Exhibit 2

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF ARIZONA

Center for Biological Diversity, et al., Plaintiffs, vs. Ryan Zinke, Secretary of the Interior, et al., Defendants.	)))))))))))	No. 4:15-cv-00019-TUC-JGZ (consolidated with Nos. 4:15-cv-00179-TUC-JGZ and 4:15-cv-00285-TUC-JGZ) DECLARATION OF CARLOS CARROLL, Ph.D.

I, Carlos Carroll, Ph.D., declare as follows:

1. I am a research ecologist with the Klamath Center for Conservation Research, and Conservation Science Advisor to the Wilburforce Foundation. My qualifications to provide information relevant to recovery of the Mexican wolf (*Canis lupus baileyi*) stems from more than two decades of work as a research scientist focused on population viability and habitat analysis for wolves and other large carnivores. I have authored over 40 research papers which have altogether received approximately 3,000 citations in the scientific literature. I served as a member of the Science and Planning Subgroup of the Mexican Wolf Recovery Team convened in 2011, and as a technical advisor to the previous Mexican Wolf Recovery Team in 2005. In the course of this research, I have authored peer-reviewed papers on the science underpinning the recovery of the Mexican wolf (e.g., Carroll et al. 2014, 2015). Further detail and documentation of my credentials is provided in my curriculum vitae, a copy of which is attached to this declaration as Exhibit 1.

2. In this declaration, I cite and discuss a number of scientific publications, referencing them by author and date as is customary in scientific literature. A bibliography of these cited references is attached to this declaration as Exhibit 2. This declaration also references tables and figures presenting my recent modeling results concerning the Mexican wolf population, which are collected and attached as Exhibit 3.

3. I submit this declaration to address two principal points concerning conservation of the Mexican wolf. Firstly, wild Mexican wolves that are not supplementally fed show statistically significant effects of inbreeding depression on litter size, and this effect was not accurately estimated in analyses associated with the 2017 Mexican Wolf Recovery Plan (USFWS 2017a).

4. Inbreeding depression, the reduced biological fitness that occurs in a population as a result of breeding of related individuals, has been identified as a major threat to persistence and recovery of the Mexican wolf (USFWS 2017a). Inbreeding continues to increase in both the wild and captive population (Fitak et al. 2018). Genetic

threats resulting from inbreeding effects on survival and fecundity have been documented in many small populations that have been studied (Frankham et al. 2017). Carroll et al. (2014) found that the strength of inbreeding depression was the fourth most important factor affecting extinction risk among simulated populations of Mexican wolves.

5. Population viability analysis (PVA) is a tool for systematically synthesizing information on factors affecting the status of threatened species, and the influence of these factors on population viability and endangerment. PVA can be an important tool in informing development of recovery criteria, especially for well-studied species such as the Mexican wolf. Inbreeding can affect fecundity (reproductive output) either by increasing the odds of failure of a pair to produce any offspring or by reducing the litter size of those litters that are produced. Previous Mexican wolf PVAs (Carroll et al. 2014) modeled effects of inbreeding depression on litter size based on a previously published analysis of data from the reintroduced, wild Mexican wolf population in the Blue Range of Arizona and New Mexico (Fredrickson et al. 2007). The wild population was not subject to extensive supplemental feeding at that time. Extensive supplemental feeding of more than half of packs in the wild population occurred starting in approximately 2009 (USFWS 2017c). It is well known that inbreeding depression is environmentally dependent, with greater inbreeding depression evident in harsher environments (Armbruster & Reed 2005, Fox & Reed 2010, Yun & Agrawal 2014). Supplemental feeding would be expected to mask inbreeding effects and allow pups that would otherwise be compromised by inbreeding to survive. Despite this issue, which was raised by participants in recovery planning workshops held in 2016 as well as by peer reviewers of the U.S. Fish and Wildlife Service's 2017 Mexican Wolf Recovery Plan (USFWS 2017a), the PVA underlying the 2017 Mexican Wolf Recovery Plan (Miller 2017) analyzed the effect of inbreeding on litter size using data from both fed and unfed packs. The analysis found no significant effects of inbreeding on litter size, and as a result, the Miller (2017) PVA incorporates inbreeding effects on the probability of producing a litter, but not as an influence on litter size.

6. Working with Dr. Richard Fredrickson and other scientists, I re-analyzed the data on litter size used in the Miller (2017) PVA to determine if the assumption underlying the Miller (2017) PVA (that inbreeding influenced the probability of producing a litter, but did not influence litter size) was correct. I used a zero-inflated Poisson model, which, by allowing simultaneous estimation of effects on both litter probability and litter size, may provide more accurate estimates than would development of separate models for litter probability and litter size, the approach used by Clement and Cline (2016) and Oakleaf and Dwire (2016).

7. Our analysis identified statistically significant effects of inbreeding on litter size in unfed packs (which constitute 68 of a total of 116 pairings recorded). A model containing inbreeding effects (Table 1in Exhibit 3) was 425% more likely than was a model not containing such inbreeding effects. This model predicted that reproductive output (a function of both the probability that a pairing produces a litter, and the size of any resultant litter) for a 6-year-old female would be reduced by 0.72 pups per pairing as inbreeding increased from 0.15 to 0.25, which is within the range of inbreeding currently shown by wolves in the wild population. These predictions are similar to those from the model of Fredrickson et al. (2007), which predicts a reduction of 0.82 pups per pairing for an increase in inbreeding from 0.15 to 0.25. The relatively good agreement of these two models strengthens confidence in their estimates, given that the 2018 analysis included almost twice as many records (68 vs. 39 pairings) as did Fredrickson et al. (2007).

8. I then compared my resulting model predictions to those from the 2017 PVA (Miller 2017) used by the U.S. Fish and Wildlife Service in developing the 2017 Mexican Wolf Recovery Plan (USFWS 2017a). Miller (2017) based predictions of litter size on a combination of two models. The model of Oakleaf and Dwire (2016), which did incorporate inbreeding effects, was used to predict the proportion of pairs which would fail to have a litter. Then, the model of Clement and Cline (2016), which does not incorporate inbreeding effects, was used to predict litter size. Predictions from the

combined model thus do show inbreeding effects but they are reduced when compared to effects predicted by either Fredrickson et al. (2007) or my 2018 analysis. Miller (2017) estimated that the reproductive output for a 6-year-old female in an unfed pack would be reduced by 0.29 pups per pairing as inbreeding increased from 0.15 to 0.25; thus the strength of the effect in Miller (2017) is only 40% that shown in my 2018 model based on data from unfed packs.

9. It is evident from these results that the strength of inbreeding effects was underestimated in the Miller (2017) PVA, as would be expected if inbreeding effects were being masked by feeding. In addition, the strong contrast between litter size in fed and unfed packs observed in the Blue Range population (according to data reported in Miller (2017)) suggests that any future reduction in intensive management intervention via feeding, consistent with the Endangered Species Act's goals for recovery of self-sustaining populations, will reduce production of pups and potentially increase extinction risk for the population. The unusually high proportion of packs (~70%; USFWS 2017c) currently receiving supplemental feeding has resulted, and will in the near future continue to result in increased population growth rate in the wild population. However, feeding does not address, but rather only temporarily masks, the deleterious effects of inbreeding, which will continue to increase in the wild population during the years when feeding is employed, and become apparent via reduced litter size and other effects at the time that feeding is discontinued or reduced.

10. The fact that the Miller (2017) PVA underlying the 2017 Mexican Wolf Recovery Plan (USFWS 2017a) underestimated the effects of inbreeding depression is important for at least two reasons. Firstly, the Miller (2017) PVA, because it underestimates the effects of inbreeding on reproductive output, as a result underestimates the need for releases from the captive population which serve to reduce inbreeding and enhance viability of the wild population. Secondly, the PVA may underestimate genetic threats that arise due to small population size and consequently

suggest as adequate population thresholds which in reality are insufficient to address genetic threats over the long term.

11. The second principal point I wish to address is that, based on my PVA modeling, the rate of releases from the captive to the wild Mexican wolf population in the Mexican Wolf Experimental Population Area (MWEPA) proposed in the Recovery Implementation Strategy issued by the U.S. Fish and Wildlife Service in connection with the 2017 Mexican Wolf Recovery Plan (USFWS 2017a) appears inadequate to alleviate genetic threats, as measured by metrics such as gene diversity (GD), mean kinship (MK), and founder genome equivalents (FGE). Given the significance of genetic issues as threats to survival of the Mexican wolf, inadequate alleviation of those threats in turn significantly decreases likelihood of successful recovery of the species.

12. The PVA associated with the 2017 Mexican Wolf Recovery Plan (Miller (2017)) clearly demonstrates that in order for Mexican wolf populations to achieve recovery, a higher rate of releases from the captive to the wild population must occur than was envisioned in the environmental impact statement associated with revisions to the Mexican wolf 10(j) rule (USFWS 2014). Additionally, my subsequent analysis, which consisted of simulations of population viability based on the model used in Miller (2017), but with even higher rates of releases (Table 2 in Exhibit 3), demonstrates that a rate of releases beyond that proposed in the recovery and implementation plans is necessary to adequately alleviate genetic threats to the wild Mexican wolf population.

13. I performed additional simulations of population viability using PVA model input files and parameters identical to those used in the "379_200_200_249_EISx2_20_20" scenario of Miller (2017), which was the primary scenario on which the 2017 Mexican Wolf Recovery Plan's criteria were based. Specifically, to ensure comparability with Miller (2017), I did not incorporate the results of the new inbreeding model described above. The analysis differed from that of Miller (2017) only in that I added two new scenarios that simulated the effects of higher rates of releases to the wild population (Table 2 in Exhibit 3). Firstly, I simulated population

viability using a release rate based on that proposed in the implementation plan (action 2.1.2, release 12 cross-fostered pups per year to the wild population in the MWEPA; USFWS 2017b), continued for 17 years to match the duration of releases in Miller (2017). Secondly, I simulated population viability using a release rate based on that proposed in the implementation plan (release 12 cross-fostered pups per year to the wild population) plus that proposed in the EISx2 scenario of Miller (2017) (14 pairs of adults released with 3 pups each for a total of 71 wolves). In these scenarios, genetic diversity in the wild population increases during the first 2 decades due to releases from the captive population, and then declines steadily through the remaining decades of the simulation.

14. Modeling results suggest that, given the other parameter values incorporated in the "379_200_200_249_EISx2_20_20" scenario of Miller (2017), only the most ambitious schedule of releases (cross-fostering plus EISx2) is able to meet commonly proposed metrics for alleviation of genetic threats. For example, Dr. Phillip Hedrick (pers. comm.) has proposed that an appropriate goal would be to bring the gene diversity of the wild population in the MWEPA 50% of the way from its level assuming no releases, to the level shown in the captive population. For the wild MWEPA population, given a genetic diversity level assuming no releases of 0.665, and a level in the captive population at 100 years of 0.785, this 50% goal would be 0.725, a level that is nearly attained by the most ambitious release strategy that I modeled (Figure 1 and Table 2 in Exhibit 3).

15. It is important to note that goals for genetic metrics relevant to recovery are typically expressed in relation to a) the gene diversity present in the "founder population", i.e. those animals originally taken from the wild to form the captive population; or b) the gene diversity currently held in the captive population. For example, the goal for Florida panther recovery was to retain 90% of the current genetic diversity for 100 years or longer (Seal and Lacy 1989). These goals are **not** typically expressed in relation to the depleted gene diversity that the captive population may hold at some future time, as they were in the recent Mexican Wolf Recovery Plan (USFWS 2017a), because

such a “shifting baseline” represents an inadequate yardstick for alleviating genetic threats. For example, Fitak et al. (2018) documented that a metric of genetic health (observed heterozygosity) is declining steadily in the captive population at a rate of 0.6-0.7%/year. Dr. Hedrick’s proposed goal to retain a gene diversity level of 0.725 in the wild population (discussed above) represents a retention of 87% of the gene diversity currently present in the captive population, so is less ambitious than the commonly proposed goal of retaining 90% of the diversity currently present in the captive population. However, achieving the goal proposed by Dr. Hedrick would still require the most ambitious release rate evaluated here (cross-fostering plus EISx2).

16. One can conceive of recovery as a three-legged stool supported by 1) releases from the captive to the wild population, 2) rapid population growth of the wild population due to, among other factors, access to sufficient suitable habitat (which is important because genetic inbreeding is accentuated the longer a population remains small), and 3) long-term population numbers sufficient to prevent continued genetic decline. A high rate of initial releases, as one of these three factors, is not sufficient but is necessary to achieve recovery. In the absence of sufficient releases, the genetic diversity that forms the basis for recovery would not be present in the wild population. PVA simulations (Miller (2017)) show that effects of decisions as to the rate of releases from captivity, although immediately evident in terms of genetic metrics, may be difficult to discern in the first two decades of recovery in terms of extinction risk. However, this initial release rate strongly influences extinction risk several decades later, as populations which received few releases show increased inbreeding depression and enter an “extinction vortex”. Since the publication of the Mexican Wolf Recovery Plan, I have performed comprehensive sensitivity analyses of the PVA model used in Miller (2017), and found that the number of initial releases from the captive to the wild population is the second most important factor determining the ultimate genetic health of the wild population.

17. A high rate of releases in initial years also increases the resilience of the population to periods of unfavorable demographic rates (i.e. high rates of human-caused mortality) that may occur in the future, by ensuring higher reproductive potential due to the presence in the wild population of pairs with lower inbreeding levels. Thus, initial releases serve as a form of "insurance" for a successful recovery outcome, particularly for species such as the Mexican wolf for which the captive population is much more genetically diverse than is the wild population.

I declare under penalty of perjury that the foregoing is true and correct. Executed this 18th day of July, 2018, at Orleans, California.

Carlos Carroll, Ph.D.

Exhibit 1

Curriculum Vitae

Carlos Carroll

Personal Information

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Professional Qualifications

2000 Ph.D., Forest Science Oregon State University, Corvallis, Oregon

1997 M.S., Wildlife Science Oregon State University, Corvallis, Oregon

1994 B.A., Biology University of California Santa Cruz, Santa Cruz, California

Employment History

2004-present	Conservation Science Advisor, Wilburforce Foundation, Seattle, WA
1999-present	Director, Klamath Center for Conservation Research, Orleans, CA
1997-1999	Research Ecologist, Conservation Biology Institute, Corvallis, OR
1995-1997	Ecologist, USDA Forest Service, Pacific Southwest Experiment Station, Redwood Sciences Laboratory, Arcata, CA

Professional Contributions and Distinctions

Member-at-Large, Society for Conservation Biology (Global) Board of Governors, 2011-2014

President, Society for Conservation Biology North America, 2014-2017

Treasurer, Society for Conservation Biology North America, 2017-present

Publications

Refereed Articles

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Exhibit 2

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Exhibit 3

Table 1. Zero-inflated Poisson generalized linear mixed model of the relationship of litter size to age of dam and inbreeding of pup. Model selection metrics including AIC (227.8) and BIC (241.2) indicated this was the most-supported model of those evaluated. Data from unfed pairs in Blue Range population (n=68 pairings of 38 different pairs).

Estimate		Pr(>|z|)
Conditional model:		
Intercept	1.611	1.27e-08
Inbreeding (F) of pup	-2.660	0.0265
Young dam (<4 years)	-0.521	0.0273
Zero-inflation model:		
Intercept	-2.4148	0.001012
Old dam (>8 years)	3.6758	0.000726
Groups: Pairs (n=38)		
Variance	3.655e-10	
Standard deviation	1.912e-05	

Table 2. Results of Vortex simulations comparing effects of release strategies on genetic metrics for the MWEPA population, under the base scenario of Miller (2017) ("379_200_200_249_[releases]20_20"). Genetic metrics for the captive population (SSP) under the EISx2 strategy provided for comparison. Totals for the implementation plan strategy assume release of 12 pups/year for 17 years (years 2-18) rather than for 16 years as stated in the implementation plan, for comparability with Miller (2017).

Release strategy	Total number of adults released into MWEPA (year 2-18)	Total number of pups released into MWEPA (year 2-18)	Genetic diversity (GD) at year 100	Mean kinship (MK) at year 100	Founder genome equivalents (FGE) at year 100
No releases	0	0	.665	.342	1.46
EIS	14	21	.684	.320	1.56
EISx2	28	42	.705	.299	1.67
Imp. plan	0	204	.712	.287	1.74
EISx2 + Imp. plan	28	246	.724	.275	1.82
Captive population (SSP)	NA	NA	.785	.214	2.34

Figure 1. Gene diversity results of PVA simulations of either Miller (2017)(EISx0, EISx1, EISx2) or by C. Carroll using the PVA model of Miller (2017) in combination with higher rates of releases from the captive to wild population (Impl. Plan, EISx2 + Impl. Plan). Results are compared against a goal proposed by P. Hedrick to address genetic threats by increasing gene diversity in the wild population to a point halfway between the level in the wild population at 100 with no releases, and the level in the captive population at 100 years.