixation, because it is unusual for abundant populations to have very low numbers of alleles. Thus, we would expect a loss of haplotypic diversity in abundant populations but fixation should be rare.

The distributions of haplotypic diversity are highly non-Normal with many populations fixed ($h = 0$) and a few with some remaining diversity. For this reason we compared the distributions using the Kolmogorov Smirnov test for goodness of fit with H_0 = the two distributions result from the same underlying distribution, and H_A = the two distributions result from different un-

derlying distributions. Because heterozygosity varies through time (Fig. 1A) we compared the 1940 distribution of h from the 100 simulations to a 1997 distribution where the simulated population remained stable, and then compared the 1940 distribution to the 1997 distribution resulting from a decline to $N_e = 100$. The stable populations maintain p-values of at least 0.8 regardless of abundance (Fig. 4, filled symbols). For all mutation rates, H_0 was rejected (at $\alpha = 0.05$) when N_e was at least 8,000. Note that the P-values for the declining scenario are generally less for the high mutation rate (Fig. 4, open triangles). High mutation rates lead to many more rare alleles that are likely to be lost during the decline, thus decreasing diversity by a greater magnitude. Note that H_0 cannot be rejected for the low mutation rates at low abundance (Fig. 4, open squares). This is because only common alleles are present and change in a relatively random fashion over the short time span of 57 yr. Of course, eventually even this small amount of remaining diversity would be lost at $N_e = 100$, but the important point here is that little has been lost so far because alleles are either fixed or occur in common frequency.

A population founded by less than 50 effective females increased the probability of fixation substantially (Fig. 5). The founder effect was very small with 80 founders and indistinguishable from the fragmentation results with 320 founders. These results show that a high probability of fixation can result either from a long-term low abundance ($N_e \leq 1{,}000$) or from an abundance twice that magnitude (2,000) if founded by a small number of individuals. Population growth rate also affected the probability of fixation. The probability of fixation was lower for the higher growth rate of $r = 0.04$ compared to $r = 0.01$ (shown in Fig. 5), but the conclusion that probabilities are elevated when the number of founders is less than 50 remains the same.

Assessing the Risk of Inbreeding

The documented effects of inbreeding on captive animals with known pedigrees is highly variable (Ralls and Ballou 1983, Ralls *et al.* 1988). Despite high and rapid inbreeding, some species show no apparent ill effects while others experience 100% juvenile mortality. This emphasizes the chance nature of both what genes remain in the population and what challenges are presented to the inbred populations. Thus, given these highly variable effects in well-documented cases, we clearly cannot conclude that the vaquita is doomed to extinction because of inbreeding depression.

Our demographic sensitivity analysis examined the feasibility of affirming the doom hypothesis through gathering more data to allow an evaluation specifically of the vaquita case. We excluded the extreme outlier cases of no effect and complete lack of juvenile survival because the results of those cases are obvious: no effect results in no effect on r, and complete lack of survival results in extinction. Adult survival rate has the strongest effect on r as seen looking across the range of plausible adult survival rates in Fig. 6a, b. The second most influential unknown is the ratio of juvenile survival rate to adult survival rate. Figure 6a, b show extremes of the range for this ratio for a

constant *AFR* (5) and oldest age (25). For example, for the highest effect of inbreeding (150% increase in juvenile mortality), the proportion of adult survival rates yielding negative population growth rate $r > 0$) goes from half with a ratio = 0.85 to all negative rates with a ratio = 0.75. We do not show results for different oldest ages because this parameter had a negligible effect on the outcome.

We show only values that are biologically plausible. It was not considered biologically plausible that a species would have a maximum population growth rate that was <1%/yr in the absence of inbreeding effects. Many of the combinations of low adult survival (0.9), high *AFR* (6) and a low ratio of first-year to adult survival (0.75) produced negative population growth rates and hence are not biologically plausible. No doubt some or all of these demographic parameters are correlated, such as a high adult survival rate with low birth rates. We did not attempt to guess at such correlations because we are not attempting to estimate the probability of various growth rates but rather to examine the sensitivity of the plausibility of a resulting negative growth rate to different unknown demographic parameters.

The age of first reproduction (*AFR*) also affected the response of population growth rate to inbreeding depression. This is expected because a lower *AFR* results in a higher growth rate, which in turn can better withstand a reduction in first-year survival. Figure 7a, b show the population growth rate (r) for ends of the range of *AFR* values for the different levels of inbreeding but for a single ratio of first-year to adult survival (0.80) and a single oldest age (25).

Discussion

Interpreting Lack of mtDNA Variation

Of 1,000 simulations done at plausible abundances and mutation rates, 293 fixations were observed, and of those 46 fixed during the recent decline. Our approach has been rather crude in that we have not assigned likelihoods to the plausible range of abundance or mutation rates. However, it is clear from Fig. 2 that fixation is far more likely to be the result of historical rather than recent origin, which is sufficient for our current exploration of whether the vaquita is doomed to extinction because of inbreeding depression.

We discuss below how having a strong likelihood of historical fixation may influence the risk of extinction for the vaquita. However, we feel perhaps the more important result of the modeling exercise is the support for historical rarity. To date there have been no data to support the natural rarity of vaquitas. Although the species was described only recently, the combination of the limited range and the fact that they can be confused with other small cetaceans found in that area has left open the question of the degree of rarity. We still cannot estimate how rare vaquitas have been because we know neither whether the species originated from a founder or fragmentation event nor how long they have been isolated. Further modeling could be done to examine the sensitivity of the range of plausible long-term abundances to different as-

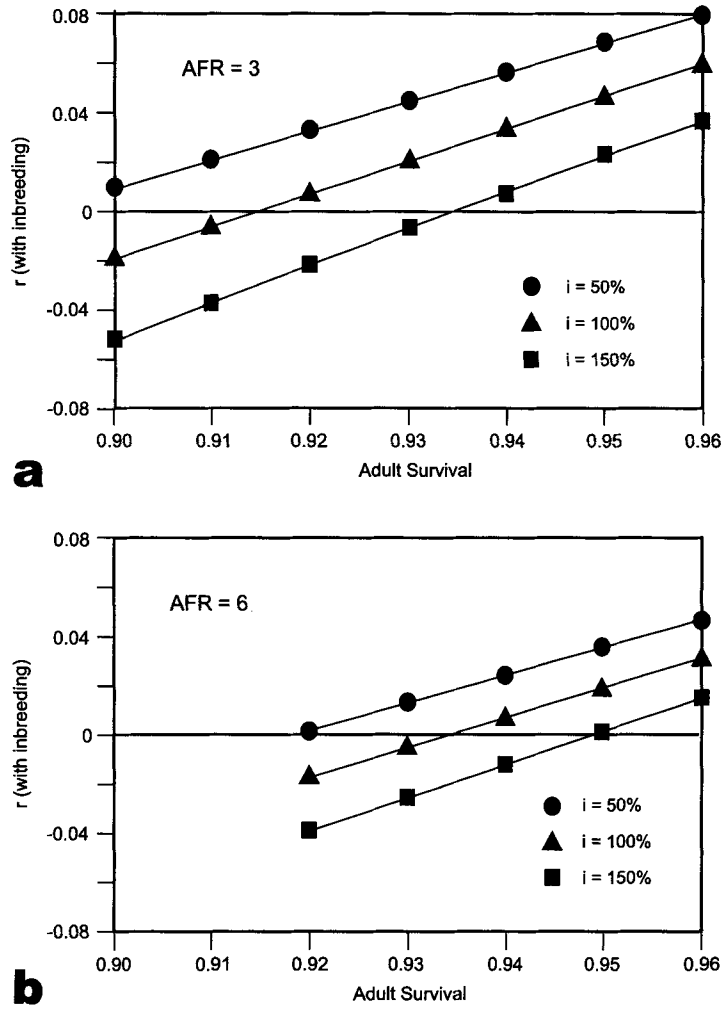

Figure 7. Growth rate assuming constant oldest age (25) and constant ratio of juvenile to adult survival (0.80) for different levels of mortality increase (i = 50%, 100%, 150%) and for extremes of different ages of first reproduction (AFR): 7a–AFR = 3 and 7b–AFR = 6.

sumptions about duration of isolation and mutation rate. However, at a minimum we can say that fixation in mtDNA is inconsistent with vaquitas having been, on average, abundant in the past. The reason the natural rarity is important beyond the genetic implications discussed below is that recovery to a level that is evidently viable for the vaquita will require less time if it is naturally rare than if it was naturally abundant. In other words, the magnitude of current depletion is lower if it is naturally rare, which engenders some much needed optimism in recovery efforts.

Genetic theory has found that the amount of heterozygosity lost from a

reduction in abundance depends both on how small the population becomes and how long the population remains at very low abundance. Kimura (1955) showed that at least initially, diversity is little impacted, while the average number of alleles is impacted more strongly. Our simulations began with the high diversity typical of a larger population of harbor porpoises. Most of that diversity was lost within the first 1,000 yr (Fig. 1A, B). For the simulations with $N_e < 2{,}000$ this diversity was eroded until all the rare haplotypes disappeared (Fig. 3). Thus, the recent decline did not strongly affect either diversity or the number of alleles for populations of low abundance. Rare haplotypes were not lost because usually they did not exist at the time the decline began. However, at higher abundances the balance between drift and mutation retained more haplotypes and thus haplotypic diversity was affected (Fig. 4). Note, however, that the probability of becoming fixed when $N_e > 2{,}000$ is low (Fig. 2).

It is also important to remember that although the hypervariable region of mtDNA is considered to be selectively neutral, the portions of the genome that determine population growth rate are not. Holsinger and Vitt (1997) noted that adaptation to future environmental changes is likely to involve alleles that are either currently (1) present in high frequency, (2) present in low frequency but maintained by natural selection, or (3) not present at all. Thus, the only alleles likely to not be important to future adaptation are rare, selectively neutral alleles. It is precisely these alleles that are most likely to be lost to genetic drift and indeed are the type of alleles lost in the high ($m = 0.001$) mutation rate simulations. This is also the type of variability that already has been lost in naturally small populations (like vaquitas, assuming plausible mutation rates). Lande (1988) and Menges (1990) noted that populations large enough to be ecologically self-sustaining are likely to remain genetically viable indefinitely. Prior to the advent of gillnet fishing in the Gulf of California, vaquitas could be considered such a population. It is important to remember, however, that alleles that are at low frequency but are maintained by selection can be lost once populations become sufficiently small that selection becomes ineffective in the face of rapid drift. The implications of loss of this type of allele (which are important in immune responses) are discussed below.

The exception to the above generalization relating early loss to unimportant alleles might be genes that code for immune responses. These genes are likely to have high mutation rates, and rare alleles could prove crucial to surviving a novel disease challenge. Loss of such potentially crucial genes certainly could make vaquitas more vulnerable. However, rather than spelling doom for the species, this observation emphasizes the need to reverse further declines as rapidly as possible. On the positive side, although rare alleles and haplotypic diversity will be lost most rapidly for genes with high mutation rates (Fig. 4), variability will also be regenerated more quickly because of that same high mutation rate.

Because data for vaquitas were lacking, our simulations required several assumptions; nevertheless, we feel our conclusions are robust. We showed that

results were the same for all plausible mutation rates. The time frame of 10,000 yr is also unimportant to the conclusion that fixation is more likely an historical rather than a recent loss. Most of the change in haplotype diversity from the starting point of 57 haplotypes occurred within the first 1,000 yr. Prolonging the time-span of the simulations would increase the probability of fixation but would not alter the fact that populations either rapidly become fixed for one haplotype or stabilize around a few common haplotypes. Thus, longer simulations would result in a broader range for N_e for which fixation was likely but would not change the probability that there was diversity in 1940 that was lost in the recent decline. Similarly, the evolutionary model chosen was unimportant; simulations based on fragmentation or founder models produced similar results. Thus, we feel our conclusion can be considered general for populations that are naturally rare, independent of assumptions about species-specific demographic parameters, many of which are difficult if not impossible to obtain.

Concern has been expressed over the studies of anatomical material that revealed that most vaquitas possess a sixth digit in their pectoral fin and some females show unusual mineral deposits in their ovaries (Ortega-Ortiz *et al.* 1993, Hohn *et al.* 1996). It is unknown whether these conditions were present in the ancestral form or whether they are uncommon traits fixed through drift or potentially selection. However, if these conditions do not decrease population growth, they do not constitute inbreeding depression even if they are the result of inbreeding. For example, in Killarney, Ireland, two female and one male sika deer (*Cervus nippon*) founded a population now numbering in the thousands. The males show bilateral asymmetry in antler size, an unusual trait probably caused by inbreeding (Hayden *et al.* 1994). The population, however, has grown at a high rate and is not, therefore, displaying inbreeding depression. Certainly, any current declines in vaquita abundance are very unlikely to be the result of inbreeding depression, because the inbreeding is the result of isolation for perhaps tens of thousands of years. Thus, although inbreeding depression may effect vaquita growth rate in the future, vaquitas are not currently doomed because of a lack of genetic variability.

However, both experimental and theoretical studies indicate that exposure of deleterious recessive alleles may not be the only effect of inbreeding. Several subspecies of wild mice with varying levels of inbreeding were used to test the purging hypothesis in a laboratory experiment (Brewer *et al.* 1990, Lacy and Ballou 1998). Surprisingly, some insular and inbred mice populations performed more poorly after further inbreeding on a number of fitness measures than did mice with lower initial inbreeding levels. Although this does not support the benefits of purging, purging may have occurred but not been detectible because of the expression of other factors that had an even higher influence on fitness components. Among the biological reasons given for the relatively poorer performance of the already inbred mice were reduced levels of heterozygosity and accumulation of slightly deleterious alleles.

It has long been argued that genetic variability (measured by heterozygosity) gives populations more genetic material with which to adapt to changing

biotic and abiotic environments (Williams 1966). In addition to giving species adaptive flexibility over evolutionary time, some genes may be highly advantageous but only in circumstances that occur periodically. For example, the advantages of small bill size in Galapágos finches are only evident in rare years when only small-seeded annual plants germinate (Gibbs and Grant 1987). Disease is another genetic challenge that maybe manifested only every few generations. Thus, genes that are only periodically useful may be lost during periods when they are not needed.

Other genes may confer only slight advantages or disadvantages. In both cases the effects of genetic drift in small populations may be greater than selection, resulting in the decrease of advantageous or increase of disadvantageous alleles and the fixation of some slightly deleterious alleles. Thus, even though the tested mice may have purged some highly deleterious alleles, they may also be more vulnerable because they have lost genes that may allow them to adapt to novel challenges, such as a captive situation.

Another potential effect of inbreeding is the loss of the potential benefits of overdominance (Charlesworth and Charlesworth 1987, Mitton 1993). This refers to heterozygotes having higher fitness than homozygotes for a given allele. Inbreeding reduces heterozygosity and therefore would lower any correlated fitness. Unfortunately, this reduction has proven hard to quantify. At this point it is unlikely that this potential effect could be quantified for vaquitas to assess its contribution to potential doom from inbreeding depression.

The simulations indicating that vaquitas were likely genetically depauperate before the onset of the recent decline in abundance increases our comfort level regarding the purging of recessive lethal alleles from the species. However, the results from the mouse studies emphasize that we cannot be complacent, because low genetic variability likely makes any population more vulnerable to changes in biotic and abiotic environments. The degree of vulnerability is impossible to predict, because we have no idea which genetic components were randomly fixed and which were lost. This variability in the genetic makeup of depauperate populations most likely explains the variable outcomes of inbreeding experiments both in wild and in captive populations (Ralls and Ballou 1983, Ralls *et al.* 1988, Lacy and Ballou 1998).

Assessing the Risk of Inbreeding

Dismissing the doom hypothesis given what we know about the response of captive mammals is straightforward; some captive animals display no detrimental effect due to inbreeding, therefore detrimental effects cannot be assumed for vaquita. Further, the demographic model shows that even if we assume a smaller range of reductions in juvenile survival rates, uncertainty about demographic parameters limits our abilities to assess the likelihood of the doom hypothesis.

Adult survival has the greatest influence on the effects of inbreeding, with low adult survival rates resulting in a higher likelihood of the doom hypothesis than high rates. What are the chances of estimating this parameter for va-

quitas? Zero. Adult survival rates have been estimated for only two small odontocetes: bottlenose dolphins (Wells and Scott 1999) and Hector's dolphins, *Cephalorhynchus hectori* (Slooten *et al.* 1992). These estimates were both made using photographic identification. Like harbor porpoises, vaquitas are difficult to photograph. The authors know of no photograph of a living vaquita suitable for photographic identification. No survival estimates have been made for similarly difficult-to-photograph but far more abundant harbor porpoises. Estimates of juvenile survival are even more difficult, because juveniles usually do not have scars or fixed adult coloration patterns. Estimating juvenile survival is out of the question for the vaquita.

Obtaining more samples of dead vaquitas in order to better estimate *AFR* will be difficult, because killing vaquitas is illegal and fishermen are aware that the vaquita problem is a serious threat to their livelihood. Also, data from previous gillnet-caught vaquitas show that this age group is not likely to be represented, for unknown reasons (Hohn *et al.* 1996). Further, great efforts to obtain such data for the purposes of better assessing the risk posed by inbreeding depression seem unfounded given the more serious problems of not being able to get estimates of adult survival rates.

Data for the effect of inbreeding depression are fewer for other demographic parameters than they are for juvenile survival. Because we could demonstrate that gathering the additional data required to better assess the doom hypothesis for vaquitas is impossible, we feel it unnecessary to examine these additional inbreeding depression effects (*e.g.,* effects on adult survival, fecundity, *etc.*).

Conclusions

For populations on the brink of extinction, Soulé (1987) noted that "there are no hopeless cases, only people without hope and expensive cases." Our simple exercises do not provide any strong evidence that vaquitas are doomed to extinction from inbreeding. In contrast, our results support the notion that the first priority of managers should be to reverse deterministic threats to persistence and increase the size of populations as rapidly as possible (Caughley 1994, Schemske *et al.* 1994, Holsinger and Vitt 1997).

There is no reason to believe that vaquitas are currently genetically compromised. The recent decline has been so rapid that most of the variability vaquitas had in 1940 should remain today. This does not mean, however, that the current abundance is viable in the long term. Franklin estimated that an N_e of 500 would be sufficient to maintain a species' ability to evolve and adapt. Lande (1995) suggested that this number should be increased to 5,000. Short term erosion of genetic variability is expected when $N_e < 50$ (Franklin 1980). Using an estimate of current abundance of 500, the effective population size could range between 50 and 167 (N_e/N = 1/10; Frankham 1995; N_e/N = ⅓; Nunney 1991, 1993). Thus, it is very important that vaquitas not be allowed to decline further and that the population be allowed to recover as quickly as possible.

There is reason to believe that the vaquita could fare better than most species experiencing the genetic problems encountered by small populations. The most parsimonious interpretation of the current lack of variability in mtDNA, according to our simulations of plausible population dynamics, is that the vaquita is a naturally rare species. Experiments with *Drosophila* indicate that roughly half of the inbreeding depression in viability results from the expression of rare recessive lethal and sublethal alleles (Simmons and Crow 1977). Given that vaquitas have successfully remained viable as a small population for many generations, there is good reason to believe that at least that part of inbreeding depression easily acted on by selection has indeed been purged. We would therefore expect increases in mortality due to inbreeding to be less dramatic in vaquitas than those observed in captive populations, where the breeding pool has been recently taken from wild populations and subjected to novel captive challenges.

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Literature Cited

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York, NY.

Barlow, J. 1986. Factors affecting the recovery of *Phocoena sinus,* the vaquita or Gulf of California harbor porpoise. US NMFS SWFC Administrative Report No. LJ-86-37. 39 pp. Available from Southwest Fisheries Science Center, P. O. Box 271, La Jolla, CA 92038.

Barlow, J., and P. Boveng. 1991. Modeling age specific mortality for marine mammal populations. Marine Mammal Science 7:50–65.

Barnes, L. G. 1985. Evolution, taxonomy and antitropical distributions of the porpoises (Phocoenidae, Mammalia). Marine Mammal Science 1:149–165.

Brault, S., and H. Caswell. 1993. Pod-specific demography of killer whales (*Orcinus orca*). Ecology 74:1444–1454.

Brewer, B. A., R. C. Lacy, M. L. Forster and G. Alaks. 1990. Inbreeding depression in insular and central populations of *Peromyscus* mice. Journal of Heredity 18: 257–266.

Brownell, R. L., Jr. 1986. Distribution of the vaquita, *Phocoena sinus,* in Mexican waters. Marine Mammal Science 2:299–305.

Caswell, H. 1989. Matrix population models: Construction, analysis, and interpretation. Sinauer Associates, Sunderland, MA.

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology 63:215–244.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237–268.

D'Agrosa, C. E. 1995. Mortalidad incidental de la vaquita (*Phocoena sinus*) en actividades pesqueras del alto Golfo de California, México, 1993–94. Tesis de Maestría

en Ciencias, Instituto Tecnológico y de Estudios Superiores de Monterrey, Guaymas, Sonora, México. 112 pp.

EHIOBU, N. G., M. E. GODDARD AND J. F. TAYLOR. 1989. Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster.* Theoretical Applications of Genetics 77:123–127.

FRANKHAM, R. 1995. Effective population size/adult population size ratios in wildlife: A review. Genetical Research 66:95–107.

FRANKLIN, I. R. 1980. Evolutionary change in small populations. Pages 135–150 *in* M. E. Soulé and B. A. Wilcox, eds. Conservation biology: An evolutionary-ecological perspective. Sinauer Associates, Sunderland, MA.

GERRODETTE, T., L. A. FLEISCHER, H. PÉREZ-CORTÉS, AND B. VILLA RAMÍREZ. 1995. Distribution of the vaquita, *Phocoena sinus,* based on sightings from systematic surveys. Report of the International Whaling Commission (Special Issue 16):273–282.

GIBBS, H. L., AND P. R. GRANT. 1987. Oscillating selection on Darwin's finches. Nature 327:511–513.

HAYDEN, T. J., J. M. LYNCH AND G. O'CORRY-CROWE. 1994. Antler growth and morphology in a feral sika deer (*Cervus nippon*) population in Killarney, Ireland. Journal of Zoology, London 232:21–35.

HOELZEL, A. R., J. M. HANCOCK AND G. A. DOVER. 1991. Evolution of the cetacean mitochondrial D-loop region. Molecular Biological Evolution 8:475–493.

HOHN, A. A., A. J. READ, S. FERNANDEZ, O. VIDAL AND L. T. FINDLEY. 1996. Life history of the vaquita. Journal of Zoology, London 239:235–251.

HOLSINGER, K. E., AND P. VITT. 1997. The future of conservation biology: What's a geneticist to do? Pages 202–216 *in* S. T. A. Pickett, R. S. Ostfeld, M. Shachak and G. E. Likens, eds. The ecological basis of conservation. Chapman and Hall, New York, NY.

JARAMILL-LEGORRETA, A. M., L. ROHAS-BRACHO AND T. GERRODETTE. 1999. A new abundance estimate for vaquitas: First step for recovery. Marine Mammal Science 15:957–973.

KIMURA, M. 1955. Solution of a process of random genetic drift with a continuous model. Proceedings of the National Academy of Sciences 41:144–150.

LACY, R. C., AND J. D. BALLOU. 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. Evolution 52:900–909.

LANDE, R. 1988. Genetics and demography in biological conservation. Science 241: 1455–1460.

LANDE, R. 1995. Mutation and conservation. Conservation Biology 9:782–791.

LANDE, R., AND G. F. BARROWCLOUGH. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–123 *in* M. E. Soulé, ed. Viable populations for conservation. Cambridge University Press, Cambridge.

MENGES, E. S. 1990. Population viability analysis for an endangered plant. Conservation Biology 4:52–62.

MITTON, J. B. 1993. Theory and data pertinent to the relationship between heterozygosity and fitness. Pages 17–41 *in* N. W. Thornhill, ed. The natural history of inbreeding and outbreeding. The University of Chicago Press, Chicago, IL.

NEI, M., T. MARUYAMA AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

NORRIS, K. S., AND W. N. MCFARLAND. 1958. A new harbor porpoise of the genus *Phocoena* from the Gulf of California and Mexican waters. Journal of Mammalogy 39:22–39.

NUNNEY, L. 1991. The influence of age structure and fecundity on effective population size. Proceedings of the Royal Society, London. Series B. Biological Science 246: 71–76.

NUNNEY, L. 1993. The influence of mating system and overlapping generations on effective population size. Evolution 47:1329–1341.

ORTEGA-ORTIZ, J. G. 1993. Descripción osteológica de la aleta pectoral de vaquita, *Phocoena sinus* (Cetacea: Phocoenidae). Tesis de Maestría, Universidad Nacional Autónoma de México, Iztacala. 46 pp.

PIELOU, E. C. 1991. After the Ice Age: The return of life to glaciated North America. Chicago University Press, Chicago and London.

RALLS, K., AND J. BALLOU. 1983. Extinctions: Lessons from Zoos. Pages 164–183 *in* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde and L. Thomas, eds. Genetics and conservation: A reference for managing wild animal and plant populations. The Benjamin/Cummings Publishing Co., Menlo Park, CA.

RALLS, K., J. BALLOU AND A. TEMPLETON. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2:185–193.

ROJAS-BRACHO, L., AND B. L. TAYLOR. 1999. Risk factors affecting the vaquita (*Phocoena sinus*). Marine Mammal Science 15:974–989.

ROSEL, P. E. AND L. ROJAS-BRACHO. 1999. Mitochondrial DNA variation in the critically endangered vaquita, *Phocoena sinus* (Cetacea: Phocoenidae). Marine Mammal Science 15:990–1003.

ROSEL, P. E., M. G. HAYGOOD AND W. F. PERRIN. 1995. Phylogenetic relationships among the true porpoises (Cetacea: Phocoenidae). Molecular Phylogenetics and Evolution 4:463–474.

SCHEMSKE, D. W., B. C. HUSBAND, M. H. RUCKLESHAUS, C. GOODWILLIE, I. M. PARKER AND J. G. BISHOP. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584–606.

SIMMONS, M. J., AND J. F. CROW. 1977. Mutations affecting fitness in *Drosophila* populations. Annual Reviews of Genetics 11:49–78.

SLOOTEN, E., S. M. DAWSON AND F. LAD. 1992. Survival rates of photographically identified Hector's dolphins from 1984 to 1988. Marine Mammal Science 8:327–343.

SOULÉ, M. E. 1987. Where do we go from here? Pages 175–183 *in* M. E. Soulé, ed. Viable populations for conservation. Cambridge University Press, Cambridge.

TORRE COSIO, J. 1995. Descripción del esqueleto, dimorfismo sexual y crecimiento alométrico en el craneo de la vaquita, *Phocoena sinus* (Cetacea: Phocoenidae). Tesis de Maestría, Instituto Tecnologico y de Estudios Superiores de Monterrey–Campus Guaymas. 183 pp.

VILLA-RAMÍREZ, B. 1976. Report on the status of *Phocoena sinus,* Norris and McFarland, 1958, in the Gulf of California. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología 47:203–208.

WELLS, R. S., AND M. D. SCOTT. 1999. Bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821). Pages 137–182 *in* S. H. Ridgway and R. J. Harrison, eds. Handbook of marine mammals. Volume 6. The second book of dolphins and porpoises. Academic Press, San Diego, CA.

WILLIAMS, G. C. 1966. Adaptation and natural selection. Princeton University Press, Princeton, NJ.

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