



U.S. Fish and Wildlife Service

April 18, 2023

Re: Proposed rule and draft environmental impact statement on establishment of a non-essential experimental population of the gray wolf in Colorado, 88 Fed. Reg. 10258 (Feb. 17, 2023).

Submitted via www.regulations.gov, docket no. FWS–R6–ES–2022–0100.

To Whom it May Concern,

Please consider the following comments by the Center for Biological Diversity and the Sierra Club on the proposed rule and draft environmental impact statement on establishment of a non-essential experimental population of the gray wolf in Colorado. Please also read and consider the attached studies that follow our comments, as an Appendix.

The Center for Biological Diversity is a national, non-profit conservation organization dedicated to protecting and recovering imperiled species and protecting their ecosystems. The Sierra Club works to protect wild places, promote responsible use of the earth's ecosystems and resources, and educate and enlist humanity to protect and restore the quality of the natural and human environments. Center for Biological Diversity and Sierra Club members and supporters in Colorado helped to pass Proposition 114, now Colorado Revised Statute 33-2-105.8, which requires the Colorado Parks and Wildlife Commission to approve a gray wolf restoration and management plan and for Colorado Parks and Wildlife to begin reintroducing wolves by December 31, 2023.

The Center and the Sierra Club were also plaintiffs in the most recent round of federal litigation – as well as other successful lawsuits on the gray wolf's listing status going back to 2003 – that in February 2022 returned the gray wolf to the endangered and threatened species lists throughout most of the contiguous states. We have abiding interests in Colorado wolf reintroduction and management fulfilling the noble goals of both Proposition 114 to “help restore a critical balance in nature”¹ and of the Endangered Species Act to “conserve the ecosystems on which threatened species and endangered species depend” and “to provide a program for the conservation of such species.”²

Notwithstanding our staunch and longstanding support for reintroduction of gray wolves in Colorado, we are troubled by critical flaws in the proposed rule and by cursory analysis in the draft environmental impact statement. The logical, legal and scientific infirmities in this rule-making and analysis are interrelated. First, the section on the “Purpose and Need For Action” in the draft EIS wrongly premises the proposed rule on a “need” to “provide management flexibility to the Service and its designated agents.”³ In fact, as we explain below, the foremost purpose

¹ C.R.S. 33-2-105.8.

² 16 U.S.C. § 1531(b).

³ U.S. Fish and Wildlife Service, Draft Environmental Impact Statement Colorado Gray Wolf 10(j) Rulemaking, 2023; p. i.

and need must be the statutory responsibility to conserve endangered species – the gray wolf and the Mexican gray wolf; moreover, the Fish and Wildlife Service is obliged in this rulemaking to conserve the ecosystems on which these wolf subspecies depend. Stemming from that fundamental misapprehension, the DEIS fails to provide a reasonable range of alternatives. And the DEIS altogether neglects to take a hard look at the likely results of either proposed action alternative nor the no-action alternative. This overall misdirection and these specific omissions facilitate the Service in its proposed course of action – removing all guardrails against excessive killings, and foreclosing establishment of a Mexican wolf population in southwestern Colorado where scientists have proven such a population will be necessary for recovery. Such a course will not conserve the gray wolf and will actively undermine the recovery of the Mexican gray wolf.⁴ If these documents were to be finalized in anything close to their present form, that would constitute violations of the Endangered Species Act, the National Environmental Policy Act, and the Administrative Procedure Act – as we explain below.

I. The True Purpose and Need for this Rulemaking Derives From the Endangered Species Act Mandates to Conserve Endangered Species and the Ecosystems On Which They Depend.

A decade and a half after Congress enacted the 1973 Endangered Species Act, it amended the law to authorize (among other changes) the Fish and Wildlife Service to designate experimental population areas with tailored management to facilitate reintroduction of threatened and endangered wildlife. President Ronald Reagan signed the amendment on October 7, 1988, creating subsection 10(j) and other changes in the Act.

Subsection 10(j) states: “The Secretary may authorize the release (and the related transportation) of any population (including eggs, propagules, or individuals) of an endangered species or a threatened species outside the current range of such species if the Secretary determines that such release will further the conservation of such species.”⁵ Moreover, implementing regulations for the Act require that, in invoking subsection 10(j) to designate and reintroduce an experimental population, “the Secretary must find by regulation that such release will further the conservation of the species.”⁶ Underlying that crucial mandate is the Act’s definition of conservation as “to use and the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures provided pursuant to this chapter are no longer necessary.”⁷

In other words, the 10(j) rule must contain measures to meet the statutory intent to recover wolves from their present state of endangerment. Moreover, the Act requires the Secretary of the Interior invoking subsection 10(j) to make a finding that the 10(j) rule is

⁴ The Fish and Wildlife Service clearly anticipates de-listing the Mexican wolf within the next decade on the basis of the manifestly unscientific delisting criteria in the 2022 revised Mexican Wolf Recovery Plan, which the Service’s own legal briefs argue need not adhere to any scientific standard; that will not withstand legal scrutiny. The Service should plan to add additional decades to its Mexican wolf to-do list, on top of the delays already built in by past and present mismanagement, if it finalizes the proposed rule without provision for introduction of Mexican wolves in the southwestern portion of the proposed new experimental population area.

⁵ 16 U.S.C. § 1539(j)(2)(a).

⁶ 50 C.F.R. § 1781.

⁷ 16 U.S.C. § 1532(3)

consistent with the Act’s purposes, which include conservation of the ecosystems on which endangered species depend.⁸ Because of that unequivocal direction from Congress, not only must the final 10(j) rule ensure the conservation of wolves in Colorado; it also must advance ecosystem conservation in Colorado – and those twin mandates must constitute the overarching purpose and need for this rulemaking. The DEIS emphasizes the wrong factors in asserting:

The purpose of this action is to respond to Colorado’s request to designate the gray wolf population that would be reintroduced to Colorado as experimental under section 10(j) and to further the conservation of the species. . . . The need for this action is to provide management flexibility to the Service and its designated agents . . . [to] reduce the regulatory impact.⁹

The DEIS’s prejudicial statement as to the purpose and need for the action has led to an insufficient range of alternatives and a blinkered analysis of wolf conservation and ecosystem conservation, which in turn facilitated development of a deeply flawed proposed rule. All these legal errors must be corrected in the final EIS, starting with the misdirectional purpose-and-need-for-action statement.

II. The Draft Environmental Impact Statement Does Not Include a Range of Alternatives Sufficient to Explore Different Management Possibilities and Their Differing Effects on Conservation, and Includes an Action Alternative That is Illegal on its Face.

Aside from the fundamental misapprehension of purpose and need described in the section above, the driving reason for the draft environmental impact statement’s broad failure to inform is that Alternative 1 (the preferred alternative) presents a single vision among several (unexplored) management possibilities that would each potentially advance conservation, and Alternative 2 would violate section 10(j) of the Endangered Species Act and for that reason is not a viable alternative.

In our August 22, 2022 letter to the Fish and Wildlife Service during the scoping period for this rule-making, the Center for Biological Diversity requested and explained the need for five provisions in the proposed rule, and requested a review of each provision: (1) A proscription on killing wolves to the extent that such killings would inhibit or slow attainment of a growing wolf population of at least 750 animals with genetic connectivity to wolf populations north and south; (2) a proscription on killing wolves to the extent that such killings would inhibit trophic cascades and specifically conservation of riparian habitats, pronghorn, swift fox, black-footed ferret, and Canada lynx; (3) a proscription on killing wolves that injure or kill livestock solely on public lands; (4) a proscription on killing wolves that kill livestock in instances in

⁸ Section 10(a)(1) allows for exceptions to the section 9 prohibition on “take” of endangered species, including allowing “acts necessary for the establishment and maintenance of experimental populations pursuant to subsection (j).” That provision serves as the basis for allowing *take* of members of experimental populations. Yet, section 10(d) constrains the granting of those exceptions: “The Secretary may grant exceptions under subsections (a)(1)(A) and (b) of this section only if he finds and publishes his finding in the Federal Register that . . . such exceptions . . . will be consistent with the purposes and policy set forth in section 2 of this Act.” Section 2’s first statement of purposes is “to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved.” Therefore, the 10(j) rule must provide a means to conserve the gray wolf’s ecosystems in Colorado.

⁹ DEIS, p. i.

which the same wolves had previously scavenged on non-wolf-killed livestock carrion; and (5) approval for introducing Mexican gray wolves into southwestern Colorado. These reasonable management provisions, each of which is necessary for conservation, were unreasonably excluded from analysis in the DEIS. Alas, had the DEIS started with a proper and lawful description of its purpose and need as rooted in conservation of endangered species and their ecosystems, this unfortunate paucity of management alternatives would have been less likely. As is, and as we describe in sections below, the Service must rapidly supplement the DEIS (and open a new public comment period) with an analysis of these provisions as a separate alternative, and also undertake a deeper analysis of the effects of Alternative 1 and remove Alternative 2 from further consideration.

Alternative 2 is unlawful because on its face it violates the section 10(j) requirement that experimental populations be “wholly separate geographically” from non-experimental populations of the same species. As preface, we acknowledge that the known distribution and numbers of breeding wolves in Colorado and Wyoming at present justify designating the state of Colorado as an experimental population area, as Alternative 1 proposes, due to the way the Service has defined what constitutes a population, and specifically because there is just one known breeding pair in Colorado and the southern portion of Wyoming has no known breeding wolves coupled with state rules that allow killing all wolves in southern Wyoming. As noted in the DEIS, these Wyoming circumstances have led to as-yet insufficient wolf colonization of Colorado habitats to establish a population as defined by the Service, and suggest an infrequency of contact between wolves in Wyoming and wolves in Colorado consistent with describing the upcoming Colorado experimental population area as wholly separate geographically.

That said, in contrast to Alternative 1, Alternative 2 would eliminate the geographic separation that section 10(j) requires between an experimental and a fully-endangered population, through (were it to be selected) bifurcating Colorado arbitrarily within areas of suitable habitat on both sides of such a (future) delineating line:

Under this alternative, wolves that establish a population naturally in the 10(a)(1)(A) permit area would be granted more protection than wolves that are reintroduced to the rest of the state. The wolf population may increase more rapidly in the state as a whole because of the protection granted in one small area, which would support wolf conservation and recovery objectives. However, wildlife do not respect invisible boundaries of administrative zones, and wolves that occur naturally in the 10(a)(1)(A) permit area would eventually disperse into the experimental population boundary based on biological needs and their social environment.¹⁰

The proposed rule and the DEIS present Alternative 2 as an actionable alternative only if a wolf population – that is, two breeding pairs that each keep alive at least two pups, during each of two successive years – is found to be naturally occurring in Colorado. As such, Alternative 2 serves merely as a stopgap in the event that wolf recolonization of Colorado proceeds such that Alternative 1 could not be carried out. Yet in such circumstances, those naturally-occurring breeding wolves would not be geographically separated from habitat that would be designated as an experimental population area, nor would there be an intervening region in which regulations

¹⁰ DEIS, p 4-7.

would effectively separate the two populations. For example, the DEIS specifies a possible wolf recolonization region in which wolves would not be designated as experimental: “A portion of the state, potentially including most of Jackson County and the western part of Larimer County (areas within Colorado big game management units 161, 6, 7, 16, 17, and 171) would be covered under a section 10(a)(1)(A) permit that the Service would issue to the State of Colorado under alternative 2.”¹¹ Yet such an outlined area would have no geographic separation from other areas of suitable wolf habitat in Colorado.

Moreover, Alternative 2 would be illegal in allowing the ‘take’ of wolves – albeit non-lethal take – on behalf of protection of native and non-native ungulates, as proposed. That is because such take would not serve to conserve endangered wolves.

For those two foregoing reasons, Alternative 2 must be removed from consideration. And its removal further illustrates the inadequacy of the range of alternatives in the DEIS.

The DEIS’s inadequacy in range of alternatives is also made apparent in that the preferred alternative -- Alternative 1 – is effectively bifurcated into two alternatives through its single diverging unresolved issue for which the Service requests comments: Whether to kill wolves for preying on native and non-native ungulates. (We discuss our opposition to killing wolves for these purposes in a section below.) The fact that the single, on-its-face legal (though not actually legal) action alternative is in fact two alternatives, suggests that the Service sought not to open the proverbial can of worms in presenting the question of which circumstances might justify the killing of wolves; the Service understood that if presented with one real choice as a separate alternative, the public would understand that other choices should be discussed as well.

Despite the Service’s disinterest in exploring real differences in management, the National Environmental Policy Act requires a multitude of real action alternatives, each thoroughly analyzed for its impacts on conservation. That is a primary reason that the Service must issue a supplemental DEIS – and it should do so as expeditiously as possible to facilitate timely issuance of a final rule prior to the December 31, 2023 deadline for Colorado authorities to reintroduce wolves in the state.

III. The Draft Environmental Impact Statement Misrepresents the Effects of the Preferred Alternative and Proposed Rule, Which Would Actually Serve to Thwart Conservation.

1. The proposed rule is unlikely to conserve the endangered gray wolf.

Conservation of wolves in the proposed experimental population area requires meaningful limits on their killing. The proposed rule acknowledges this glaringly-obvious contingency in the possible success of wolf recovery: “[I]f population levels and controllable sources of mortality are adequately regulated, the life-history characteristics of wolf populations provide natural resiliency to high levels of human-caused mortality.”¹² Yet, Alternative 1 does not regulate adequately the controllable sources of wolf mortality. Alternative 1 contains no limits in the killing wolves in circumstances in which wolves prey on livestock at least twice in one calendar year; nor does the draft Colorado Wolf Restoration and Management Plan that

¹¹ DEIS, p. 4-27.

¹² 88 Fed Reg. 10269 (Feb. 17, 2023).

would, when finalized, guide the state’s decisions on the killing of wolves. Given the contiguity, particularly during the winter, of elk and cattle herds in western Colorado, most wolves will encounter livestock on a regular basis. Unless livestock owners are required to take affirmative steps to prevent predation, almost every wolf will kill at least two head of stock each year. As the preferred alternative in the proposed rule, Alternative 1 delegates to the state and/or tribes management decisions, while acknowledging that scientists “cautioned that model predictions may be inaccurate because they did not account for the presence of livestock and the potential use of lethal removal to mitigate conflicts, which may affect the likelihood of establishment of gray wolves as well as their year-to-year survival and distribution on the landscape.”¹³ That big caveat entirely undercuts the reliability of the Service’s reassuring sounding conclusion:

Based on our demonstrated ability to reintroduce and successfully establish wolves to the NRM that reached recovery goals, the availability of habitat suitability and prey availability in the proposed reintroduction area (see *Habitat Suitability/Prey Availability* section, above), the demonstrated resiliency of gray wolves in the United States, and the ongoing development of a comprehensive Gray Wolf Restoration and Management plan in Colorado, the best available scientific data indicate that the reintroduction of gray wolves into suitable habitat in Colorado supports the likely success of establishment and survival of the reintroduced population, and the proposed experimental population has a high likelihood of becoming established within the foreseeable future.¹⁴

Citing in the proposed rule and draft environmental impact statement the robust wolf numbers achieved through the Service’s reintroduction of wolves as an experimental non-essential population to the northern Rocky Mountains is not predictive as a model to project Colorado’s upcoming wolf demography under Alternative 1. That is because of a difference in land use and a difference in management:

First, the northern Rockies is blessed with two extensive areas with little or no domestic livestock grazing – 2.2 million acres in Yellowstone National Park and a similar extant in central Idaho encompassing the Frank Church – River of No Return Wilderness Area, each an order of magnitude larger than any livestock-free area found in Colorado, for example Yellowstone is an order of magnitude greater in size than the 267,000 acres of Rocky Mountain National Park (and that comparison does not even account for the far-greater proportion of Yellowstone National Park and in particular central Idaho compared to Rocky Mountain National Park, comprising winter range for the elk that constitutes wolves' primary prey). Within those livestock-free areas in the northern Rockies, wolf numbers increased, and from within them dispersing wolves emanated, even as wolf numbers were (and are) perennially reduced through killings in response to livestock predation almost everywhere else in the northern Rockies.

Second, the Service’s November 1994 northern Rocky Mountain wolf reintroduction final rule prohibited the baiting of wolves through this language that was not found in the final rule on Mexican wolf reintroduction nor proposed in the instant proposed rule:

¹³ 88 Fed. Reg. 10267 (Feb. 17, 2023).

¹⁴ 88 Fed. Reg. 10268 (Feb. 17, 2023).

The Service and authorized agencies of the Service would use the following conditions and criteria to determine the status of problem wolves within the nonessential experimental population area . . . No evidence of artificial or intentional feeding of wolves can be present. . . . If livestock carrion or carcasses are not being used as bait for an authorized control action on Federal lands, it must be removed or otherwise disposed of so that they will not attract wolves.”¹⁵

That provision prohibiting the intentional or unintentional (i.e. negligent) attracting of wolves through livestock carrion prevented an uncounted number of wolves from being drawn to the vicinities of vulnerable livestock, where they might have otherwise have begun preying on such stock, and then would have become subject to agency killings. Unfortunately, no such provision is found in the instant proposed rule nor in Colorado Parks and Wildlife’s draft Wolf Restoration and Management Plan.

The draft environmental impact statement acknowledges that, under alternative 1, legal killings of wolves could impede the ability to establish a self-sustaining population and projects that “[w]olf numbers in Colorado during the first five years are likely to be similar to reported wolf numbers in Oregon (average of 37 wolves in 2009–2013) and Washington (average of 27 wolves in 2008–2012)” because “lethal control actions in Colorado are likewise anticipated to be similar to Oregon and Washington, during their respective initial monitoring years, where 3 percent and 2 percent, respectively, of the known wolf numbers were lethally controlled.”¹⁶ Nonetheless, the DEIS is optimistic that “[i]n the long term, the allowable take provisions under alternative 1 would be unlikely to reduce the number of wolves in Colorado because wolf populations are able to sustain relatively high rates of human-caused mortality.”¹⁷

The DEIS’s short-term projection based on Oregon and Washington numbers, and the DEIS’s accompanying long-term reassurance about the demographic effects of Alternative 1 on wolves, are each misplaced. Colorado’s wolves will likely prey on livestock and be subject to ensuing federal and state wolf killing (in addition to private illegal killings) at rates similar to those experienced by Mexican gray wolves during their initial reintroduction to Arizona and New Mexico. The Service should turn to its files on wolf management, numbers and genetic diversity in its Mexican wolf reintroduction program to reach a more realistic and sobering conclusion. The experience with the Mexican wolf reintroduction is more akin to the upcoming Colorado reintroduction in part because the Apache National Forest in Arizona where the wolves were first reintroduced is a similar high elevation landscape to areas in western Colorado where reintroduction will take place; in each region, livestock and elk are often in close proximity during the winter, which makes wolves more likely to encounter livestock (as well as carrion from dead livestock). In contrast, in much of eastern Oregon and eastern Washington where wolves first dispersed from Idaho, elk and livestock are not found so close together. And contrasting with the statistics that the DEIS reports of an average of 37 and 27 wolves each year during the first five years of colonization in, respectively, Oregon and Washington – in the case of the Mexican wolf over the first five years of reintroduction, 1998 through 2002, the average number of wolves was just under 22. And contrasting with the Service’s and Colorado Parks and

¹⁵ 59 Fed Reg. 224 (Nov. 22, 1994).

¹⁶ DEIS, p. 4-5.

¹⁷ DEIS, p. 4-5.

Wildlife's assumption that the numbers of wolves in Colorado will reach 200 before too many years, over the course of 25 years from 1998 through 2022, the average number of wolves in the wild across Arizona and New Mexico has averaged just under 84; it took 25 years for wolf numbers in these two states combined to rise above 200.

Delegating authority on all aspects of wolf management including the killing of wolves to state and tribal officials, and in particular to Colorado Parks and Wildlife, will likely keep the wolf population in Colorado suppressed for an even longer period than the Mexican wolf population remained demographically stagnant in Arizona and New Mexico. That is because, parsing out year-by-year results in the latter two states, one finds that from 2003 to 2009, during which period the Arizona Game and Fish Department brought into existence and chaired the Mexican Wolf Adaptive Management Oversight Committee (AMOC), with authority on wolf removals, the Mexican wolf population in the wild in Arizona and New Mexico declined from 55 to 42 – an almost 24% decrease in wolf numbers. The Colorado Parks and Wildlife and its draft wolf restoration and management plan, operating in similar landscapes, similarly calling for the removal of wolves that prey on livestock, and similarly containing no measures to require non-lethal protection of livestock from wolves, if given an opportunity will similarly suppress the number of wolves.

In the case of the Mexican gray wolf, during the period of effective state management through the AMOC, the Mexican wolf's genetic diversity declined dramatically. From a founding population of seven Mexican wolves variously captured in the wild in Mexico (six) and Arizona (one) between the 1960's (estimated) and 1980, the population in the reintroduced population has had its genetic diversity reduced to 2.1 founder genome equivalents today¹⁸ – the equivalent genetic diversity that would stem from a theoretical 2.1 founders instead of the actual seven founders. Most of that catastrophic loss of genetic diversity occurred from 2003 through 2007 when the U.S. Department of Agriculture's Wildlife Services division, acting in response to AMOC decisions, removed 39 wolves from the wild due to conflicts with livestock. Those included eleven wolves shot by the government¹⁹ and an additional eight who died as a consequence of their having been captured.²⁰

Given the identical lack of any restrictions on wolf killings in response to predation on livestock, and the state control that would be granted under authority of Alternative 1, loss of genetic diversity from the founding population will occur in Colorado as well. Such losses will only rarely be mitigated by introgression of northern wolves' genes into the Colorado wolf population, given the widespread wolf killing and lack of regulations restricting such killing throughout 84% of Wyoming, and encompassing the entirety of southern Wyoming. A small, isolated population of wolves in Colorado, subject to killing and with limited and declining genetic diversity, would likely become extirpated and likely also not be able to contribute meaningfully to the representation, redundancy and resilience that the Service cites (but didn't

¹⁸ U.S. Fish and Wildlife Service, Mexican Wolf Experimental Population Area Initial Release and Translocation Proposal for 2023, Nov. 2022, p. 2.

¹⁹ Sycamore Pack alpha female 592, Saddle Pack alpha male 574, Saddle Pack male pup 1007, Ring Pack alpha male 729, Hon-Dah Pack alpha male 578, Nantac Pack alpha female 873, Nantac Pack alpha male 993, San Mateo Pack alpha male 796, Durango Pack alpha female 924, and lone male wolves 859 and 864.

²⁰ Francisco Pack alpha female 511, Hon-Dah Pack alpha female 1027, and six Hon-Dah Pack pups – four male and two female.

bother to really examine) as its anticipated benefit to overall conservation of endangered gray wolves from Alternative 1.

In contrast to the comparative geographic and regulatory factors and actual record with Mexican wolves that we explained immediately above, the DEIS analysis is cursory:

In the long term, it is not expected that allowable take under alternative 1 would have a measurable impact on the population. Over time, the wolf population in Colorado is expected to settle at a density that is naturally regulated locally by wild ungulate prey availability and distribution (Mech and Barber-Meyer 2015) and territoriality (Cariappa et al. 2011), and regulated extrinsically by social carrying capacity statewide (2022a). Nonlethal take (harassment) would become integrated into livestock husbandry best management practices. Ongoing management actions (lethal and nonlethal) would occur under alternative 1, but they are not expected to have population-level impacts statewide. Given the amount of ecologically suitable habitat and prey availability in Colorado (Carroll et al. 2006; Ditmer et al. 2022), the wolf population is expected to increase at rates similar to other established populations in the long term (i.e., 20 percent per year; Fuller et al. 2003).²¹

Yet, the experience with Mexican wolves demonstrates that many livestock owners prefer the government would kill wolves, than that they personally engage in harassing wolves yet keeping them alive. There is no reason to believe that exercise of non-lethal harassment would meaningfully constrain the number of wolves that would be killed. That is why, as we explain below, it is imperative that livestock owners be required to engage in meaningful measures to deter wolf predation on their stock.

The DEIS nonsensically states that “Alternative 1 promotes an adaptive management approach for wildlife managers to support both wolf conservation goals and ungulate populations, and to implement deterrent tools (lethal and nonlethal take) that reduce the potential for livestock depredation.”²² But absent any limits on wolf killing and any requirement for deterrence, Alternative 1 does not promote any such adaptive management. Notably, “adaptive management” was the buzzword for the Mexican Wolf Adaptive Management Oversight Committee’s decision-making, and served to gloss over the attendant reduction in Mexican gray wolf numbers and genetic diversity from 2003 to 2009 (when a successful settlement agreement by conservation groups that had sued the Service ended the delegation of authority to AMOC and restored decision-making to the Service).

The DEIS and proposed rule also state that a critical difference between the fates of wolves previously extirpated from Colorado, and future wolves’ likely fates under Alternative 1, is intentionality: “Purposeful eradication is no longer a tool used for wolf management. Based on the elimination of purposeful eradication, and the fact that gray wolves are protected under State and Federal laws, we do not anticipate the original cause of wolf extirpation from Colorado to be repeated.”²³ Yet, stated intentions in management often have less consequence in wolf

²¹ DEIS, p. 4-5.

²² DEIS, p. 4-6.

²³ 88 Fed. Reg. 10267 (Feb. 17, 2023).

conservation than underlying motivations, as in the example of the eerily-similar Mexican wolf reintroduction, and going as far back as 1928 when the Service's predecessor agency the Bureau of Biological Survey officially changed policy to reject extermination – and yet 17 years later in 1945 killed off Colorado's (and likely the West's) last U.S.-born wolf.²⁴ During that time period, as imminently in the future under Alternative 1, the reason for the killings was to eliminate threats to livestock. And in 1928 – as in 2023 – the culture and prevailing world view of almost all of the staff members of the wolf management government agency, first Bureau of Biological Survey and soon under delegation the Colorado Parks and Wildlife, internally was at odds with and disagreed with its official policy of wolf non-extermination.²⁵ However else it might be described, and notwithstanding official rhetoric, CPW's wolf restoration and management plan is not a plan that will lead to a self-sustaining wolf population in Colorado. Alternative 1 violates the conservation mandated of the Endangered Species Act in giving full reign to CPW to carry out its wolf plan.

2. The proposed rule will not conserve the wolf's ecosystems.

Unfortunately, the draft environmental impact statement only barely mentions the scientific findings on wolves' positive effects on their ecosystems elsewhere, in particular wolves' influences on other species of animals and plants through trophic cascades. And the fact that the DEIS does not contain meaningfully different alternatives precludes its analysis of how wolves' roles in ecosystems would be affected by different types of management. The supplemental DEIS that is required through this DEIS's inadequacy should evaluate how the authorized killing of wolves under different circumstances – reflected in different alternatives -- would affect their ecosystems. In particular, the killing of wolves would harm four species that are negatively affected by high coyote densities – since wolves kill coyotes, suppress their numbers, and limit their densities; with greatly constrained levels of wolf-killing, those species -- blackfooted ferret, swift fox, Canada lynx and pronghorn. Other rare and imperiled species would also benefit from wolves and conversely be harmed by unconstrained killing of wolves, including wolverines that feed on carrion provided by wolves, and Chapin Mesa milkvetch whose range is constrained in part by high levels of elk herbivory – along with other sensitive native plants in Colorado affected by high densities and/or sedentary behaviors of elk, cattle and other ungulates, and animals affected by overgrazing of riparian areas.

In the absence of any such analysis, it is nonetheless clear that the proposed rule that allows unlimited killing of wolves that prey on livestock and at the direction of Colorado Parks and Wildlife, the species briefly listed above, and others, will be harmed. It is imperative that in

²⁴ Robinson, M.J. 2005. *Predatory Bureaucracy: The Extermination of Wolves and the Transformation of the West* (University Press of Colorado); pp. 198, 285-286.

²⁵ During the campaign for wolf reintroduction, CPW unofficially sought to tilt opinion against Proposition 114 through announcing the presence of a naturally-occurring pack in Colorado shortly after the announcement of Proposition 114 garnering enough signatures to make the ballot, and issuing anti-wolf statements in a variety of formats including community meetings. After voters approved Proposition 114, CPW's northwest regional manager J.T. Romatzke sought to undercut implementation; yet after Romatzke returned from a period of leave, CPW assigned the whistleblower who reported Romatzke to continue to work under his supervision. The whistleblower felt compelled to resign from CPW and departed the state. CPW's bias toward the livestock industry and against wolves will mean that its decision-making on when to kill wolves will not be constrained by the conservation intent of federal law (nor of C.R.S. 33-2-105.8 which seeks to "help restore a critical balance in nature"). That is one reason that the final 10(j) rule should constrain the circumstances in which wolves can be killed.

the required supplemental DEIS, a new alternative for consideration serve to limit wolf killing to the extent of enabling the benefits of wolves to accrue to the wolf's ecosystem.

3. The proposed rule will thwart conservation of the Mexican wolf.

The proposed rule – through limiting the listed species to be reintroduced into the experimental population area to the gray wolf and specifically through excluding the separately listed subspecies, Mexican gray wolf -- would undermine Mexican wolf conservation. Moreover, the draft environmental impact statement's analysis of this outcome was undercut through omission of the best available science which (notwithstanding the opposite conclusion in the politically-derived and counter-scientific 2022 Mexican Wolf Recovery Plan) shows that a Mexican wolf population must be established in the southern portion of the southern Rocky Mountains, in southwestern Colorado and northwestern New Mexico, to effectuate eventual recovery. That imperative is because the partial restoration and longterm retention of Mexican wolf genetic diversity depends on establishment of three interconnected populations including in the southern Rockies and the Grand Canyon ecosystem, and totalling at least 750 animals.

Moreover, those two northern populations must have connectivity to northern gray wolves, to facilitate an infusion of northern genes that are now needed by the inbred wild Mexican wolves. It is vital that a future wolf population in the southern portion of the southern Rocky Mountains be established through introduction of Mexican wolves, and not through reintroduction (even somewhat further north in Colorado) of northern gray wolves along with range expansion southward. The contrasting genetic contribution from northern gray wolves to Mexican gray wolves in their current wild U.S. range based on whether Mexican wolves are introduced to southwestern Colorado or alternately whether reintroduced northern wolves colonize southwestern Colorado, shows what is at stake in attempting the necessary diversification of the Mexican wolf wild population's gene pool without swamping the genome with too many specifically-northern genes. Introducing Mexican wolves to the San Juan Mountains would move the eventual central zone of subspecific intergradation several hundred miles northward – to central Colorado – as opposed to not doing so and eventually having northern wolves disperse southward to establish a central zone of intergradation somewhere in central New Mexico and/or Arizona. To simply the explanation through likely examples, in the former instance northern-derived genes would be mediated by matings of intergraded wolves with pure Mexican wolves in southern Colorado, and some of the pups from those matings would eventually breed with Mexican wolves in southern New Mexico and Arizona. In the latter instance, half-and-half intergraded wolves might breed with pure Mexican wolves in southern New Mexico and Arizona, and deliver twice as many northern genes. Introducing a population of Mexican wolves through releases in the San Juan National Forest or nearby would ensure the necessary diversification of the Mexican wolf's gene pool and also ensure that the subspecies is not irrevocably harmed through excessive introgression of northern-derived genes.

IV. Other Elements of the Proposed Rule Would Also Prove Inimicable to Conservation.

If the Fish and Wildlife Service does approve Alternative 1 in the final rule, at a minimum it should make three essential changes. We stress that such changes would not obviate the harms described above from the proposed rule, nor even sufficiently mitigate those harms

biologically or legally; but the changes would make the situation somewhat better in certain restricted situations affecting conservation, than in the alternate.

Those changes in the preferred alternative would prohibit the killing of wolves to protect wild ungulates (in this instance not a change per se but a choice suggested in the odd bifurcation of Alternative 1); restrict the sources of wolves released in Colorado to those captured outside of national parks and wolf-protective Tribal lands but within the states of Wyoming, Montana and Idaho, and also include Mexican gray wolves to be released from captive facilities; and restrict removals and relocations back of wolves who leave the experimental population area to the sole circumstance of preventing too much introgression of northern wolves genes into the U.S Mexican wolf population and only in such instances after a science-based finding that the prospective introgression of northern genes from the wolf to be removed would be detrimental to conservation of Mexican wolves.

1. The final rule should not allow the killing of wolves to protect wild ungulates.

Killing wolves to protect wild ungulates for hunting and for hunting outfitters would add to other sources of wolf mortality and exacerbate the harms already proposed in the killing of wolves to protect livestock. Such killing of wolves for this purpose would also thwart the ecosystem conservation mandate of the Endangered Species Act, insofar as wild ungulate numbers and distribution should be affected by wolf predation in order to benefit riparian areas and scavenging animals such as wolverines that rely to a significant extent on wolf predation for their own food sources.

These harms would prove particularly egregious and contrary to conservation if wolf killing were to be allowed to protect non-native moose and non-native mountain goats, both of which may be harming native plant communities and rare plant species in Colorado where the flora did not evolve in the presence of moose or mountain goats. The final rule should in no circumstances allow the killing of wolves to protect wild ungulates.

2. The final rule should restrict the source populations of wolves slated for release in Colorado to those in unprotected parts of Wyoming, Montana and Idaho, and Mexican gray wolves captive facilities.

Unless there is no alternative, wolves destined for translocation to Colorado should not be captured from national parks, Tribal lands where they receive high levels of protection, or from areas of Oregon and Washington in which protections remain significantly higher than in most of Idaho, Wyoming and Montana where wolf hunting is virtually unlimited. They should only be captured from Idaho, Montana and Wyoming outside of such protected areas, unless such captures are prohibited by all of those states. That is because it would thwart the spirit of conservation and humaneness to take protected wolves and place them in a place and a circumstance in which their legal protections have largely been removed.

In addition, Mexican gray wolves from the captive breeding facilities in the U.S. and Mexico should be released in southwestern Colorado. Similarly to the logic expressed above

regarding northern gray wolves, Mexican wolves should not be taken from the wild for release in Colorado, but rather released from captivity as well-bonded family groups.

3. The final rule should only allow removal of wolves who leave the experimental population area and their attendant relocation back within the experimental population area for the sole purpose of preventing too much introgression of northern wolves genes into the U.S Mexican wolf population and only in such instances when the best available science strongly indicates that the prospective northern wolf genes would be detrimental to Mexican wolf recovery.

The above-captioned criterion for removal of wolves who leave the experimental population area, and their relocation back, would prohibit the removal of wolves for the purpose of curtailing conservation – for example in Utah where authorities oppose wolf conservation. It would require that wolf removals for the purpose of benefiting the Mexican wolf, actually do so, or such removals would not be allowed.

V. At Least One Additional Alternative Should be Analyzed in a Supplemental Draft Environmental Impact Statement and Selected in the Final Rule, To Ensure Conservation of Gray Wolves, Mexican Gray Wolves, and Their Ecosystems.

The needed supplemental draft environmental impact statement and final rule should respectively analyze and consist of the following provisions: (1) A proscription on killing wolves to the extent that such killings would inhibit or slow attainment of a growing wolf population of at least 750 animals with genetic connectivity to wolf populations north and south; (2) a proscription on killing wolves to the extent that such killings would inhibit trophic cascades and specifically conservation of riparian habitats, pronghorn, swift fox, black-footed ferret, and Canada lynx; (3) a proscription on killing wolves that injure or kill livestock solely on public lands; (4) a proscription on killing wolves that kill livestock in instances in which the same wolves had previously scavenged on non-wolf-killed livestock carrion; and (5) approval for introducing Mexican gray wolves into southwestern Colorado.

These first four provisions, which would protect wolves, are necessary in part because CPW's draft wolf restoration and management plan would create financial and institutional incentives within CPW and the state legislature tending against CPW forbearance of wolves that kill livestock. For example, the technical working group that CPW appointed to help draft the plan acknowledges that sufficiently high financial payments "may also disincentivize conflict risk reduction solutions and improved management practices" but simultaneously refused to recommend any incentives to reduce conflict risk and improve management – and no such protective incentives are in the draft plan. Specifically, the technical group would not endorse "requirements that non-lethal risk reduction techniques be used prior to depredation to be eligible for compensation."²⁶ The latter measure would create an incentive to make livestock less vulnerable to wolves, and ultimately would serve to limit the payment of public funds to compensate for wolf-killed livestock. But CPW's advisory groups advised against implementing the measure. Therefore, the final rule will have to constrain the ensuing killing.

²⁶ Colorado Wolf Restoration and Management Plan Technical Working Group (TWG) to Colorado Parks and Wildlife (CPW), Final Summary of Technical Considerations on Compensation for Wolf Damage to Livestock. February 2022; pp. 4 & 10.

The other reason that the 10(j) final rule should constrain killing of wolves in response to wolves preying on livestock is that livestock grazing is ubiquitous in Colorado on both private and public lands. National forests and public lands managed by the Bureau of Land Management likely do not contain any regions without livestock that are large enough that even a single pack of wolves could maintain a full-year home range. The national parks and monuments in the state are similarly too small to contain wolf packs within their boundaries.

If, as is likely, every wolf pack in Colorado will regularly encounter livestock, and if as called for in draft state wolf plan, any wolf that kills two or more livestock during a calendar year could itself be killed, and if moreover CPW is attitudinally inclined to kill wolves and may be fiscally motivated to do so insofar as it may be called upon to contribute to compensation for livestock losses, all else being equal it is likely that the wolf population will be unable to recover. Wolf numbers would be small and perpetually suppressed, the population would be cut off from other gray wolf populations to the north and south, risking inbreeding, and wolves would not be numerous enough to significantly influence other animals and plants and cause trophic cascades, thereby falling short of ecosystem conservation. To forestall these deleterious effects, the experimental, non-essential population of wolves in Colorado will require additional legal protections through the 10(j) rule.

1. The final rule and supplemental draft environmental impact statement should define, measure, and promote achievement of wolf conservation goals.

In order to effectively conserve the future experimental population of wolves in Colorado, the final rule should define conservation goals, including the number of wolves inhabiting Colorado, and other aspirational conditions, that would represent a population no longer in danger of extirpation. The supplemental draft environmental impact statement should explain the basis for these conservation goals. This section of our letter provides guidance on those numeric and associated conservation goals. Wildlife populations suffer losses of viability through genetic drift and inbreeding depression stemming from their small sizes and/or fragmented configurations. The number of animals necessary for viability has been determined in a variety of ways and with refinements in methodologies, as summarized below.

Relatively early in the quest to determine minimal viable population sizes, in 1995, a scientist calculated that effective populations of around 5,000 organisms (i.e. that number of reproducing animals -- not equaling the total number of animals) would be necessary to stave off deleterious mutations leading to inbreeding and other genetic ills.²⁷ In 2003, five scientists noted that population size is a major determinant of extinction risk and used population viability analysis to estimate minimum viable populations for 102 species, with a mean estimate of 7,316 organisms and median estimate of 5,816 adults. They advised that conservation programs for wild vertebrate populations conserve habitat capable of supporting approximately 7,000 adults in order to ensure long-term persistence.²⁸

²⁷ Lande, R. 1995. Mutation and conservation. *Conservation Biology*, 9(4):782-791.

²⁸ Reed, D.H., J.J. O'Grady, B.W. Brook, J.D. Ballou, R. Frankham. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation*, 113:23-34. They defined a minimum viable population size as one with a 99% probability of persistence for 40 generations.

Then a 2007 meta-analysis of population viability studies for 212 disparate species, determined that the median minimal viable population was 4,169 organisms.²⁹ And in 2009 researchers found that “thousands (not hundreds) of individuals are required for a population to have an acceptable probability of riding-out environmental fluctuation and catastrophic events, and ensuring the continuation of evolutionary processes.”³⁰

A 2011 critique challenged the universal applicability of a single number as a threshold for population viability. This set of scientists agreed that in many instances “multiple populations totaling thousands (not hundreds) of individuals will be needed to ensure long-term persistence” and “realistic MVPs might well be in the thousands for many life histories,” but argued that “uncertainty around any guideline figure would be of a similar order of magnitude” because “estimates both among and within species show striking variation for many reasons. The fundamentally contingent nature of MVPs means that we cannot support a universally applicable MVP threshold.”³¹ Noting the importance of population trend in extinction risk, they determined that had a previous meta-study (Brook et al 2006) of 1,198 populations, which found a median minimal viable population number at 1,181 organisms, instead excluded populations with a significant declining trajectory, and solely analyzed populations exhibiting stable or increasing numbers, then the median for viability would drop to just 355 organisms.³²

To explore how these estimations may be relevant to a wolf population in Colorado, first consider that wolf numbers in Colorado will be increasing with reintroduction, so a population comprising 355 breeding animals could be presumed viable, if only barely so. The Fish and Wildlife Service must also take into account that not all wolves in a family pack breed; generally, just two animals breed. With a presumed average pack size of eight (not estimable to precision before wolves reinhabit the landscape), that means that 25% of the wolves contribute genetically, serving as an effective population, i.e. those animals that successfully breed and raise young. Thus, for 355 wolves to serve as an effective population, four times that many wolves would have to be part of the population overall – equaling 1,420 wolves.

However, it is possible that 1,420 is above the wolf carrying capacity of Colorado’s ecosystems. A 1994 congressionally-chartered wolf reintroduction feasibility study found that 1,128 wolves could live on Colorado’s seven national forests based on the biomass of elk and deer.³³ The assessment did not assess carrying capacity on other lands.

To stay within the ballpark of that roughly-estimated and very old calculation of carrying capacity – 1,128 wolves – and to ensure genetic viability, the proposed rule should set as a preliminary and tentative recovery goal (subject to future recovery planning) for the Colorado

²⁹ Traill, L.W., C.J.A. Bradshaw, B.W. Brook. 2007. Minimal viable population estimates: a meta-analysis of 30 years of published reports. *Biological Conservation*. 139:159-166.

³⁰ Traill, L.W., B.W. Brook, R.R. Frankham, C.J.A. Bradshaw. 2010. Pragmatic population viability targets in a rapidly changing world. *Biological Conservation*, 143:28-34; p. 28.

³¹ Flather, C.H., G.D. Hayward, S.R. Beissinger and P.A. Stephens. 2011. Minimum viable populations: is there a ‘magic number’ for conservation practitioners? *Trends in ecology and evolution*, 26(6):307-316; p. 314.

³² Flather et al (2011), p. 311.

³³ Bennet, L.E. 1994. Colorado gray wolf recovery – a biological feasibility study. Final report to U.S. Fish and Wildlife Service; p. 112, table 23.

wolf population a minimum presence over three successive years of 750 wolves, with a growing population each year and with connectivity through natural migration to and from wolves in the northern Rocky Mountains and Mexican gray wolves in the Southwest, such that at least one immigrant wolf into Colorado from each of these regions, north and south, successfully reproduces and raises at least two pups to age two in Colorado, every two years, for at least six years. That would amount to a minimum effective (i.e. contributing genetically) population of just 187 wolves within the state, but the connectivity to wolf populations outside of Colorado would make it much less likely that genetic drift and inbreeding would doom Colorado's wolves.

The 10(j) rule should set a timeline toward achievement of this numeric goal – 750 wolves – and the environmental impact statement should enumerate the expected annual growth in the wolf population necessary to achieve the numeric goal by the expected year. We suggest that reaching 750 wolves should be achievable within 15 years (i.e. by end of 2038) – amounting to an annual average increase in wolf numbers through releases and reproduction of 50 wolves per year; the first few years are likely to have significantly smaller annual increments while the last few years should each manifest significantly larger increments that will hopefully represent sustained reproduction in the wild.

The 10(j) rule should ensure annual progress toward the numeric goal, and the EIS should logically explain that assurance, such that the killing of wolves will not preclude the population attaining and sustaining at least 750 wolves in the state within 15 years. The 10(j) rule and EIS should also ensure that wolf-killing will not preclude the successful immigration and subsequent successful reproduction and multiple pup-rearing of at least one wolf emanating from the northern Rocky Mountains and one from the Southwest (i.e. respectively from the north and south), every two years.

2. The final rule should define, measure, and promote achievement of ecosystem conservation goals.

As shown above, the final rule must further the conservation of the ecosystems on which gray wolves depend. And just as in the last subsection we demonstrated that the killing of wolves could not be allowed to prevent establishment of a viable, connected population, so in this subsection we show that the killing of wolves cannot be allowed to thwart the conservation of the wolf's ecosystems. Specific components of the wolf's ecosystem – such as requisite numbers or density of prey animals – may be readily identifiable as what wolves depend on. But the law calls not for conservation of components of ecosystems; it calls for the conservation of ecosystems. In the case of gray wolves, their ecosystems are best identified through the positive trophic cascades that wolves are known to precipitate. *Trophic cascades* is the scientific term for the ripple effects on diverse animal and plant species stemming from predatory behaviors. Those ripple effects consist of the influencing of various animals' behaviors, distributions and abundance, and even of the distribution and abundance of plants, in ways that ultimately favor the persistence of all animals and plants in an ecosystem through maintaining the specific conditions to which each species has evolutionarily adapted.

Largely due to the research opportunities afforded through reintroduction of wolves to Yellowstone National Park in 1995, in addition to other investigations, scientists have attributed

multiple examples of trophic cascades to wolves. The widespread extirpation of wolves throughout the course of the development of the U.S. disrupted trophic cascades nationwide, including in Colorado. Wolves must be restored in sufficient abundance and density on the landscape to help to re-precipitate trophic cascades, the operations of which are central to the conservation of the gray wolf's ecosystems. A 2011 review by 34 scientists concluded that "Top-down forcing must be included in conceptual overviews if there is to be any real hope of understanding and managing the workings of nature."³⁴ At the end of this section, we advise two measures of ecosystem conservation that represent the scope of (though hardly the entirety of) the trophic cascades to be expected from wolves managed such as to conserve their ecosystems. We explain the basis for those two metrics of ecosystem conservation below.

In Yellowstone National Park, biologists discovered that alongside some of the larger waterways where steep terrain blocks elks' ability to spot approaching wolves, cottonwood and willow trees have proliferated where previously elk browsing kept them from maturing; the elk had learned to avoid such areas where wolves can surprise them. These riparian trees provide food and dam construction materials for beaver that are far more common than before wolf reintroduction.³⁵ The dams that the beavers construct provide habitat for a wide variety of fish, amphibians, and reptiles.³⁶

A scientist associated with the Colorado Natural Heritage Program in Colorado (who is also on the emeritus faculty of the Department of Environmental Science and Engineering at Colorado School of Mines) wrote in the Colorado Native Plant Society newsletter in 2020 that wolves reintroduced to Colorado could have similar beneficial effects on the degraded vegetative communities that he is familiar with in Colorado:

The most noticeable damage occurs in aspen groves and in riparian willow carrs, particularly if elk use these resources year-round or if unmanaged livestock overgraze these systems. When healthy, both ecosystems support a high diversity of sub-dominant plant species, as well as diverse animal communities including mammals, birds, and invertebrates. This diversity declines with prolonged heavy grazing. In Rocky Mountain National Park, excessive grazing of alpine tundra plants by elk may have contributed to the decline of ptarmigan numbers.

Aspen groves—typically clones in which the trees are interconnected by a common root system—produce shoots, or suckers, from the root system to expand the grove or to replace ageing or diseased trees. When the shoots are heavily browsed by elk and other herbivores, the groves fail to mature. Elk also gnaw the bark of aspen trees during late winter and early spring, and that can lead to infection of the tree by various diseases. Both situations in concert can eventually lead to the death of the entire clone.

³⁴ Estes, J.A., et al. 2011. Trophic downgrading of planet earth. *Science* 333:301–306; p. 306.

³⁵ Ripple, W.J., and R.L. Beschta. 2016. Riparian vegetation recovery in Yellowstone: the first two decades after wolf reintroduction. *Biological Conservation* 198:93–103.

³⁶ Ripple, W.J., and R.L. Beschta. 2012. Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. *Biological Conservation* 145:205–213.

Riparian willow carrs, particularly those in broader valley bottoms, are commonly occupied by beavers. Willows and beavers are codependent. Beavers use the willows for food and building materials for their dams and lodges. Beaver dams raise the water table, providing shallow ground water that willows need. When willows are browsed heavily by elk, beavers leave due to over-competition with elk, beaver dams are no longer maintained, and streams and rivers begin to run straight and fast. This results in downcutting of the channel, a drop in the water table, and further demise of the willows. Sedges, grasses, and smaller shrubs that help to maintain channel stability are also affected by heavy elk browsing.³⁷

However, conserving such riparian areas in the face of such vegetative changes would only occur with sufficient numbers of wolves in the state:

The reintroduction of wolves to Colorado is likely to have mostly subtle and indirect effects on our native plant communities. Those effects will probably not occur until wolf populations increase to ecologically effective numbers and stabilize.³⁸

Killing wolves to the extent that there are not enough of them to be ecologically effective would thwart the needed trophic cascades that would help to conserve the vital riparian portions of Colorado's ecosystems. Such an absence of wolf-caused trophic cascades was documented in the Apache National Forest of east-central Arizona where endangered Mexican gray wolves were reintroduced as an experimental population in 1998, and should serve as a warning to Colorado of the adverse ecological effects of limiting wolf numbers. The reason for that absence was insufficient wolf numbers – illustrating precisely the effect of killing too many wolves. Researchers in 2009 selected three mixed-conifer sites on the Apache National Forest to characterize long-term age structure of aspen and to check for the possible occurrence of a tri-trophic cascade involving Mexican wolves, elk and aspen, as the same researchers had previously documented was occurring in Yellowstone National Park. The abstract to the study describes the sites and the long-term and more recent effects of elk herbivory within them:

These mixed-conifer sites included (a) a refugium site, (b) an old-growth site, and (c) a site thinned in 1991–1992. The refugium site was inaccessible to elk and cattle whereas the old-growth and thinned sites were accessible to elk, but not cattle. Age structure results indicated that aspen recruitment (i.e., the growth of sprouts/seedlings into tall saplings, poles, and eventually trees) at the refugium site had been ongoing over a period of many decades. In contrast, aspen recruitment at the old-growth and thinned sites decreased significantly ($p < 0.05$) during the two most recent decades when elk populations, as indexed by annual harvest levels, were relatively “high”. From 2000 to 2008, only 2.9 Mexican wolves per 1000 elk were present on the Apache National Forest compared to 9.3 western gray wolves (*Canis lupus occidentalis*) per 1000 elk in Yellowstone National Park where tri-trophic cascades involving wolves, elk, and aspen have been reported. The low number of Mexican wolves relative to their primary prey (elk) suggests that an ecologically effective density of wolves has not become established

³⁷ Emerick, J. 2020. Reintroduction of wolves to Colorado: could this affect our native plant communities? *Aquilegia* 44(3):21-23; pp. 21-22.

³⁸ Emerick, J. (2020), p. 22.

in east-central Arizona. Furthermore, the lack of recent aspen recruitment in stands accessible to elk indicates an absence, to date, of a tri-trophic cascade.³⁹

These researchers attributed that absence to there not being enough wolves on the landscape:

Soule et al. (2003) indicate an ecologically effective density of highly interactive species (such as wolves in terrestrial ecosystems) is one that would maintain critical ecological interactions and help ensure against ecosystem degradation. With regard to a tri-trophic cascade involving wolves, elk, and aspen, this would be a predator density sufficient to mediate the behavior and/or density of native herbivores thus regulating potential impacts to plant communities. The relatively low ratio of Mexican wolves per 1000 elk on the Apache National Forest, in comparison to gray wolves in northern Yellowstone (Fig. 6a) and other areas of north America (Fig. 6b), appears to indicate that Mexican wolves have not achieved an ecologically effective density in Arizona.⁴⁰

They concluded that Mexican wolves “appear to not yet have attained an ecologically effective density as we were unable to document a trophic cascade (i.e., improved aspen recruitment). Unless wolf densities increase, it is possible that the potential ecological benefits of these apex predators in the mixed-conifer forests of east-central Arizona will not be achieved.”⁴¹

The context to the Mexican wolves not having attained an ecologically effective density in 2009 when that study was conducted is that 2009 represented the demographic nadir in a four-year downturn in the number of Mexican wolves in the wild in Arizona and New Mexico. After reintroduction in 1998, the population had peaked at 59 animals in 2006. But the previous year, 2005, decision-making authority over removing wolves from the wild in Arizona was effectively delegated from the U.S. Fish and Wildlife Service to the Arizona Department of Game and Fish. Between 2005 and 2009, federal employees removed 71 wolves from the population. As a consequence, by 2009 the bi-state wolf population had been reduced by over 28% to 42 animals including 27 in Arizona. That year, there were only two successful breeding pairs (a male wolf and a female wolf that produced two or more pups that survived through December 31 of the year of their births) in the combined states of Arizona and New Mexico. In December 2009, the Fish and Wildlife Service entered into a settlement agreement with conservation organizations including the Center for Biological Diversity in which the federal agency committed to no longer allowing the states to decide on removing wolves. The population began to grow again in ensuing years. Learning from wolf mismanagement in the Southwest and the impacts on aspen, it is critical that the 10(j) rule for Colorado wolf reintroduction not authorize or enable the killing of wolves to the extent that riparian areas are not conserved. More broadly, it is vital that Colorado Parks and Wildlife not be given unfettered authority over killing wolves similar to the authorities conferred on (and abused by) Arizona Department of Game and Fish between 2005 and 2009.

³⁹ Beschta, R.L. and W.J. Ripple. 2010. Mexican wolves, elk, and aspen in Arizona: Is there a trophic cascade? *Forest Ecology and Management* 260:915–922; p. 915.

⁴⁰ Beschta, R.L. and W.J. Ripple (2010), p. 921.

⁴¹ Beschta, R.L. and W.J. Ripple (2010), p. 921.

The process of trophic cascades also plays out through wolf attacks on coyotes, which the larger wolves treat as territorial rivals. As a result, other mammals on which the coyotes prey benefit in the presence of wolves. For example, after wolves were reintroduced to Yellowstone National Park and expanded their range southward, pronghorn in Grand Teton National Park rebounded from a significant decline that had been caused by coyote hunting of the pronghorn fawns.⁴² Similarly, red foxes and gray foxes in Minnesota and Wisconsin are more prevalent in areas with wolves than in areas with few or no wolves and a corresponding abundance of coyotes.⁴³ And in the absence of wolves in Utah, in order to minimize dangerous proximity to coyotes, kit foxes avoid suitable habitats and thereby lessen their access to prey.⁴⁴ In Yellowstone, rodents such as voles thrive where there are wolves.⁴⁵ By killing coyotes in Wisconsin, the presence of wolves led to an increase in snowshoe hare numbers, which in turn may benefit Canada lynx.⁴⁶ Many of these same species in Colorado would similarly benefit from the presence of wolves, including endangered Canada lynx.⁴⁷ So might black-footed ferrets and swift foxes as well – for the same reason; the latter two are also preyed upon by coyotes.⁴⁸

Again, the example in reintroduction of the Mexican wolf to Arizona and New Mexico demonstrates how management under a flawed 10(j) rule can thwart ecosystem conservation. A 2018 M.S. thesis examined through the placement of 18 camera traps in three plots in eastern Arizona – cameras which over the course of 4,517 trap nights took 38,820 photographs, 1,224 of which were independent photos of wildlife species⁴⁹ – whether Mexican wolves can influence the distribution of elk, mule deer, coyotes and gray foxes. Two of the plots were within wolves' core home ranges while the third was in an area without sustained wolf activity. Detection rates for coyotes, gray foxes, elk and mule deer were not statistically different in the plots, “which means there is no indication that the Mexican wolves are influencing the distribution of coyotes, gray foxes, elk, or mule deer in the region.”⁵⁰

The thesis suggested three possible explanations – the first of which is likely most influential and which we quote here:

⁴² Berger, K.M., E.M. Gese, and J. Berger. 2008. Indirect effects and traditional trophic cascades: a test involving wolves, coyotes and pronghorn. *Ecology* 89:818–828.

⁴³ Levi, T., and C.C. Wilmsers. 2012. Wolves–coyotes–foxes: a cascade among carnivores. *Ecology* 93:921–929.
Flagel, D.G., G.E. Belovsky, M.J. Cramer, D. E. Beyer, Jr., and K. E. Robertson. 2017. Fear and loathing in a Great Lakes forest: cascading effects of competition between wolves and coyotes. *Journal of Mammalogy* 98:77–84.

⁴⁴ Kozłowski, A.J., E.M. Gese, and W.M. Arjo. 2012. Effects of intraguild predation: evaluating resource competition between two canid species with apparent niche separation. *International Journal of Ecology*. 2012:12. doi:10.1155/2012/629246

⁴⁵ Miller, B.J., H.J. Harlow, T.S. Harlow, D. Biggins, and W.J. Ripple. 2012. Trophic cascades linking wolves (*Canis lupus*), coyotes (*Canis latrans*) and small mammals. *Canadian Journal of Zoology* 90:70–78.

⁴⁶ Flagel et al (2017).

⁴⁷ Ripple, W.J., A.J. Wirsing, R.L. Beschta and S.W. Buskirk. 2011. Can restoring wolves aid in lynx recovery? *Wildlife Society Bulletin*, 35(4):514–518.

⁴⁸ Ripple, W.J., A.J. Wirsing, C.C. Wilmsers, and M. Letnic. 2013. Widespread mesopredator effects after wolf extirpation. *Biological Conservation* 160:70–79.

⁴⁹ Hoskinson, J.S. 2018. Mexican gray wolves and the ecology of fear: a comparative assessment of community assemblages in Arizona. Thesis submitted to the faculty of the Department of Ecology and Evolutionary Biology in partial fulfillment of the requirements for the degree of Master of Science in the graduate college of the University of Arizona; p. 17.

⁵⁰ Hoskinson (2018), p. 6.

One plausible explanation for the lack of significance may be due to the Mexican gray wolf population size and range. In 2016, the Mexican gray wolf population was estimated to be around 113 wolves occupying 41,735 km², primarily in New Mexico (US Fish and Wildlife Service, 2016) whereas the wolf population of approximately the same size in Yellowstone National Park occupied 8,989 km² (Smith et al 2017). For a landscape of fear to be instituted in coyotes, gray foxes, elk, and mule deer, these species have to encounter the Mexican wolves, in either a competitive or preyed upon manner, often enough for the Mexican wolves to be incorporated into each species' optimal foraging strategy (Lima and Dill 1990, Brown, Laundré, and Gurung 1999) as happened in the Yellowstone National Park system (Ripple and Beschta 2004a). If these species were to encounter the Mexican gray wolf on a consistent basis, it would be reasonable to assume a modified distribution of these species, as measured by detection rate, such that a lower detection rate is observed for coyotes, elk, and mule deer, but a higher detection rate for gray foxes, in areas consistently occupied by Mexican wolves. If, however, a Mexican gray wolf is encountered only occasionally, not consistently, then the risk of predation/competition by the Mexican gray wolf would not be incorporated into the species' optimal foraging strategy. Therefore, in this case, we should not see differences in distribution of these species as measured by detection rate. According to our analyses there is no statistically significant difference in detection rate between plots with Mexican wolves and plots without, thus we suggest that a landscape of fear has not been instituted in the Blue Range Wolf Recovery Area.⁵¹

For additional context on the 113 Mexican wolves that were estimated to survive in Arizona and New Mexico in 2016, which throughout that year (as has the wolf population in every other year in this century) inhabited almost exclusively the Gila National Forest, Apache National Forest and Fort Apache Indian Reservation, it is worth noting that the carrying capacity of the two national forests was calculated at 468 wolves based on the biomass of elk and deer on those forests, and not counting other animals that the wolves may eat such as jackrabbits and javelina.⁵² In other words, even if all those 113 wolves had lived in the two national forests, and even if they eschewed preying on smaller animals and only fed on deer and elk, their numbers were fewer than 25% of the carrying capacity, which helps explain why they apparently did not influence the distribution of elk, mule deer, coyotes and gray foxes in the thesis study quoted above. Again, the lesson for development of the Colorado gray wolf 10(j) rule must be that the killing of wolves cannot be allowed to curtail the abundance, distribution or density of wolves to the extent that wolves do not serve to meaningfully limit coyote numbers.

Two metrics to assess conservation of the gray wolf's ecosystems follow logically from that summary of trophic cascades above. Establishing these metrics in Colorado would also entail baseline research prior to restoration, and ongoing research as the experimental wolf population (hopefully) makes progress toward recovery.

⁵¹ Hoskinson (2018), pp. 19-20.

⁵² Paquet, P.C., Vucetich, J., Phillips, M.L., and L. Vucetich. 2001. Mexican wolf recovery: three year program review and assessment. Prepared by the Conservation Breeding Specialist Group for the United States Fish and Wildlife Service. 86 pp.; p. 47.

The population of wolves should be large enough and sufficiently well-distributed throughout western Colorado so as to influence the behavior and/or distribution of elk sufficient to restore or nearly restore (with an explanation as to why wolves cannot fully restore) the natural riparian and hydrological functioning of significant stretches (that the Service should identify in the final rule after taking public comment on the draft supplemental EIS) of the state's rivers, streams and other wetland habitats.

Secondly, a population of wolves should be large enough and sufficiently well-distributed throughout Colorado so as to reduce the number and/or change the distribution of coyotes sufficiently to increase populations and/or aid the sustainability of each of the following four species: pronghorn, swift fox, black-footed ferret, and Canada lynx.

These two ecological goals – improvement of riparian habitats in specific waterways, and increases in numbers and/or sustainability of pronghorn, swift fox, black-footed ferret and Canada lynx – should constrain authorizations for the killing of wolves to ensure that wolf mortality would not impede attainment of those goals.

3. Conservation of a Colorado wolf population will require public lands to serve as refugia.

The final rule should not allow the killing of wolves if the reason for such contemplated wolf-killing was in response to wolves killing livestock on public lands. Such a measure would largely serve to turn the public lands in Colorado – national forests, national grasslands, national parks, national monuments, Bureau of Land Management holdings, and state-owned lands – into refugia in which conservation of the experimental population would be prioritized. The measure reflects that most of the wolf habitat in Colorado is on the public lands, and that conservation of the wolf's ecosystem would take place primarily (though not entirely) on public lands.

Such a measure also satisfies equitable considerations. Private landowners presumptively enjoy the expectation of greater rights on their private lands than any one person would expect on public lands owned by all. If wolves kill livestock on private lands, within the limits of satisfying recovery goals, and within the limits set out in the following section, the Fish and Wildlife Service should have the authority to allow the killing of the wolves that are directly responsible. The Service should not assume or confer any such authority – nor should any other entity – in instances of wolves killing livestock on public lands. The fact that such wolves would be legally protected would serve as a powerful incentive for livestock owners using the public lands to appropriately and non-lethally protect their livestock from wolves.

4. Wolves who scavenge on non-wolf-killed livestock carrion should be immune to being killed.

The final rule should require that on both private and public lands, ranchers properly dispose of carcasses of livestock that die from non-wolf causes, so as to render the carrion entirely unavailable or unpalatable to wolves and thereby prevent scavenging and ensuing predation on live stock.

Livestock die before they are destined for slaughter for a variety of causes, including from illness, birthing problems, eating poisonous plants, and predation by mountain lions, black

bears and coyotes. Once wolves scavenge on carrion from domestic animals, they may stay closer to the area where they found such food, and therefore may be more likely to end up preying on vulnerable nearby livestock.

A 2001 independent scientific review of Mexican wolf reintroduction program recommended that the U.S. Fish and Wildlife Service require livestock owners to take responsibility for carcass removal or disposal, noting that at least three packs had been removed from the wild because they first scavenged on dead livestock left on national forest land.⁵³ The American Society of Mammalogists reiterated this recommendation for Mexican wolf recovery in 2007.⁵⁴ In 2011, researchers who examined wolf scavenging on livestock carrion and their predation behaviors in Alberta, Canada, recommended “sanitary disposal of dead livestock to prevent wolves from becoming accustomed to feeding on livestock.”⁵⁵

Oregon and Washington both provide some degree of protection for wolves by premising potential removal actions on the wolves not having been baited. In Oregon, the wolf management plan requires that “[n]o identified circumstances exist that are attracting wolf-livestock conflict” before wolves can be targeted for removal.⁵⁶ In Washington, the management plan similarly premises the killing of wolves on “no evidence of intentional feeding or unnatural attraction of wolves by the livestock owner.”⁵⁷

In reintroducing northern Rocky Mountain gray wolves to Yellowstone National Park and central Idaho in 1995, the Fish and Wildlife Service premised the removal of livestock-predating wolves on their not having been attracted to livestock in the place where they were predating:

The Service and authorized agencies of the Service would use the following conditions and criteria to determine the status of problem wolves within the non-essential experimental population area . . . (2) No evidence of artificial or intentional feeding of wolves can be present. Improperly disposed of livestock carcasses located in the area of depredation will be considered attractants.⁵⁸

For restoration of wolves to Colorado, in order to prevent wolves from scavenging, livestock carcasses can be doused in gasoline and burned, when there is snow on the surrounding ground or otherwise it is safe. With road access, carcasses can be removed or buried by backhoe. A carcass can even be exploded into pieces too small to attract a wolf via dynamite. But perhaps the most versatile technique is to dump lime, which can be brought in on horseback, on the carcass to immediately decompose it. Colorado Parks and Wildlife and other agencies can help livestock owners to accomplish these tasks. The expenses to the public would be lower than in

⁵³ Paquet et al (2001), pp. 67-68.

⁵⁴ Reintroduction and conservation of the Mexican gray wolf, eighty-seventh annual meeting American Society of Mammalogists. 2007. *Journal of Mammalogy*, 88(6):1570–1576, 2007; p. 1573.

⁵⁵ Morehouse, A.T. and M.S. Boyce. 2011. From venison to beef: seasonal changes in wolf diet composition in a livestock grazing landscape. *Front Ecol Environ* 2011; doi:10.1890/100172; p. 1.

⁵⁶ Oregon Wolf Conservation and Management Plan, p. 51.

⁵⁷ Wiles, G. J., H. L. Allen, and G. E. Hayes. 2011. Wolf conservation and management plan for Washington. Washington Department of Fish and Wildlife, Olympia, Washington, p. 88.

⁵⁸ 59 Fed. Reg. 60272 (Nov. 22, 1994).

reimbursing ranchers for their losses after wolves potentially begin to hunt livestock as a possible consequence of having scavenged.

The final rule should create conditions conducive to the vast majority of owners of domestic livestock choosing to remove or render inedible the remains of their animals that die of non-wolf causes before any wolves scavenge on them; in essence, such a standard should be required in the final rule. To ensure enforcement, as well as equity, wolves that scavenge on domestic livestock that died of non-wolf causes should not be killed for killing livestock afterwards. Such a measure would provide an incentive for each rancher to not permit the conditions (through leaving carrion for wolves to scavenge) by which any wolf or wolves would acquire legal immunity that might negatively affect other livestock owners in the community.

5. Mexican gray wolves should be introduced into southwestern Colorado.

The final rule should direct establishment of the experimental population through acquiring animals from two sources. Gray wolves taken from areas in Wyoming, Idaho and/or Montana where these animals are subject to widespread killing by people, should be captured alive and released in west-central and northwestern Colorado. And Mexican gray wolves taken from captivity should be released in the San Juan Mountains of southwestern Colorado, as recommended by scientists who advised that Mexican wolf recovery will require a population of this subspecies in the southern Rocky Mountains, which would have connectivity to the existing population at the southeastern edge of the Mogollon Plateau in Arizona and New Mexico, and also would have connectivity to a conceivable future population of Mexican wolves in the Grand Canyon ecoregion. These three connected populations would provide the genetic diversity and resilience to ensure recovery.⁵⁹

Occasional interbreeding of wolf subspecies in Colorado would benefit the existing Mexican wolf population in southwestern New Mexico and southeastern Arizona, which would be connected through long-distance dispersing wolves. The benefit would come in the form of diversifying an impoverished gene pool of the current Mexican wolf population, without doing so to such an extent as to swamp with northern wolf genes the gene pool of the Mexican wolf where it exists in the wild in the Southwest today.⁶⁰

Conclusion

The final environmental impact statement must contain more alternatives and more specificity and be based on more recent and more reliable science and the experience with Mexican wolves. The preferred rule as written is unlawful and cannot be selected as the final rule. The final rule must meet the requirements of the Endangered Species Act and specifically must further the purposes of the Act to conserve endangered wolves and their ecosystems. Constraining the killing of wolves through the standards and the metrics that we have explained

⁵⁹ Carroll, C., R.J. Fredrickson and R.C. Lacy. 2014. Developing Metapopulation Connectivity Criteria from Genetic and Habitat Data to Recover the Endangered Mexican Wolf. *Conservation Biology* 28(1):6–86

⁶⁰ Hedrick, P., R. Wayne and R. Fredrickson. 2018. Genetic rescue, not genetic swamping, is important for Mexican wolves. *Biological Conservation*. [Issue and page-number unknown.] Hedrick, P. 2016. Genetic and recovery goals for the Mexican wolf. *Biological Conservation*. And “Genetic Aspects of Mexican Wolf Expansion into Colorado,” commentary by Dr. Phil Hedrick (February 18, 2023).

in this letter, and introducing Mexican gray wolves into southwestern Colorado, must be undertaken to fulfill that mandate.

Thank you for your consideration.

Sincerely,

A handwritten signature in black ink, appearing to read "Michael J. Robinson". The signature is fluid and cursive, with a large, prominent initial "M" and "R".

Michael J. Robinson, Senior Conservation Advocate
Center for Biological Diversity

P.O. Box 1727
Silver City, New Mexico 88062

APPENDIX IN PAGES TO FOLLOW

Mutation and Conservation

RUSSELL LANDE

Department of Biology, University of Oregon, Eugene, OR 97403, U.S.A., email russ@aylmer.uoregon.edu

Abstract: *Mutation can critically affect the viability of small populations by causing inbreeding depression, by maintaining potentially adaptive genetic variation in quantitative characters, and through the erosion of fitness by accumulation of mildly detrimental mutations. I review and integrate recent empirical and theoretical work on spontaneous mutation and its role in population viability and conservation planning. I analyze both the maintenance of potentially adaptive genetic variation in quantitative characters and the role of detrimental mutations in increasing the extinction risk of small populations. Recent experiments indicate that the rate of production of quasineutral, potentially adaptive genetic variance in quantitative characters is an order of magnitude smaller than the total mutational variance because mutations with large phenotypic effects tend to be strongly detrimental. This implies that, to maintain normal adaptive potential in quantitative characters under a balance between mutation and random genetic drift (or among mutation, drift, and stabilizing natural selection), the effective population size should be about 5000 rather than 500 (the Franklin-Soulé number). Recent theoretical results suggest that the risk of extinction due to the fixation of mildly detrimental mutations may be comparable in importance to environmental stochasticity and could substantially decrease the long-term viability of populations with effective sizes as large as a few thousand. These findings suggest that current recovery goals for many threatened and endangered species are inadequate to ensure long-term population viability.*

Mutación y conservación

Resumen: *La mutación puede afectar críticamente la viabilidad de poblaciones pequeñas al causar la depresión de endocría, mantener la variación genética potencialmente adaptativa en caracteres cuantitativos, y por medio de la erosión de la condición por acumulación de mutaciones levemente perjudiciales. En el presente estudio revisé e integré trabajos empíricos y teóricos recientes sobre mutaciones espontáneas y su papel en la viabilidad de las poblaciones y la planificación para la conservación. Se analizó tanto el mantenimiento de la variabilidad genética potencialmente adaptativa en los caracteres cuantitativos como el papel de las mutaciones perjudiciales en el incremento de riesgo de extinción de las poblaciones pequeñas. Experimentos recientes indican que la tasa de producción de varianza genética cuasineutral y potencialmente adaptativa en los caracteres cuantitativos es de un orden de magnitud menor que la varianza mutacional total debido a que las mutaciones con efectos fenotípicos pronunciados tienden a ser fuertemente perjudiciales. Esto implica que a efecto de mantener el potencial adaptativo normal en los caracteres cuantitativos bajo un balance entre mutación y deriva génica al azar (o, entre mutación, deriva génica y selección natural estabilizadora), el tamaño poblacional efectivo debe ser de aproximadamente 5000 y no 500 (numero de Franklin-Soulé). Resultados teóricos recientes sugieren que el riesgo de extinción debido a la fijación de mutaciones levemente perjudiciales podría ser comparable en importancia a la estocasticidad ambiental y podría reducir substancialmente la viabilidad a largo plazo de las poblaciones con un tamaño poblacional efectivo de solo unos pocos miles. Estos descubrimientos sugieren que las metas de recuperación para muchas especies en peligro y amenazadas son inadecuadas para asegurar la viabilidad poblacional a largo plazo.*

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Introduction

Mutation is the ultimate source of all genetic variation (Dobzhansky 1970). Different kinds of genetic variation can critically affect population viability, especially in small populations. Deleterious (partially) recessive mutations, such as recessive lethal alleles, contribute to inbreeding depression in fitness, which increases the risk of extinction. Mildly detrimental mutations accumulate and can become fixed by random genetic drift, gradually eroding fitness and increasing extinction risk. Quasineutral, potentially adaptive genetic variance in quantitative characters maintained by mutation becomes diminished by inbreeding and random genetic drift, reducing the ability of a population to adapt and persist in a changing environment.

Measurements of the rates at which different types of mutations arise spontaneously have been used in conjunction with population genetic theories to suggest minimum population sizes for different goals in species conservation, such as avoiding inbreeding depression, maintaining potential for adaptive evolution, and avoiding genetic erosion of fitness from the accumulation of detrimental mutations (Franklin 1980; Soulé 1980; Lynch & Gabriel 1990; Lynch et al. 1993). I review and integrate recent experimental and theoretical work on spontaneous mutation and discuss how this affects our understanding of conservation goals for threatened and endangered species. Following a brief review of the genetic basis of inbreeding depression, I summarize information on spontaneous mutations affecting fitness and quantitative (polygenic) characters. Drawing from recent experimental and theoretical results, I then reconsider the role of mutation in the maintenance of potentially adaptive genetic variance in quantitative characters and the risk of population extinction from fixation of new detrimental mutations.

Inbreeding Depression

Decrease in the mean value of a character upon inbreeding is known as inbreeding depression, which results from the segregation of partially recessive mutations within populations. In historically large outbreeding populations, rapid inbreeding decreases the mean of characters—such as body size—and of major fitness components—such as seed yield in corn or litter size in mice and pigs—by a few percentage points to 10% or more for every 10% increase in the inbreeding coefficient (Falconer 1989; Ralls & Ballou 1983). In most naturally outbreeding species, close inbreeding—as by brother-sister mating—results in rapid extinction of a high percentage of lines (Soulé 1980).

A typical human or *Drosophila* fly carries one or two nearly recessive lethal or sublethal mutations in heterozygous form, while in some species of coniferous

trees individuals may be heterozygous for five to ten recessive lethal equivalents (Sorenson 1969; Dobzhansky 1970). Habitual inbreeding decreases this hidden (recessive) genetic load by eliminating recessive lethal and sublethal mutations from the population when they are expressed in homozygous form. Predominantly self-fertilizing plant species therefore manifest reduced levels of inbreeding depression for fitness (Lande & Schemske 1985; Charlesworth & Charlesworth 1987).

The genetic basis of inbreeding depression for fitness has been studied more carefully in *Drosophila* than in other species. Using special genetic techniques available in *Drosophila*, chromosomes have been sampled intact, without recombination, from wild populations and tested in homozygous and in heterozygous form. The distribution of egg-to-adult viability in chromosomal heterozygotes is approximately normal, with a standard deviation of several percent of the mean. In chromosomal homozygotes, egg-to-adult viability has a strongly bimodal distribution, with a lethal and sublethal mode and a quasnormal mode that has a smaller mean and larger variance than that of chromosomal heterozygotes (Dobzhansky 1970). Genetic and statistical analysis shows that about half of the inbreeding depression in viability is caused by rare, nearly recessive lethal and sublethal point mutations, with the remainder attributable to numerous mildly detrimental mutations of small, more nearly additive effect (Simmons & Crow 1977). There is little evidence that overdominance (heterozygote advantage) contributes substantially to inbreeding depression (Charlesworth & Charlesworth 1987, 1990; Crow 1993).

The amount of inbreeding depression manifested by a population depends on the rate of inbreeding and the opportunity for selection to purge recessive lethal and semilethal mutations; this important point is neglected in some recent models that therefore overestimate the impact of inbreeding depression on population viability (Senner 1980; Halley & Manasse 1993; Mills & Smouse 1994). Gradual inbreeding by incremental reductions in population size over several or many generations allows selection to eliminate at least the lethal and sublethal mutations when they become homozygous (Falconer 1989). In contrast, the component of inbreeding depression from more nearly additive mutations of small effect may be difficult or impossible to purge by inbreeding, and even habitually self-fertilizing plants show considerable inbreeding depression manifested as heterosis (increased fitness) upon crossing different lines (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Charlesworth et al. 1990; Hedrick 1994).

Mutation Rates

In *Drosophila melanogaster* about 5000 lethal-producing loci mutate at an average rate of $\bar{\mu} = 2 \times 10^{-6}$ per allele per generation, giving a gametic mutation rate of

10^{-2} per generation. Lethal and sublethal mutations are not completely recessive, decreasing the fitness of heterozygotes by about 2% on average (Simmons & Crow 1977). In large populations, selection keeps such strongly deleterious mutations at low equilibrium frequencies ($\approx 4 \times 10^{-4}$). The expected frequency of slightly dominant lethal and sublethal mutations in a population of constant size is approximately independent of population size, provided that the effective population size, N_e , is large enough for selection to keep them at low frequency ($N_e \gg 2$) (Crow & Kimura 1970:448-449). N_e refers to an ideal population of constant size, reproducing by random union of gametes, that gives the same rate of random genetic drift as an actual population of size N . Because of temporal fluctuations in population size, greater than Poisson variance in reproductive success, and unequal sex ratio, N_e is generally substantially less than N (Wright 1969; Crow & Kimura 1970; Lande & Barrowclough 1987).

Mildly detrimental mutations in *Drosophila melanogaster* have a heterozygote disadvantage of about one-third to one-half that of homozygotes (Mukai et al. 1972; Crow & Simmons 1983). Upper bounds for the average homozygous effect of mildly detrimental mutations are 4%-5% for viability (Simmons & Crow 1977; Crow & Simmons 1983) and about twice as large for fitness (Houle et al. 1992). These estimates are upper bounds because they neglect variation in selection coefficients among new mutations, which would decrease the estimated average effect by a factor of $1/(1 + c^2)$. c is the coefficient of variation (standard deviation/mean) of the effects of mildly detrimental mutations on fitness (Crow & Simmons 1983), which is likely to be substantial, perhaps of order 1 (Keightley 1994; Lande 1994). We therefore assume that mildly detrimental mutations have additive effects with an average heterozygous fitness loss of 2.5%. Such slightly deleterious mutations arise frequently, with total genomic mutation rates on the order of $U \approx 1$ per generation (Mukai et al. 1972; Mukai 1979), and they have a much higher probability of fixation than do mutations of large effect if N_e exceeds a few individuals.

The rate of phenotypic divergence by random genetic drift among replicated, highly inbred lines, and/or the rate of response in the mean phenotype to artificial selection on a highly inbred line, can be used to measure the genomic mutation rate and the production of genetic variance by spontaneous mutation in quantitative characters. For typical quantitative traits of plant and animal morphology, in excess of one in a hundred gametes contains a new mutation with a small effect on any character (Sprague et al. 1960; Russell et al. 1963; Hoi-Sen 1972). Thus, the (diploid) genomic mutation rate summed over all loci producing mutations that affect a particular character, $U = 2\sum\mu_i$, is typically about 0.02 per generation. For a variety of quantitative characters in plants and animals, the additive genetic variance created

each generation by spontaneous mutation, V_m , is roughly 10^{-3} times the environmental (nongenetic) variance in the character that would be expressed in a vigorous inbred line, V_e (Lande 1975; Lynch 1988). Assuming that mutational changes are symmetric (equally often increasing and decreasing the character), the average squared mutational effect is then about $\alpha^2 = 0.05V_e$. Recent experiments with *Drosophila* indicate that nearly half of these mutations, especially those with large phenotypic effects, are highly detrimental (recessive lethal), and that the rate of production of quasineutral mutational variance per generation, denoted as \tilde{V}_m (which is likely responsible for much of the standing variation in quantitative traits of natural populations), is roughly an order of magnitude lower, $\tilde{V}_m \approx 10^{-4}V_e$ (Mackay et al. 1992; López & López-Fanjul 1993a, 1993b). Thus, in comparison to the total set of mutations affecting a typical quantitative character, the genomic mutation rate is about half as large for quasineutral mutations, $U = 0.01$, and the mean squared mutational effect is about one-fifth as large, $\alpha^2 = 0.01V_e$.

Maintenance of Potentially Adaptive Genetic Variance

Franklin (1980) and Soulé (1980) proposed that $N_e = 500$ is sufficient for long-term maintenance of genetic variability in quantitative characters, and this number was quickly adopted as the basis of management plans for captive as well as wild populations (Lande 1988). The Franklin-Soulé number was derived by assuming a balance between mutation and random genetic drift and by using experimental measurements of mutability of quantitative characters to determine an effective population size sufficient to maintain typical amounts of heritable variation and adaptive potential (Lande & Barrowclough 1987). Explicit connections between additive genetic variance, adaptation, and population persistence were later analyzed by Lynch and Lande (1993), Bürger and Lynch (1994) and Lande and Shannon (1995).

Mutation in a Finite Population

Quantitative characters of morphology, physiology, and behavior are of great importance in adaptation to natural environments. The rate of evolution of the mean phenotype in response to directional natural selection is proportional to the additive genetic variance (the heritable portion of the genetic variance responsible for the resemblance between relatives) when selection acts on a single character (Falconer 1989), or to the additive genetic variance-covariance matrix when selection acts on a set of correlated characters (Lande & Arnold 1983).

Let V_g be the (purely) additive genetic variance in a quantitative character. Assuming a wide range of possi-

ble allelic effects at each locus, with a constant rate and distribution of mutational changes to altered allelic effects on the character, the input of additive genetic variance from mutation each generation is a constant, V_m . In a diploid randomly mating population, the expected rate of loss of heterozygosity, or additive genetic variance in a quantitative trait, due to random genetic drift in the absence of selection is $1/(2N_e)$ per generation (Wright 1931; Lande & Barrowclough 1987). Measuring time, t in generations, the expected value of the additive genetic variance, \bar{V}_g , under random genetic drift and mutation obeys the dynamics

$$\frac{d\bar{V}_g}{dt} = -\frac{\bar{V}_g}{2N_e} + V_m \quad (1)$$

At equilibrium between mutation and random genetic drift, the expected genetic variance is $\bar{V}_g = 2N_e V_m$. The heritability of a character is the proportion of the total phenotypic variance due to additive effects of genetic variation. For quantitative characters of morphology, physiology, and behavior, excluding major components of fitness such as viability and fecundity, heritabilities usually range between 0.2 and 0.8 (Mousseau & Roff 1987; Falconer 1989). Assuming a typical heritability of 0.5, so that $\bar{V}_g = V_e$, using $V_m = 10^{-3}V_e$ and solving for the effective population size gives $N_e = 500$.

If we incorporate the finding that only about 10% of the spontaneous mutational variance is quasineutral (López & López-Fanjul 1993a, 1993b), we should substitute $\bar{V}_m \cong 10^{-4}V_e$ for V_m into the above formulas, and the Franklin-Soulé number would have to be increased by a factor of 10, to $N_e = 5000$.

Mutation and Stabilizing Selection in a Finite Population

Natural selection on quantitative characters (other than major components of fitness) generally favors an intermediate optimum phenotype that may fluctuate with time. Weak stabilizing selection on a quantitative character can be described by a bell-shaped (Gaussian) curve giving the fitness per generation as a function of the individual phenotype. The strength of stabilizing selection is measured by V_s , the "width" (or variance analog) of the fitness function. Stabilizing selection acting directly on a given character and indirectly on correlated characters (Lande & Arnold 1983) decreases the phenotypic variance of a typical trait within a generation by a few to several percent (Johnson 1976; Endler 1986). The total strength of (direct + indirect) stabilizing selection on a typical character is therefore about $V_s \cong 25V_e$.

The expected additive genetic variance maintained by mutation in a finite population for a quantitative character under stabilizing selection is accurately described by the formula

$$\bar{V}_g = \frac{2N_e V_m V_g(\infty)}{2N_e V_m + V_g(\infty)} \quad (2)$$

$V_g(\infty)$ is the additive genetic variance that would be maintained at mutation-selection equilibrium in an infinitely large population (Bürger & Lande 1994). Two approximations for the amount of additive genetic variance maintained in an infinite population under the polygenic mutation model described above are known as the house-of-cards model and the Gaussian allelic model.

The house-of-cards model assumes a large number of loci, n , and low mutation rates per locus, μ , such that with a typical strength of stabilizing selection each locus has one common "wild-type" allele in high frequency and rare mutant alleles with relatively large effects. Under the house-of-cards approximation, $V_g(\infty) \cong 4n\mu V_s$ (Turelli 1984; Keightley & Hill 1988; Bürger et al. 1989; Houle 1989).

The Gaussian allelic model assumes a relatively small or moderate effective number of loci, N_E , with high per-locus mutation rates such that each locus has several or many segregating alleles per locus, with an approximately Gaussian distribution of small phenotypic effects. Under the Gaussian allelic approximation, $V_g(\infty) \cong \sqrt{(2n_E V_m V_s)}$, where n_E is typically around 5 or 10 (Kimura 1965; Lande 1975, 1981).

In the house-of-cards approximation, the additive genetic variance maintained in an infinite population with $V_s = 25V_e$ and $U = 2n\mu = 0.02$ is $V_g(\infty) = 1.0V_e$. In the Gaussian allelic approximation, with $V_s = 25V_e$, $V_m = 10^{-3}V_e$, and $n_E = 10$, a somewhat lower additive genetic variance is maintained in an infinite population, $V_g(\infty) = 0.71V_e$. These correspond, respectively, to heritabilities of 0.50 and 0.42, both of which are in the typical range for quantitative characters (0.2 to 0.8; Mousseau & Roff 1987; Falconer 1989).

If we exclude recessive lethal mutations and include only the quasineutral, potentially adaptive mutations, the additive genetic variance maintained in an infinite population under the house-of-cards approximation becomes $V_g(\infty) = 0.50V_e$ and under the Gaussian allelic approximation, assuming n_E remains at 10, the additive genetic variance becomes $V_g(\infty) = 0.225V_e$. These correspond, respectively, to the reduced heritabilities of 0.33 and 0.18. It should be noted, however, that mutation is only one of several factors contributing to the maintenance of potentially adaptive genetic variance, including gene flow among differentiated populations, and density- and frequency-dependent competition (Felsenstein 1977; Slatkin 1979). The segregation of rare excessive lethal mutations contributes only a small fraction of the additive genetic variance in most quantitative characters (Falconer 1989).

Figure 1 shows the expected additive genetic variance maintained by mutation as a function of N_e for typical quantitative characters under the two approximations. The effective population size at which the expected additive genetic variance is equal to a proportion p of that

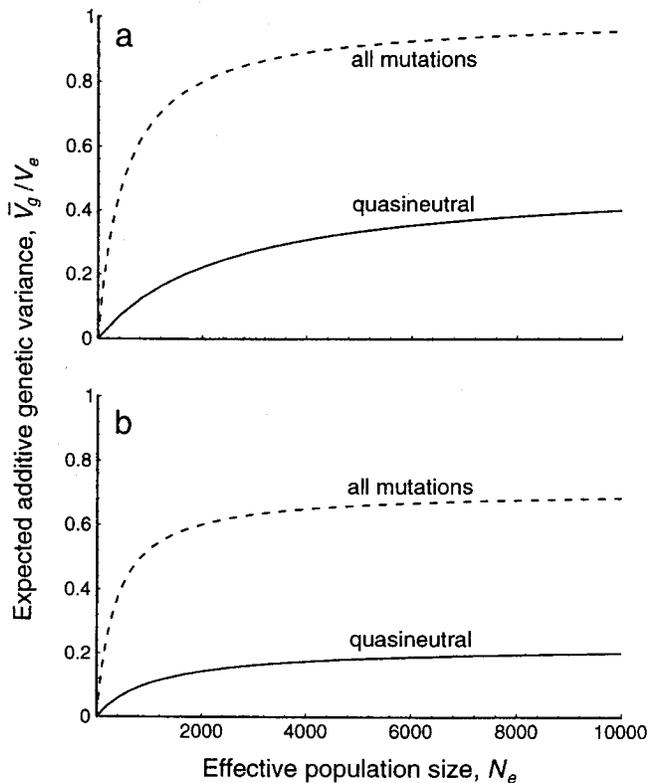


Figure 1. Expected additive genetic variance, \bar{V}_g , relative to environmental variance, V_e , maintained by mutation in a typical quantitative character under stabilizing selection in a finite population: house-of-cards approximation (a) and Gaussian allelic approximation (b). Dashed lines include all mutations; solid lines include only quasineutral mutations, excluding recessive lethals.

maintained in an infinitely large population is from equation 2, $N_e^* = [p/(1-p)]V_g^{(\infty)}/(2V_m)$. Table 1 gives numerical values of N_e^* corresponding to different values of p under both approximations. For example, under the house-of-cards approximation, including all mutations (ignoring recessive lethal effects), a population with $N_e = 1000$ is expected to maintain 67% of the additive genetic variance maintained in an infinite population; under the Gaussian allelic approximation, a population with $N_e = 707$ is expected to maintain 67% of the additive genetic variance maintained in an infinite population. Including only quasineutral mutations N_e would have to be five times larger (5000) in the house-of-cards approximation and 3.15 times larger in the Gaussian allelic approximation (2236) to maintain 67% of the additive genetic variance in an infinite population.

The house-of-cards approximation is most accurate for loci with relatively large effects and low mutation rates, and the Gaussian allelic approximation is most accurate for loci with relatively small effects and high mutation rates. Because both types of loci probably contribute to

variance of quantitative characters, the actual amounts of additive genetic variance maintained by mutation are likely to be between these two approximations. Excluding recessive lethal mutations, and whether or not we include stabilizing selection, it therefore appears that the effective population size necessary to maintain a high proportion of the potentially adaptive, additive genetic variance that would occur in a large population requires effective population sizes an order of magnitude larger than the original Franklin-Soulé number, increasing the management goal from $N_e = 500$ to $N_e = 5000$.

Extinction from Fixation of New Mutations

Small populations risk extinction from a variety of genetic and demographic factors, including inbreeding depression as well as the fixation of new detrimental mutations. For large populations that have been reduced suddenly to a small size, inbreeding depression based on segregating detrimental mutations carried by the founders constitutes an important risk of rapid extinction (Soulé 1980). For populations that are reduced in size more gradually, the fixation of new detrimental mutations poses a more serious risk of eventual extinction. In this section I examine how the risk of extinction from fixation of new mutations scales with population size and how this compares with the risks from stochastic demographic factors.

Most mutations are either quasineutral or detrimental. In populations with effective sizes larger than a few individuals, fixations of new, mildly detrimental mutations are far more important in causing loss of fitness and eventual extinction than are new, lethal and semilethal mutations. This is because strongly detrimental mutations have an exceedingly low chance of fixation in comparison to selectively neutral mutations, which have a relatively high chance of fixation but do no damage to the population. Lande (1994) analyzed the fixation of new, mildly detrimental mutations in a randomly mating population of constant size. Mildly detrimental mutations were assumed to have additive effects on Malthu-

Table 1. Effective population sizes, N_e , required to maintain an expected additive genetic variance equal to a given fraction p of that in an infinite population including either all mutations or only quasineutral mutations (excluding recessive lethals).

Mutations	Approximation	p			
		0.50	0.67	0.75	0.80
All	house-of-cards	500	1000	1500	2000
	Gaussian allelic	354	707	1061	1414
Quasineutral	house-of-cards	2500	5000	7500	10,000
	Gaussian allelic	1118	2236	3354	4472

sian fitness (population growth rate), with a fitness decrement of s per heterozygote and $2s$ per mutant homozygote. He found that the magnitude of selection coefficient that minimizes the mean time to extinction is about $\hat{s} = 0.4/N_e$, which is close to the border between neutrality and selection defined by Wright (1931, 1969), $s \leq 1/(2N_e)$. For this reason, and because they arise at much higher spontaneous rates than do recessive lethal mutations, mildly detrimental mutations on the border of neutrality are the most damaging to population viability.

Risks of Extinction from Genetic Stochasticity

To analyze the risk of population extinction from fixation of new mutations, and to compare this to extinction risks from demographic factors, Lande (1994) modeled a randomly mating population with no demographic or environmental stochasticity. Population size was assumed to remain constant as long as the mean Malthusian fitness, r (the intrinsic rate of increase of the population), is positive. This model accounts only for unconditionally detrimental mutations. Stochastic fixation of mildly detrimental mutations gradually erodes the mean Malthusian fitness until r becomes negative—the population then can no longer replace itself and declines relatively rapidly to extinction (Lynch et al. 1993). Lande (1994) derived analytical approximations for the mean time until the population becomes genetically inviable (or extinct) in the situation where all mildly detrimental mutations have the same selection coefficient, s against heterozygotes and $2s$ against homozygotes. Lynch et al. (1995a) analyze this situation in more detail and perform computer simulations to check the analytical results. Lande (1994) also analyzed the influence of variance in selection coefficients among new mutations. The results on the scaling of mean time to extinction with effective population size are as follows.

With a constant selection coefficient against new mutations, s , the mean time to extinction, \bar{t} , is a nearly exponential function of effective population size, N_e . Because the mean time to extinction is such a rapidly increasing function of N_e (see Fig. 2), with values of s around a few percent, this indicates that the fixation of new mutations poses little risk of extinction for populations with effective size about 100 individuals (Lande 1994). This conclusion is in agreement with extensive simulation results of Charlesworth et al. (1993). However, the inclusion of variance in selection coefficients in the model drastically decreases the mean time to extinction.

With variance in s , the mean time to extinction increases as a power of population size. If s is exponentially distributed, then \bar{t} is asymptotically proportional to N_e^2 . This more gradual increase in \bar{t} with population size, shown in Figure 2, indicates a much greater risk of

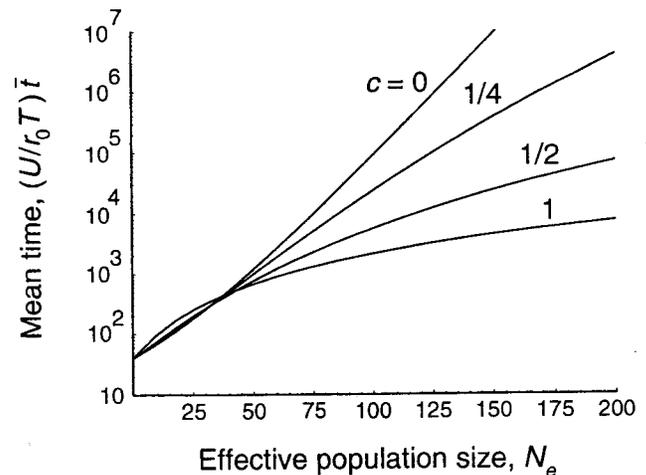


Figure 2. Mean time until extinction, in generations, as a function of effective population size, N_e , due to fixation of new mutations. U is the genomic rate of mildly deleterious mutations, r_0 is the initial Malthusian fitness (intrinsic rate of increase), and T is the generation time. Selection coefficients against mildly deleterious mutations follows a gamma distribution with coefficient of variation c . For $c = 1$, the selection coefficients are exponentially distributed. Mutations have additive effects on Malthusian fitness, with average selection coefficient $\bar{s} = 0.025$ against heterozygotes. (From Lande 1994.)

extinction than in the case of a constant selection coefficient. For reasonable variance in s (a coefficient of variation of about $c = 1$), it appears that fixation of new, slightly deleterious mutations poses a considerable risk of extinction for populations as large as a few thousand individuals. Numerical examples appear in Table 2. These results, in conjunction with data on the rate and magnitude of mildly detrimental mutations in *Drosophila melanogaster*, indicate that the long-term viability of even moderately large populations, with effective sizes of a few thousand (and actual sizes of several or many thousands), may be substantially reduced by the fixation of new mutations.

RELATIVE RISKS FROM GENETIC AND DEMOGRAPHIC FACTORS

It is illuminating to compare the risks of population extinction from genetic stochasticity, due to fixation of new mutations, with the risks from demographic and environmental stochasticity. The relative risks of population extinction from different stochastic factors can be assessed by comparing asymptotic scaling relationships describing how the average time to extinction, \bar{t} , increases with equilibrium population size (or carrying capacity) under each stochastic factor alone. Although the actual extinction risk for a population of a given effec-

Table 2. Scaled mean times to extinction, $(U/r_0T)\bar{t}$, in generations (from Lande 1994).

Effective Population Size, N_e	$(U/r_0T)\bar{t}^*$	
	constant s	variable s
2	44	49
5	52	65
10	69	99
20	128	193
50	1179	677
100	8.8×10^4	2149
200	9.7×10^8	7690
500	4.1×10^{21}	4.4×10^4
1000	1.1×10^{43}	1.7×10^5
2000	1.4×10^{86}	6.7×10^5

* U is the genomic rate of mildly deleterious mutations, r_0 is the initial Malthusian fitness (intrinsic rate of increase), and T is the generation time. The selection coefficient, s , against mildly deleterious mutations either is constant or has an exponential distribution with $\bar{s} = 0.025$. For most species, the scale factor r_0T/U is likely to be in the range of about 1 to 10.

tive size depends on details of life history, ecology, and genetics, the relative risks associated with different factors are, for sufficiently large populations, determined predominantly by the relative rates at which \bar{t} increases with population size under each factor alone.

Under demographic stochasticity alone, due to chance events in individual birth and death, \bar{t} increases nearly exponentially with population size. Under either environmental stochasticity or random catastrophes, which affect the birth and death rates of all individuals in a population in approximately the same way, \bar{t} increases as a power of population size (Lande 1993). Thus, the risk of extinction from fixation of new mutations with a constant selection coefficient appears comparable to that of only the weakest demographic factor, demographic stochasticity. In contrast, with reasonable variance in selection coefficients, the fixation of new detrimental mutations poses an extinction risk potentially comparable to that of the strongest factor, environmental stochasticity (Table 3).

Nevertheless, Table 2 and Figure 2 show that in very small populations that have survived the inbreeding depression caused by founder effects and still retain a substantially positive r , environmental stochasticity is more likely than genetic stochasticity to cause extinction because the mean time to extinction due to fixation of new deleterious mutations is on the order of 100 generations or more. Of course, for populations on the brink of extinction, with r near zero, all risk factors and their interactions may be important.

LIMITATIONS OF THE MODELS

Several factors could modify the mean persistence times of populations in this simple model. The time required

Table 3. Asymptotic scaling laws for mean time to extinction, \bar{t} , as a function of the equilibrium population size, N , or effective population size, N_e , for demographic and genetic risk factors.

Risk Factor	Proportional Scaling of \bar{t}^*
Demographic Stochasticity	$(1/N)e^{2Nr/V_1}$
Environmental Stochasticity	$N^{2\bar{s}}V_e^{-1}$
Fixation of New Mutations	
constant s	$(1/N_e)e^{4N_e s}$
variance in s	N_e^{1+1/c^2}

* r is the expected population growth rate (or mean Malthusian fitness); V_1 is the demographic variance in Malthusian fitness among individuals; \bar{s} is the mean population growth rate; V_e is the (temporal) environmental variance in population growth rate; $c = \sigma_s/\bar{s}$ is the coefficient of variation of s among new mutations.

for mildly detrimental mutations to become fixed and the final decline of the population after r has become negative are both short in comparison to the mean time to reach genetic inviability, provided that the initial intrinsic rate of increase per generation (r_0T) is substantially positive and N_e is not very small (Lynch et al. 1995b). Partial recessivity of detrimental mutational effects would increase the probability of fixation and decrease the mean time to extinction. Synergistic epistatic interactions among mildly detrimental mutations, which has been documented for viability in *Drosophila melanogaster* (Mukai 1969), slows the loss of mean fitness if all mutations have the same selection coefficient (Charlesworth et al. 1993), but substantial variance in selection coefficients among new mutations is likely to minimize this effect (Lande 1994). Large populations may never become genetically inviable due to fixation of mildly detrimental mutations because selection against them is more efficient and the erosion of mean fitness may be counteracted by reverse and advantageous mutations. Finally, compensatory mutations at different loci are common, especially for quantitative characters under stabilizing selection (Fisher 1958:44-48), and even strongly deleterious mutations with major morphological effects can be gradually compensated for by the evolution of minor modifiers (Lewontin 1974:91).

Nevertheless, there may be a large class of mildly detrimental mutations that cannot be readily compensated, such as deletions of nonessential but fitness-enhancing loci that may compose the majority of single-copy genes (Cavallier-Smith 1985). The theory remains valid if the genomic mutation rate is multiplied by the proportion of mildly detrimental mutations that are unconditionally deleterious. If the fitness effects of a substantial fraction of all mildly detrimental mutations are unconditional, this would not change the result that with variance in selection coefficients the mean time to extinction is asymptotically proportional to a low power of N_e ; one would still conclude that the risk of population extinction from fixation of new mutations is potentially of

comparable importance with that from environmental stochasticity.

Discussion

Mutation has been used in two different ways in the evaluation of population viability and the design of conservation plans. Quasineutral, potentially adaptive mutations have been used to establish minimum effective population sizes for the maintenance of typical levels of additive genetic variance in quantitative characters, which are known to be of great importance in adaptive evolution. Harmful mutations also have been used to set minimum effective population sizes for avoiding the immediate consequences of inbreeding depression and for preventing the erosion of fitness by accumulation of mildly detrimental mutations.

Recent experiments accumulating spontaneous mutations in *Drosophila melanogaster* indicate that the rate of production of quasineutral, genetic variance in quantitative characters is an order of magnitude smaller than the total mutational variance in the characters, because mutations with large effects tend to be strongly detrimental (recessive lethals). This suggests that the effective population size of about 5000, rather than the Franklin-Soulé number of 500, is necessary to maintain normal levels of potentially adaptive genetic variance in quantitative characters under a balance between mutation and random genetic drift. Including stabilizing selection toward an intermediate optimum phenotype does not much affect this conclusion. Of course, $N_e = 5000$ should not be regarded as a magic number sufficient to ensure the viability of all species, because of differences among characters and among species in genetic mutability and differences in environmental fluctuations and selective pressures to which populations are exposed. Maintenance of potentially adaptive genetic variation in single-locus traits (such as major disease resistance factors), which have mutation rates on the order of 10^{-6} per allele per generation, may require much larger effective population sizes, on the order of 10^4 or 10^5 (Lande & Barrowclough 1987; Lande 1988).

In stable populations, mildly detrimental mutations on the border of neutrality do the most damage to population viability because they arise far more frequently and have a much higher chance of fixation than strongly deleterious mutations. Accounting for the high variance in selection coefficients likely to exist among new mutations, the fixation of new, mildly detrimental mutations may be comparable in importance to environmental stochasticity and could substantially reduce the long-term viability of populations with effective sizes as large as a few thousand. In contrast to the rapid extinction caused by inbreeding depression in populations suddenly re-

duced to a few individuals (Soulé 1980), the fixation of mildly detrimental mutations in a stable population, even of very small size, produces only a gradual erosion of fitness that is likely to take many generations to cause extinction.

When listed, threatened and endangered species typically have actual population sizes on the order of 100 (plants) to 1000 (animals) (Wilcove et al. 1993), and population recovery goals frequently are not much larger than at the time of listing (Tear et al. 1993, 1995; Schemske et al. 1994). The above results cast doubt on whether populations of many threatened and endangered species will maintain adequate evolutionary potential and long-term genetic viability unless they recover to much larger sizes. Effective population sizes generally are substantially lower than actual population sizes because of fluctuations in population size, high variance in reproductive success, and unequal sex ratios (Wright 1969; Crow & Kimura 1970; Lande & Barrowclough 1987); maintaining effective population sizes of several thousand in the wild therefore will usually require average actual population sizes on the order of 10^4 or more. Synergistic interactions among different genetic and demographic factors contributing to the risk of population extinction (Gilpin & Soulé 1986) are likely to cause the minimum population sizes for long-term viability of many wild species to be much larger than 10^4 .

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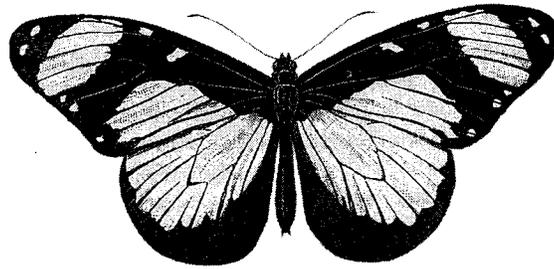
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Minimum viable populations: is there a 'magic number' for conservation practitioners?

Curtis H. Flather¹, Gregory D. Hayward^{2,3}, Steven R. Beissinger⁴ and Philip A. Stephens⁵

¹ USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO 80526, USA

² USDA Forest Service, Alaska Region, Anchorage, AK 99503, USA

³ Department of Zoology & Physiology, University of Wyoming, Laramie, WY 80271, USA

⁴ Department of Environmental Science, Policy & Management, University of California, Berkeley, CA 94720, USA

⁵ School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE, UK

Establishing species conservation priorities and recovery goals is often enhanced by extinction risk estimates. The need to set goals, even in data-deficient situations, has prompted researchers to ask whether general guidelines could replace individual estimates of extinction risk. To inform conservation policy, recent studies have revived the concept of the minimum viable population (MVP), the population size required to provide some specified probability of persistence for a given period of time. These studies conclude that long-term persistence requires ≥ 5000 adult individuals, an MVP threshold that is unaffected by taxonomy, life history or environmental conditions. Here, we re-evaluate this suggestion. We find that neither data nor theory supports its general applicability, raising questions about the utility of MVPs for conservation planning.

Conservation planning and the viability of populations

Establishing species conservation priorities and quantitative management objectives is enhanced by the ability to estimate the extinction risk faced by populations. In particular, two topical, linked concepts in conservation (triage and return on investment) often require an estimate of the risk faced by a population and the financial costs of strategies to mitigate that risk [1]. Early work on estimating extinction risk focused on population viability analysis (PVA) and related methods for estimating a threshold population size below which extinction risks were deemed unacceptably high (the so-called 'minimum viable population', MVP) [2–4] (Box 1). Formal application of these methods requires extensive, high-quality data, usually drawn from intensive, long-term studies [5–10]. Even with quality data, extinction probabilities will often be estimated with considerable uncertainty, unless populations are rapidly growing or declining [11], and forecasts of population fates should be restricted to short time horizons [10]. Unfortunately, for many species, especially those of conservation concern, quality long-term data on which to base estimates of persistence remain limited [12–14].

The need to make rapid decisions about conservation targets, often in the absence of necessary data, has prompted interest in identifying robust, general guidelines for MVPs [15,16]. Taking advantage of growing access to population and life-history data for large numbers of species, several recent papers [17–20] explore the credibility of a lower limit to robust MVPs. Despite apparent caution about overinterpreting the strength of evidence, the most recent review [20] asserts that '[t]he bottom line is that both the evolutionary and demographic constraints on populations require sizes to be at least 5000 adult individuals.' A popular science summary of the article goes further, christening 5000 adults 'a magic number' that applies to 'mammals, amphibians, insects, plants and the rest' [21]. The conservation implications of this claim are profound, because it asserts that a population threshold of 5000 must be reached or exceeded, regardless of taxon (plant, invertebrate or vertebrate) or environmental context (either short-term stressors or more fundamental properties of the local environment).

Given the importance of managing for viable populations, it is essential that conservation biologists engage in robust debate regarding MVP. Our intention here is to focus on the analyses and conclusions from recent studies that advocate a universal threshold for MVP [17–20]. We begin by considering whether ecological principles support the notion of a universally applicable MVP threshold and by outlining crucial conservation policy outcomes of recent MVP papers [17–20]. Using data from three of the key papers [17–19], we identify aspects of analysis and interpretation that do not support the existence of a universally applicable estimate of MVP. Finally, we offer suggestions for how conservationists might proceed in the absence of such an estimate.

A universal threshold for MVP?

Traill *et al.* [20] argue that conservationists working in developing countries lack the resources to estimate MVPs accurately for conservation targets and, thus, that there is 'a compelling argument to develop rules of thumb for population size extinction-risk thresholds.' By contrast,

Corresponding author: Stephens, P.A. (philip.stephens@durham.ac.uk)

Box 1. History and estimation of a MVP

The MVP concept emerged in 1981 from Shaffer's [2] pioneering paper that defined a minimum viable population as 'the smallest isolated population having a 99% chance of remaining extant for 1000 years despite the foreseeable effects of demographic, environmental, and genetic stochasticity, and natural catastrophes.' The criteria for evaluating viability (the time frame and associated extinction risk) were 'tentatively and arbitrarily' chosen by Shaffer, recognizing that risk criteria were within the purview of society as well as science. Operationally, time horizons of 50–100 years and extinction risk of 5% became the most frequently used criteria.

Shaffer [2] outlined five possible approaches for determining MVPs: experiments, biogeographic patterns, theoretical models, simulation models and genetic considerations. Experimentally manipulating the size of replicated populations and then following their trajectories is rarely possible in nature. Examining biogeographic patterns of distribution can lead to estimates of minimum area requirements, densities, or population sizes versus occupancy (i.e. incidence). However, because of the indirect tie to extinction, this approach is rarely used. Theoretical models can be used to predict the time required for a population of a given size to go extinct (see [26]), but the idiosyncratic or contextual situation that characterizes most wild populations precludes the application of such models to real-world conservation. Genetic considerations consisted of comparing an estimate of the effective size (N_e) of a population to the 50/500 'rule' of conservation genetics (i.e. an N_e exceeding 50 for short-term and 500 for long-term survivability). However, the 50/500 values of N_e are simply viability goals for maintaining genetically diverse populations; they provide little direct connection with extinction risk.

The remaining method, simulation modelling (also known as PVA), is the most general and popular approach to estimating MVPs.

A stochastic population projection model is constructed from estimates of the mean and variance of demographic rates (from studies of individuals) or from population growth rates (i.e. r or λ , estimated from time series of counts or indices). Simulation models project populations into the future using Monte Carlo methods, incorporating chance events (e.g. demographic and environmental stochasticity, genetic effects of inbreeding and catastrophes) as well as other processes that affect the population, to produce extinction probabilities at specified time periods in the future. The minimum viable population size is found by iteratively changing the initial population size to find the smallest size that has a 95% chance of remaining extant at the end of the time period evaluated in the simulation.

The initial promise of MVP estimates as conservation yardsticks faded as conservation biologists realized that estimates of extinction risk from PVA models were often imprecise, inaccurate, contingent upon threats currently acting, and affected by model structure, study duration and other uncontrolled factors [5,71–73]. Many conservation biologists recognized that PVA models were best used for ranking relative extinction risk [5,50]. The focus on MVP was reversed to emphasize the importance of PVAs for understanding the relative probability of persistence for populations in a variety of scenarios. This approach, which focused on understanding population drivers and processes, was of broader utility to land managers and conservation practitioners. When used to evaluate multiple scenarios, PVA can bring together Caughley's [25] small and declining population paradigms in a tool that helps practitioners search for solutions to conservation problems, rather than focusing only on a static, small population paradigm answer (MVP) [74].

we argue that there are compelling reasons to suspect that no single MVP is likely to apply adequately to all populations because extinction risks are often context dependent (Box 1), and manifest from a complex interaction between life history, environmental context and threat [22–24] that can be difficult to detect with noisy data [5,10]. Theory strongly suggests that the size of a population is only marginally relevant to the extinction risk when the rate of decline is rapid and continuous [25,26]. Characteristics of both a 'fast life style' associated with small body size and short generation times [27], and the 'slow life style' represented by large organisms with long generation times [24,28] can make some species and lineages more or less likely to go extinct, affecting any estimation of MVP. Moreover, extinction risks differ between lineages threatened by habitat loss, lineages threatened by human persecution and introduced novel predators [22,24] and lineages threatened by loss of food resources [29]. Thus, theory indicates that populations of equal size will vary greatly in their extinction risk depending on their life histories, long-term population growth rates, habitat quality and current threats.

The findings of recent MVP papers [17–19] are at odds with theoretical expectations. A brief overview of the approaches and findings used in these three recent papers is given in Table 1. These papers have been characterized by largely measured tones (see also [30]), with the authors using generally careful analyses to expose sources of uncertainty in estimates of MVP. Against that backdrop, the authors' emergent conclusions [20] are surprising. In particular, the assertion that practitioners 'must manage for biologically relevant MVPs [of] at least 5000 adult individuals' [20] is made without reference to the specifics of the

situation of any population. The suggestion that funding could be allocated on the basis of the numerical distance of a population from 5000 adults [20], if strictly implemented, does not adequately acknowledge that some populations might persist safely at lower population sizes, whereas others might need to be considerably larger to ensure persistence. The concern that '[w]hile scientists debate MVP variance, the extinction crisis deepens' [20], discourages further discussion of the issue. However, if a generally applicable MVP is to guide policy and funding allocation, then a robust debate culminating in a workable consensus is essential. Such a debate has yet to occur, but will need to focus on the value judgements inherent in estimating MVPs, as well as on the methods underlying MVP estimates. In the next section, we direct our attention to the latter.

What do data on MVP tell us?

Using 5000 individuals as a rule-of-thumb for the MVP of a population (the robust conservation threshold advocated by Traill *et al.* [20,21]) would disregard substantial uncertainty in existing estimates of MVPs that suggests that 5000 is likely to be a very poor estimate for any specific population. Analyses underlying the derivation of the 5000 benchmark are complex and, inevitably, analytical decisions were required to make disparate data comparable for a wide range of species and from a large number of sources. Here, we focus on three important issues. Technical details are provided to support our arguments. We demonstrate that uncertainty and contingency in the data have not been accounted for adequately, and that a failure to find taxonomic or ecological differences in measures of central tendency among highly variable data does not, in and of

Table 1. Data sets and analyses used to estimate MVPs, and results and conclusions from papers advocating the use of generally applicable MVP thresholds

Data analysed	MVP analyses	Results and conclusions	Refs
Demographic data on 102 vertebrate species; estimates of the frequency and magnitude of catastrophes, and default assumptions about the consequences of inbreeding depression	Standard PVA software package (VORTEX [81]) used to estimate MVPs conferring a 99% probability of persistence for 40 generations; estimates standardized to their expected values if 40 generations of demographic data were available (see Box 2)	No statistically significant difference detected among MVPs across taxa; overall mean standardized MVP = 7316. '[C]onservation programs, for wild populations, need to be designed to conserve habitat capable of supporting approximately 7000 adult vertebrates to ensure long-term persistence'	[17]
Time series data (acquired from the Global Population Dynamics Database [82]) on 1198 populations of plants, vertebrates and invertebrates	Information theoretic approaches used to assess support among five population growth models ranging in complexity from a random walk to a θ -logistic [80,83]. Numerical simulations based on each model determined initial population size (i.e. the MVP) conferring 99% probability of persistence for 40 generations or 90% probability of persistence for 100 years. Average MVP determined for each criterion by model averaging [84]	Median MVP = 1181 and 1377 for 40-generation and 100-year criteria, respectively; 'a striking lack of predictability in MVP'; 'although MVPs provide a useful rule of thumb for species conservation, they should not be used as precise conservation targets'	[18]
287 published MVP estimates, covering 212 species	Meta-analysis; all published estimates of MVP standardized to those expected if the generating model had sought a 99% probability of persistence for 40 generations, and had included both inbreeding depression and the potential for catastrophes	Model including desired probability and time-frame for persistence, the inclusion of inbreeding effects, and the inclusion of catastrophes explained 6.3% of the deviance among published MVP estimates. Median standardized MVP = 4169; 'a species' or population's MVP is context-specific, and there are no simple short-cuts to its derivation'	[19]

Box 2. Data standardization and uncertainty

Environmental stochasticity is a major driver of the dynamics of many populations. To model the effects of environmental stochasticity, it is necessary to have good estimates of variance in vital rates, which, in turn, require data collected over many years to sample the extent of environmental variation [5,75]. Short studies will often underestimate the range of vital rate variation, providing optimistic estimates of population stability and viability [76]. As a result, Reed and colleagues found that raw estimates of MVP (termed MVPA) produced by VORTEX were strongly affected by the study length (in generations; SLG) from which data were drawn [17]; they 'corrected' MVP estimates to the value expected from 40 generations of population data (MVPC) using the process depicted in Figure 1. Essentially, the correction amounts to extrapolating an estimated MVP at point a along a line parallel to the regression (Equation (1)):

$$\ln(MVPA) = \beta_0 + \beta_1(\ln[SLG]) + \varepsilon_i \quad (1)$$

until it intersects a study length of 40 generations at point c .

Two aspects of this process are problematic. First, the correction approach assumes that a regression of estimated $\ln(MVP)$ against $\ln(SLG)$ for any individual population would have an independent intercept, but a common slope defined by the overall regression of 102 different populations. Thus, the correction method is predicated on the belief that collecting an equal amount of additional data on individual populations (i.e. increasing the study lengths by a given number of generations) would increase each estimated MVP by the same absolute amount. No theory exists to support this assumption.

The second concern is that the regression equation used to standardize MVPs to a span of 40 generations included only two investigations with >15 generations of data. Assessing the consequences of this is problematic. Although theory exists to guide the assessment of prediction intervals around a standard regression (Figure 1), such theory cannot be applied in a straightforward manner

to data points assumed to lie on independent regression lines, with a shared slope but independent intercepts. Owing to this complexity, we consider only that component of uncertainty in MVPC estimates [17] that is associated with error in the estimated regression slope.

We examined the uncertainty in MVPC based on the statistics associated with re-fitting Reed *et al.*'s regression model to the data presented in their appendix [17]. If we assume that MVPA and SLG are known, such that the variance associated with these quantities is zero, then the variance associated with each corrected estimate i of MVP (MVPC) is given by Equation (2):

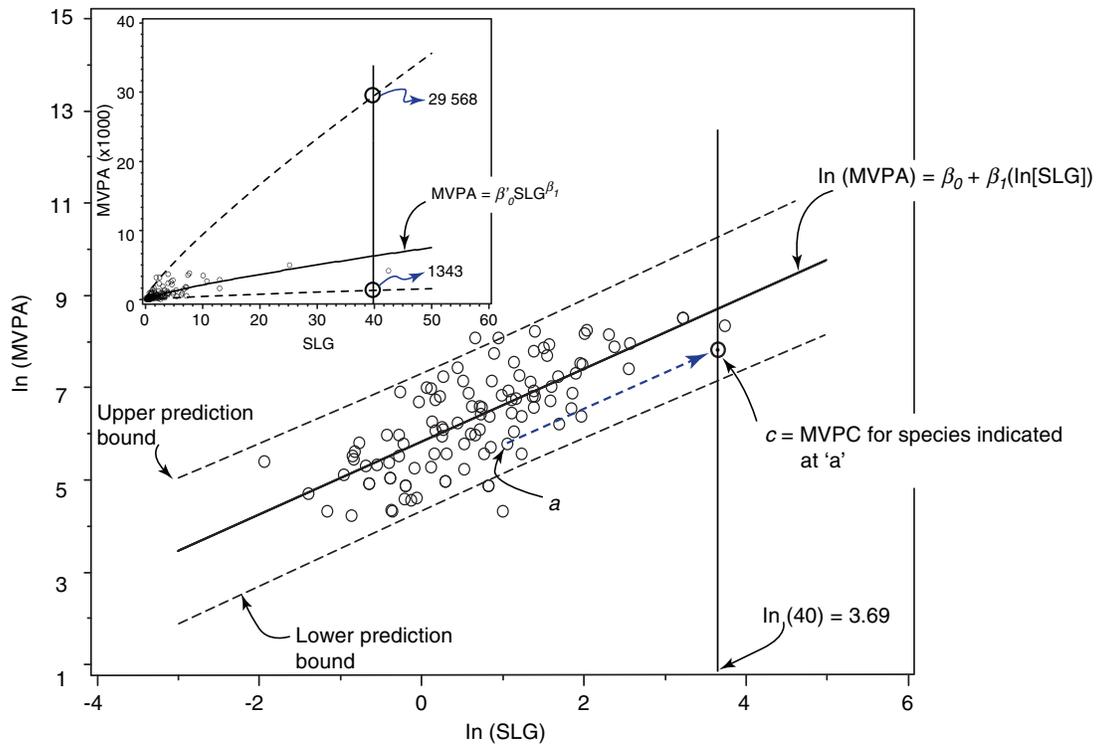
$$\text{Var}[\ln(MVPC_i)] = \text{Var}(\beta_1)(\ln[40/SLG_i])^2 \quad (2)$$

The 95% confidence interval for each corrected value, which we call an extrapolation interval, can then be estimated using Equation (3):

$$\ln(MVPC_i) \pm 1.96\sqrt{\text{var}(\ln[MVPC_i])} \quad (3)$$

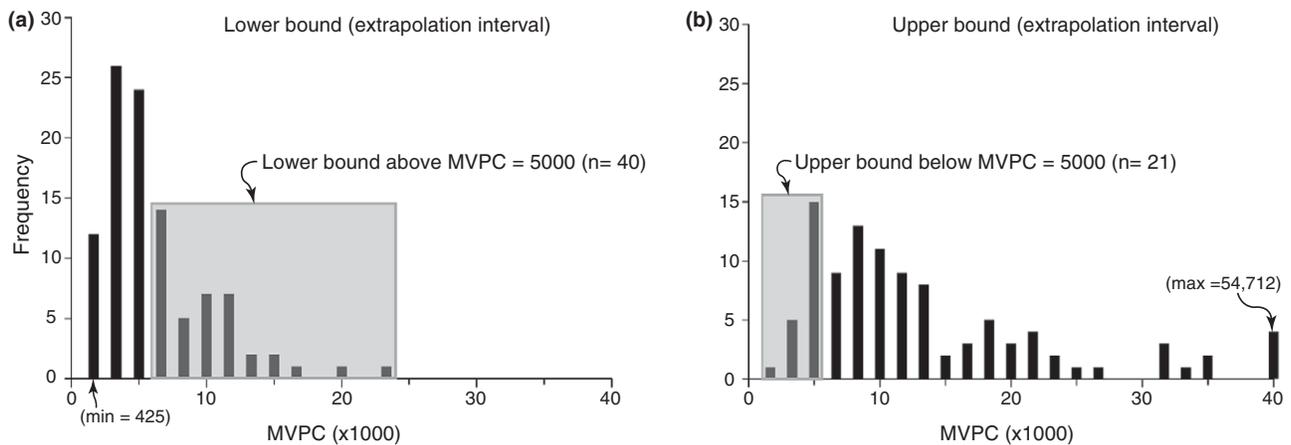
We observed that the extrapolation intervals for the 102 species analysed by Reed *et al.* [17] failed to include the universal MVP of 5000 adult individuals in 60% of the cases (Figure II).

Although this analysis illustrates the high degree of uncertainty surrounding efforts to estimate persistence over the long term with limited empirical data, uncertainty is still greatly underestimated here. For example, assigning an SLG to each study in [17] assumes that generation length is a fixed life-history property within species. However, it is well known that estimating mean generation time is a challenge among species with overlapping generations [77,78], and examples illustrate that intraspecific generation length estimates can vary substantially (e.g. Ethiopian wolf generation time estimates range from 3 to 8 years [79]). Thus, the uncertainty bounds associated with the extrapolation process reported here are, at best, minimum estimates.



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Figure I. Correcting reported MVPA to that expected if the study used to estimate VORTEX parameters had been conducted for 40 generations (MVPC). The process is illustrated in the transformed log scale and back-transformed arithmetic scale (inset). SLG represents the study length (in generations) of the investigation used to parameterize the model of any individual population in VORTEX. The standardized MVP for species *a* is found at point *c*. Conventional lower and upper prediction intervals are displayed in log and arithmetic scales.



TRENDS in Ecology & Evolution

Figure II. Frequency distributions of the lower (a) and upper (b) bounds of the extrapolation interval (based on Equation (3)) for the MVPC for the 102 species used by Reed *et al.* [17]. The union of the sets where the lower bound exceeds 5000 and the upper bound is less than 5000 defines the number of species ($n = 61$) whose extrapolation interval does not include MVPC = 5000.

itself, provide evidence for a generally applicable MVP threshold.

Standardization and extrapolation

Reed *et al.* observed a strong effect of study duration on MVP estimates [17]. Furthermore, variation in vital rates among species with different life histories (e.g. large- versus small-bodied animals) was reduced when scaled to a common generation length [31]. To account for these observations,

MVP estimates were ‘corrected’ to MVPC, the value expected if 40 generations of population data had been available [17–19]. The standardization process by which this was achieved, together with the statistical shortcomings of that approach, are summarized in Box 2. Reconsidering uncertainty in the data, we estimated that the lower and upper bound of the corrected (standardized) MVP for each individual population studied ranged from a minimum of 425 individuals to a maximum of 54 712 individuals (Box 2,

Figure II). This outcome suggests to us, as it has to others, that the size of populations required for long-term persistence ‘...are generally believed to be highly circumstance and species specific, depending on the environment and life history characteristics of the species’ [31].

A similar process was used by Traill *et al.* to standardize the MVPs estimated through many disparate modelling exercises [19]. A statistical model was used to standardize MVP estimates to control for varying methodology in the literature (Table 1). As with the standardization process applied by Reed *et al.* (Box 2), this amounts to assuming that the effect of a given factor (e.g. the inclusion of inbreeding effects) across populations would apply with the same absolute magnitude within each population. Although it would be testable, no theory exists to support this notion. Even accepting the standardization process, it is telling that the fitted model explained only 6.3% of the deviance among MVP estimates; remaining variability is presumably attributable to inherent differences among the focal populations and their environmental context (see following section). The explanatory power of the fitted model does little to justify the claim [20] that, ‘[d]ifferences between published MVP estimates, even for the same species, can also be explained by the different survival probabilities and timescales used.’

Determinism, outliers and environmental context

Reed *et al.* [17] investigated whether MVPs were higher than is usually acknowledged. To eliminate data from populations subject to strong deterministic declines (probably arising from anthropogenic effects), they excluded populations with ‘strong negative growth rates’ [17]. By contrast, Brook *et al.* [18] were actively interested in the relationship between population growth rate and MVP. Consequently, the 1198 populations in their analysis included 561 populations with negative growth rates [including species in very steep decline, such as the Steller sea lion (*Eumetopias jubatus*), for which $r = -0.72$]. Unsurprisingly, the population growth rate was found to have an important influence on estimated MVP [18], with the highest MVPs associated with situations characterized by low growth rates combined with high population variance. That MVPs are useful only in cases where strong deterministic effects have been removed [25] does not appear to have influenced the selection of species assessed by Brook *et al.* [18] or Traill *et al.* [19]. Indeed, if only the relatively stable populations (i.e. those with $-0.02 \leq r \leq 0.02$) are considered, the median MVP estimate reported by Brook *et al.* shifts from 1181 ($n = 1198$) to 355 ($n = 408$) based on the 40-generation criterion. Therefore, the inclusion of populations known to be in decline owing to strong deterministic threats suggests that median estimated MVPs are overly pessimistic (see Box 3), as shown by Lande in his analytical treatment of this question [26].

Some indication of the potential importance of environmental context can be gained by considering the data presented by Traill *et al.* [19]. These data enable one to focus on the effects of extrinsic factors (rather than intrinsic ecology) by looking at species for which multiple estimates of MVP have been produced. From Traill *et al.*’s supplementary data [19], we found 52 species that each

had two to nine independent MVP estimates. Even after standardization, MVPs varied substantially within species (see Table S1 in supplementary material online). The grizzly bear (*Ursus arctos*) had the greatest number of independent estimates ($n = 9$) with standardized MVPC ranging from 395 (MVPC_{min}) to 44 259 (MVPC_{max}). Other notable species with at least four independent estimates included the wolf (*Canis lupus*; MVPC_{min} = 248; MVPC_{max} = 6332), Asian elephant (*Elephas maximus*; MVPC_{min} = 266; MVPC_{max} = 4737), mountain gorilla (*Gorilla gorilla*; MVPC_{min} = 630; MVPC_{max} = 11 919), and red-cockaded woodpecker (*Picoides borealis*; MVPC_{min} = 422; MVPC_{max} = 20 868). Overall, maximum MVP estimates were many times greater than minimum estimates for the same species, often exceeding two orders of magnitude (MVPC_{max} / MVPC_{min}: median = 15.4). The reported range of MVPC estimates also failed to include 5000 individuals in 42% of the cases ($n = 22$), with 19 cases failing because MVPC_{max} < 5000, and three cases failing because MVPC_{min} > 5000; these results illustrate well the strong dependence of MVP estimates on the environmental context of a population (see also Figure 1).

Density dependence and stability

Density dependence is profoundly important to the regulation of many natural populations, and fundamental to the recovery of populations from perturbation. The form of density dependence can exert a strong influence on extinction probabilities associated with different population sizes [32,33]. Papers analysing MVPs [17–19] have differed in their treatment of density dependence, leading to some contradictory interpretations of the importance of the phenomenon (Box 4). Clearly, a failure to include density dependence appropriately is likely to inflate estimates of MVP substantially. The difficulty of inferring the form of density dependence, even from relatively long population time series, highlights that meaningful estimates of MVP are likely to be derived only from very long, and therefore rare, data sets [8].

Re-evaluating support for a generally applicable MVP threshold

Reconsideration of the underlying data indicates that uncertainty in MVP estimates is substantial, regardless of the analytical approach used in recent treatments [17–19]. The vast uncertainty associated with MVP estimates for single populations or groups of populations probably underlies the tendency to find no evidence for statistical differences of MVPs among taxa [17] or strong life-history predictors of MVP size [18,19]. An alternative, more robust interpretation of that finding is that there is no significant difference in MVPs *between* taxa simply because there is such enormous variation in MVPs *within* taxa. Indeed, variation among populations is perhaps the most striking finding of recent analyses; for example, within-species estimates of standardized MVP varied more than 100-fold for the whooping crane (*Grus americana*), Eurasian beaver (*Castor fiber*), muskox (*Ovibos moschatus*), and others ([19] see Table S1 in supplementary material online). Thus, regardless of the taxon to which a population belongs, any ‘rule of thumb’ MVP is likely to be a poor estimate of

Box 3. Outlier MVPs

In our re-examination of the Brook *et al.* supplemental data [18], it was apparent that their original Figure 1 truncated observations at $\ln[\text{MVP}] = 20$ (~0.5 billion individuals). A replotting of their figure based on all observations in their supplemental data ($n = 1198$) revealed that the number of species with MVPs that exceeded 0.5 billion individuals varied by the population growth model fitted to the time-series data (Figure 1), with higher frequencies of exceptionally large MVPs associated with models lacking density dependence (Figure 1a,b). These large MVP estimates could be an artefact of the method used to estimate MVP, for it seems difficult to argue that species such as the rough-legged hawk (*Buteo lagopus*), northern harrier (*Circus cyaneus*), or rook (*Corvus frugilegus*) require >1 billion individuals (model-averaged estimate) to remain viable, unless the studies supplying the empirical data were on populations subject to strong deterministic threats or severe catastrophic events. To remove

the potential bias caused by what could be termed ‘methodological outliers’, we deleted any observations with an estimated $\ln[\text{MVP}] > 20$. This filtering shifted the median MVP estimate from 1181 ($n = 1198$) to 462 ($n = 756$) using the 40-generation criterion. If we further restricted our consideration to those species with relatively stable populations ($-0.02 \leq r \leq 0.02$), then the median MVP was further reduced to 280 individuals ($n = 339$).

Our point here is not to argue that MVP targets should be lower than those advocated by others [17–20], but to highlight two observations: (i) median MVP estimates are sensitive to the set of species used in their estimation; and (ii) there appear to be legitimate circumstances where reported MVPs can be overly pessimistic. Both of these observations indicate that summary statistics applied to empirically derived estimates of MVP are characterized by a degree of sensitivity that is inconsistent with the notion of a robust universal MVP.

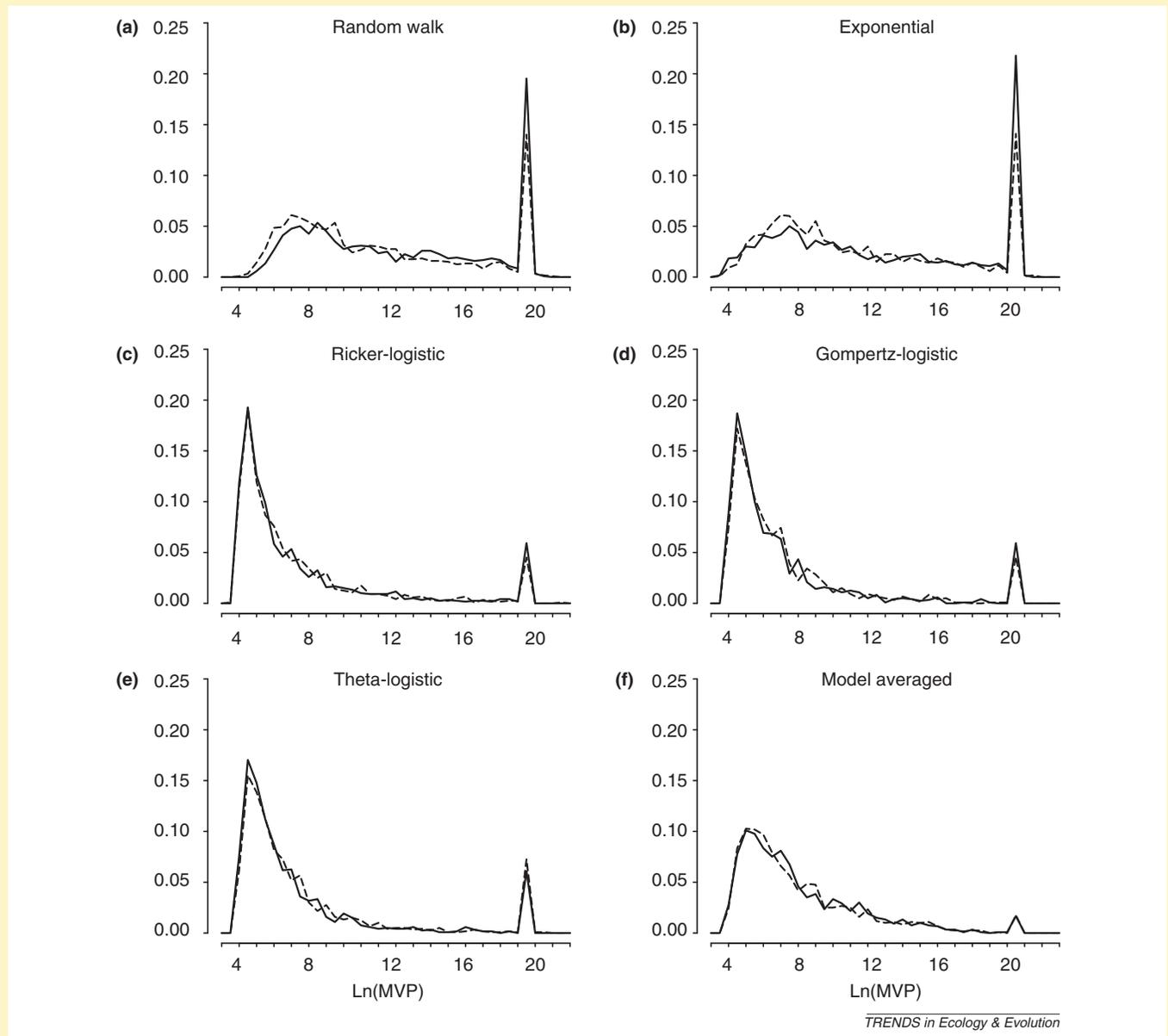


Figure 1. Full frequency distributions of $\ln[\text{MVP}]$ from Brook *et al.* supplemental data [18] among five population growth models (a–e) and model-averaged (f) estimates using Brook *et al.*'s 40-generation (dashed line) and 100-year (solid line) viability criteria. Potential methodological outliers are associated with a frequency spike at $\ln(\text{MVP}) \approx 20$.

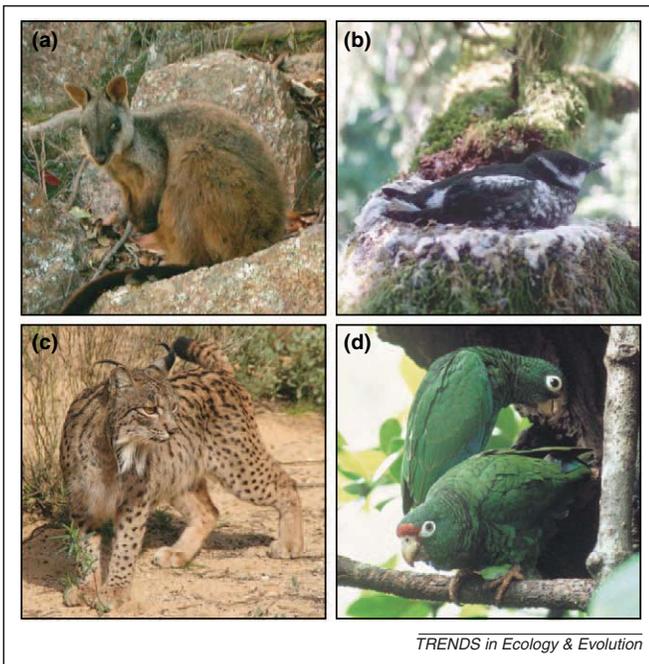


Figure 1. The viability of a population of given size is strongly related to its environmental context as well as its life history. This can confound efforts to set a guideline figure at which a population ceases to have long-term viability. For example: (a) the brush-tailed rock wallaby (*Petrogale penicillata*) is thought to number in the region of 10^4 – 10^5 individuals but is declining steadily, owing to the effects of introduced predators and competitors; (b) the marbled murrelet (*Brachyramphus marmoratus*) of the Pacific Northwest USA is thought to number in the order of 10^4 individuals but is endangered, nevertheless, by a range of threats (habitat loss and fragmentation, increasing populations of nest predators and depletion of food resources at sea); (c) the Iberian lynx (*Lynx pardinus*), numbering approximately 10^2 individuals, is unlikely to be viable in the long term (owing to prey depletion, habitat loss and fragmentation and high rates of unnatural mortality); and (d) globally, mature Puerto Rican parrots (*Amazona vittata*) number only 10 – 10^2 but, nevertheless, the species has shown an increasing trend over recent decades. Reproduced, with permission, from Glen Fergus (a); Thomas Hamer, Hamer Environmental L.P. (b); Programa de Conservación Ex-situ del Lince Ibérico (<http://www.lynxexsitu.es>) (c); and James W. Wiley and Noel F. R. Snyder (d).

the MVP of that population, highlighting the improbability that a universal threshold for MVP would be useful to conservation practitioners.

Alternatives to a 'magic number' for viability

Our reconsideration of recent MVP papers suggests that there are good reasons for managers and conservation practitioners to regard advocacy of a universal MVP threshold with considerable scepticism. That, in turn, prompts two questions. First, if published estimates of MVP are insufficient to identify the characteristics of a population or organism that will determine its approximate MVP, can one identify those attributes? Second, if one is unable to identify (and, therefore, plan for) a generally applicable minimum number of individuals to conserve, what are the consequences for conservation?

The concept of viable minima is of interest not only to conservation biologists. Minimum population densities are closely tied to several aspects of ecology, such as understanding consumer–resource relationships, the use of energy within ecosystems and the relative roles of factors that limit population persistence, whether genetic, behavioural or energetic. Although relationships between population density and body size have long been a focus of macroecology [34], theory has not yet been used to predict the lower limits to this relationship [35]. Viable minima are also closely tied to Allee effects [36] and, in particular, to the concept of 'Allee thresholds' (the point at which mean per-capita population growth rates become negative) [37]. This suggests that alternative, active fields of ecology could yield insights into the factors that predispose a population to be tolerant of low densities and (given an understanding of space use) small population sizes. It would be useful for researchers in those areas to consider how their work might relate to traits affecting MVP.

Box 4. MVPs and density dependence

Reed *et al.* recognized the importance of density dependence and included it where its impact on vital rates had been measured, or where there were strong indications of its operation [17]. Although they gave no details of the number of their studied populations for which adequate data on density dependence were available, we can gain a sense of their empirical challenge by examining the frequency distribution of study lengths for the populations considered. Study duration estimates for each population that we derived from Reed *et al.*'s appendix [17] show that over a quarter of the 102 populations used had been studied for one generation or less, half had been studied for less than two generations, and only one population had a study length that exceeded 25 generations (Box 2, Figure 1). With these limitations on the temporal extent of available data, establishing plausible patterns of density dependence would be difficult for most, if not all, of the populations for which MVP was estimated.

A more rigorous approach was taken by Brook *et al.*, who analysed evidence for different forms of density dependence in population time series [18]. They found strong evidence that the inclusion of negative density dependence had an important bearing on MVP estimates. MVPs determined from shorter time series (which lacked strong evidence of density dependence) were more pessimistic (i.e. larger) than those based on longer time series (from which, evidence of

density dependence was typically strong); overall, the estimated MVP was approximately an order of magnitude larger for short than for long time series [18].

Brook *et al.*'s [18] findings suggest an intriguing contrast with the earlier findings of Reed *et al.* [17]. Specifically, although Reed *et al.* found that longer studies led to more pessimistic MVP estimates, it appears that when density dependence is incorporated, the insights gained from longer studies provide reasons for greater optimism (smaller MVP estimates). Although some authors have subsequently expressed doubts about the model used to determine the form of density dependence [80], the emergent message remains that a failure to include density dependence is likely to inflate estimates of MVP substantially.

Given the clear importance of density dependence, it is perhaps surprising that Traill *et al.* found no evidence that the way in which density dependence was incorporated into models had a bearing on the estimated MVP [19]. Nevertheless, it is likely that the role of density dependence is more complex than could be detected by Traill *et al.*'s statistical approach. In particular, there are good reasons to expect that the form of density dependence would interact with other aspects of modelling and environmental context to influence MVP estimates.

If, at present, it is not possible to identify a universally applicable MVP, what might be the consequences for conservation? Traill *et al.* have linked uncertainty over MVPs to the current extinction crisis [20]. Thus, it is reasonable to ask to what extent the failure to agree on a universally applicable MVP contributes to the current extinction crisis? It seems more probable that: (i) extinctions occur because of a failure to identify and treat the causes of population declines [25,38], not because populations are assumed to be safe when, in fact, they are not; and (ii) failures to treat the major causes of decline are often related to political or economic issues [39–42], rather than to a lack of adequate scientific information on population viability.

What, then, is the utility of estimates for MVPs? In spite of the enthusiasm with which the MVP concept was initially embraced by conservation biologists, we can distil from literature only two advantages of having an estimate of MVP. First, an MVP can serve as a useful tool to persuade policy-makers that extinction is a possibility and action is required [43,44]. In this way, PVA and other quantitative criteria are used to classify taxonomically diverse species into threat categories under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [45], identifying populations that merit further scrutiny. In these cases, policy-makers should interpret estimates of extinction risk and its accompanied uncertainty for various scenarios, rather than comparing recent estimates of population size to an estimate of a 'secure' MVP population size. Second, MVPs can be used to determine conservation targets, either proactively or reactively (*sensu* [46]). Proactive targets set minimum size thresholds that, if reached, would place populations in unacceptable danger. Reactive targets suggest objectives for population recovery programs (e.g. delisting decisions). Value judgements are likely to differ in the two cases. Reactive MVPs could be used in prioritization, for example, as a tool for determining probable return on investment and, thus, as an aid to ecological triage [20]. Specifically, Traill *et al.* suggest prioritizing spending based on the distance a population is below a generalized target size of 5000 adults, in conjunction with the cost and likelihood of elevating the population to that target [20], '...to abandon hopeless-case species in favour of greater returns elsewhere' [21]. Given the poor support for a universal MVP, this approach lacks credibility.

In keeping with numerous other authors (e.g. [9,45,47–54]), we recognize the value of PVA in bringing together relevant information on a population, formalizing understanding of the important processes, exposing gaps in knowledge, and serving as a focus for both scientists and policy-makers. However, in agreement with those same authors, we recommend against using the outcomes of such modelling exercises to set conservation targets unless there are strong reasons to believe that modelling outcomes are robust and defensible for the focal population. Embracing a single, estimated MVP threshold would release biologists from the obligation to assess the situation of the focal population, thereby forfeiting the diagnostic benefits that emerge from doing so [25]. Much of modern conservation is directed towards landscape- or ecosystem-level processes [55–57]. However, in situations in which populations remain

the focus of conservation action, particularly when trying to salvage populations that are already in trouble, we suggest that there is no substitute for diagnosing and treating the mechanisms behind the decline of a population [25,38], actions that are unlikely to be informed by using a 'magic number' to set a target for conservation.

Conclusions

We applaud recent efforts [17–20] to encourage more quantitative approaches to evaluating population viability than a reliance on the oft-cited 50/500 MVP rule of conservation genetics [58,59]. The findings that MVP estimates are sensitive to the duration over which data were gathered and that meta-analytic comparisons among MVP estimates require rigorous standardization emphasize the need to obtain good estimates of demographic variability. We also suspect (as have others long before [60]) that multiple populations totalling thousands (not hundreds) of individuals will be needed to ensure long-term persistence. Nevertheless, MVP estimates both among and within species show striking variation for many reasons. The fundamentally contingent nature of MVPs means that we cannot support a universally applicable MVP threshold.

Ecology has been characterized as a science built on 'contingent generalizations' [61,62]. Such contingency has long been acknowledged in the PVA literature [2] and continues to foil attempts to generalize about crucial levels of habitat or abundance [16,63], even among populations of a single species [64]. Uncertainty, even when dealing with populations of the same species, suggests that generalizing among species is a dangerous undertaking. Failing to account for uncertainty is a common problem in conservation [65], and can lead to biased expectations and to the misdirection of scarce conservation resources [66].

The MVP concept is a key example of one of the hardest questions faced in conservation biology: how much is enough? Key national legislations governing endangered species decisions (e.g. the US Endangered Species Act, Canada's Species at Risk Act, and Australia's Environmental Protection and Biodiversity Act), as well as efforts by national and international organizations committed to species conservation [67], use MVP concepts that can revolve around listing decisions (has the species declined to a number that is threatened with extinction?), delisting decisions (has it recovered enough that it is no longer so threatened?), extinction risk categorizations, and determining the number and size of protected areas that a threatened species needs. MVP analyses and PVA modelling can be used to assist in these decisions, but their value is constrained by large uncertainty in model outcomes [68]. Realistic MVPs might well be in the thousands for many life histories, but uncertainty around any guideline figure would be of a similar order of magnitude. The extinction of the passenger pigeon (*Ectopistes migratorius*), perhaps the most abundant land bird in North America during the 1800s (numbering 3–5 billion individuals [69]), stands as a sobering reminder that population size alone is no guarantee against extinction. As others have remarked, 'population viability analysis is an inexact science,' [17] and there is 'no single 'magic' population size that guarantees' population persistence [70].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tree.2011.03.001.

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Beyond PVA: Why Recovery under the Endangered Species Act Is More than Population Viability

SHAYE WOLF, BRETT HARTL, CARLOS CARROLL, MAILE C. NEEL, AND D. NOAH GREENWALD

Recovery criteria under the Endangered Species Act are the objective, measurable targets for determining whether the recovery of listed species has been achieved. Existing criteria have been criticized as inconsistent and poorly supported. Recent proposals for improving those criteria have recommended framing them around population viability analysis (PVA) and setting criteria on the basis of extinction risk thresholds. Used in isolation, however, a PVA-centered approach is prone to limiting the scope of recovery, is too data intensive to be useful for most species, and risks misrepresenting normative recovery thresholds as objective. We recommend a framework based on the three Rs—the ecological principles of representation, resiliency, and redundancy—which makes use of multiple analytical approaches for setting recovery targets, including PVA when appropriate. We argue that the three Rs framework better fulfills the ESA's comprehensive recovery mandates for achieving geographic representation, ecosystem conservation, and threats abatement while overcoming data and budget limitations pervasive in recovery planning today.

Keywords: recovery plan, recovery criteria, Endangered Species Act, endangered species, population viability analysis

The fundamental purpose of the US Endangered Species Act (ESA) is not only to prevent extinction but also to recover species to the point that they are no longer threatened or endangered. To achieve these purposes, the act requires development of recovery plans “for the conservation and survival” of listed species (16 U.S.C. § 1533(f)(1)). These plans must specify the “objective, measurable criteria” by which it is determined that recovery has been achieved (16 U.S.C. § 1533(f)(1)(B)). Over the past 20 years, numerous reviews have identified shortcomings in recovery criteria, showing that they often lack a clear biological rationale; are inconsistently applied across species in ways that do not appear to be based in biology; are not sufficiently objective and measurable; and are set below the levels necessary for long-term persistence, ecological viability, and evolutionary capacity (Tear et al. 1995, Foin et al. 1998, Clark et al. 2002, Gerber et al. 2002, Neel et al. 2012, Himes Boor 2014).

To overcome these shortcomings, recent publications have recommended a more central use of population viability analysis (PVA) in recovery planning to provide objective, measurable recovery criteria (Doak et al. 2014, Himes Boor 2014). PVA is a quantitative model-based approach that uses demographic and abundance data to estimate the probability of extinction or a related measure of population viability such as quasiextinction (Beissinger and Westphal

1998, Morris and Doak 2002). Himes Boor (2014) recommended that PVA be used to provide the organizing framework for recovery plans. In this approach, PVA modeling results are used to develop recovery criteria that define a species with a chosen level of extinction risk. Moreover, the author suggests that PVA offers the only means to develop objective, measurable recovery criteria (Himes Boor 2014).

We agree that the failure to define and consistently employ a clear, transparent, science-based protocol for implementing recovery contributes to many of the problems with recovery criteria. We disagree, however, that PVA alone provides an adequate or practical overarching framework to overcome current shortcomings in recovery planning. First, a recurrent problem is the failure of criteria to fulfill the ESA's statutory mandates for recovery, which is not easily cured by a PVA-centered framework. Second, because of pervasive data limitations, PVA is too data-intensive to be possible or reliable for many listed species. Finally, PVA-derived recovery criteria are based on normative thresholds of extinction risk and do not inherently provide more scientifically robust or transparent criteria than do other methods.

Instead, we recommend a recovery-planning framework based on the conservation biology principles of representation, resiliency, and redundancy—the *three Rs*—for reducing extinction risk and maintaining self-sustaining

populations (Shaffer and Stein 2000). The three Rs framework is comprehensive enough to fulfill the ESA's recovery requirements for geographic representation, ecosystem conservation, and threats abatement. The framework can make use of PVA for determining recovery criteria but can also use other analytical approaches when PVA is not appropriate, given the resource-limited, data-poor environment typical of recovery planning. Although the existing recovery planning guidance document (NMFS 2010), written by the US Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (hereafter, *the services*) generally recommends the use of the three Rs, it provides no instruction on how these principles should be implemented. To realize the benefits of a three Rs approach, additional guidance to effect this approach is urgently needed.

Below, we discuss the recurrent failure of recovery criteria to fulfill the ESA's statutory requirements for recovery. We first review legal and policy considerations that are often ignored or discounted in discussions of recovery planning, because they set the stage for comparing a PVA-centered with a three Rs framework. We then discuss the limitations of a PVA-centered framework for providing an overarching framework for recovery. Finally, we present specific recommendations for developing objective, measurable criteria from the three Rs framework that meet the ESA's recovery mandates.

The ESA's recovery mandates

Any framework for recovery planning must fulfill the ESA's statutory recovery requirements, be based on the best available science, and be legally defensible as interpreted by the courts. The act requires the services to "conserve" threatened and endangered species and the ecosystems on which they depend (16 U.S.C. § 1531(b)), where "conserve" is defined as bringing to the point where the protections of the act are no longer needed (16 U.S.C. § 1532(3)). A recovered species is one that is no longer endangered or threatened, meaning that it is not currently "in danger of extinction... throughout all or a significant portion of its range" nor is it "likely to become" so "in the foreseeable future" (16 U.S.C. § 1532(6) and (20)). The act also requires that threats be eliminated or managed for a species to be considered recovered (16 U.S.C. § 1533(a)(1)). Finally, recovery plans must contain the "objective, measurable criteria which, when met, would result in a determination... that the species be removed from the list"; site-specific management actions; and time and cost estimates (16 U.S.C. § 1533(f)(1)(B)). In summary, recovery under the ESA requires a sufficiently low extinction risk (e.g., the species is not likely to become in danger of extinction in the foreseeable future) over an appropriate geographic extent (i.e., all significant portions of range), coupled with ecosystem conservation and threats abatement. The ESA does not define quantitative thresholds for achieving these goals, but both the statute and courts provide some guidance on what these components must encompass.

Geographic representation

The third in a series of federal laws designed to protect imperiled species, the Endangered Species Act of 1973 was the first to include a geographic component to endangerment by allowing a species to be protected if it was threatened or endangered in a "significant portion of its range," even if it was secure elsewhere. The 1966 and 1969 predecessors protected only species at risk of worldwide extinction (i.e., the Endangered Species Preservation Act of 1966, Pub. L. 89-669, and the Endangered Species Conservation Act of 1969, Pub. L. 91-135). Congress noted that this was "a significant shift" in how the services should evaluate species for listing, because it allowed the protection of species that were secure in some portions of their range but severely imperiled or extirpated elsewhere (H.R. Rep. No. 412, 93rd Cong., 1 Sess. (1973)). Consideration of a significant portion of a species' range is therefore important for determining recovery. If an *endangered* species is at risk throughout all or a significant portion of its range, it follows logically that a *recovered* species must be secure throughout all significant portions of its range (Vucetich et al. 2006, Carroll et al. 2010). Although "significant portion" and "range" are not explicitly defined under the ESA, the act's provisions for habitat acquisition, reintroduction, translocation, and the designation of critical habitat in areas unoccupied at the time of listing, as well as relevant case law (*Defenders of Wildlife v. Norton*, 258 F.3d 1136 (9th Cir. 2001)), indicate that Congress intended for recovery to be geographically broad in scope and to include the species' historic range in its consideration (Carroll et al. 2010).

The services have resisted definitions of "significant portion of its range" that would require them to uniformly consider the historic range in listing and recovery decisions (USFWS and NMFS 2014). However, failure to set recovery criteria for geographic representation in a consistent and biologically justified way can lead to significant disparities. For example, the USFWS recently proposed to delist the gray wolf (*Canis lupus*) in the lower 48 United States, even though it occupies only 5% of its historic range (Neel et al. 2012), but did not propose to delist the bald eagle (*Haliaeetus leucocephalus*) until populations were recovered in all five recovery regions that encompassed virtually all of its historic range (USFWS 2007). Given that both of these species were biologically viable at the global level when they were listed in the 1970s, there are no scientific reasons why the geographic scope of recovery varied so greatly.

Ecosystem conservation

Congress made clear that ecosystem conservation for listed species is fundamental: "The purposes of this Act are to provide a means whereby the ecosystems on which endangered species and threatened species depend may be conserved" (16 U.S.C. § 1531(b)). Ecosystem-focused criteria are important not only for ensuring sufficient habitat

quantity, quality, and connectivity, but also for restoring the ecological function of species by maintaining abundance at a level that provides a particular ecosystem function (Soulé et al. 2005, Estes et al. 2010). If ecological function is not considered, a species could be declared *recovered* even while remaining functionally extinct. Restoring a species' ecological role is particularly important for strongly interactive species that are key to ecosystem structure and function such as keystone species, foundation species, ecosystem engineers, and top predators (Soulé et al. 2005, Carroll et al. 2006). A recent example is the ecosystem delisting criterion for the northern sea otter (*Enhydra lutris kenyoni*) in southwestern Alaska, which requires otter abundance to reach population levels that bring about a shift of more than half of otter habitat to a kelp-dominated state (USFWS 2010).

Threats abatement

A species can only be considered *recovered* when the five threat factors set forth by the ESA, including the loss of habitat or range, exploitation, disease, inadequacy of protective regulations, and other factors (16 U.S.C. § 1533(a)(1)) are eliminated or managed so that the species can persist without ESA protection. Recovery criteria that fail to address the threat factors and “measure whether threats... have been ameliorated” have been found unlawful (*Fund for Animals v. Babbitt*, 903 F. Supp. 96 (D.D.C. 1995) and *Defenders of Wildlife v. Babbitt*, 130 F. Supp. 2d 121 (D.D.C. 2001)). As was noted in the recovery planning guidance document (NMFS 2010), demographic recovery alone does not indicate that a species is secure from underlying threats. Listed species can meet population-based recovery criteria because of intensive management interventions even though major threats remain. For example, the California least tern (*Sterna antillarum browni*) has exceeded its numeric recovery goals by a factor of six, in large part because of predator control at nest sites and fencing to reduce human disturbance, but the root threats remain, and populations are highly dependent on intensive management (USFWS 2006). Likewise, Kirtland's warbler (*Setophaga kirtlandii*) has achieved population recovery criteria through brown-headed cowbird (*Molothrus ater*) control and prescribed burns to maintain jack pine habitat, whereas most underlying threats remain (USFWS 2012a). The USFWS has recommended downlisting for both species rather than delisting, because threat abatement has not been realized. In contrast, the Aleutian Canada goose (*Branta canadensis leucopareia*) was delisted in 2001 following the removal of nonnative foxes from its nesting islands, protection of its wintering habitat, and hunting closures, which removed the main threats to the species (USFWS 2001). These examples illustrate that recovery requires that threats be abated through improved extrinsic conditions (e.g. invasive species removed) or through the adoption of adequate regulatory mechanisms to address human behavior.

The services' recovery planning guidance

The recovery planning guidance document (NMFS 2010) affirms the act's broad mandate for recovery, defining it as “the process by which listed species and their ecosystems are restored and their future is safeguarded to the point that protections under the ESA are no longer needed.” The guidance document (NMFS 2010) specifically directs recovery plans to take a “comprehensive approach” to recovery that includes threat abatement and ecosystem recovery and in which the species' historic and current range are considered. It states that recovery plans must “ensure the health of its habitat and ecosystem functions rather than the narrower view of looking at the species only.” However, the guidance document (NMFS 2010) only requires that recovery criteria meet two standards: They must address the threats facing the species, although the document provides no specific direction on how to do so, and they must be measurable and objective, although the document provides little clarity as to what these terms mean. Therefore, there is clearly a need for the services to provide more-specific guidance on how recovery criteria should fulfill the ESA's recovery mandates.

PVA as a framework for recovery

Himes Boor (2014) recommended that recovery criteria be based on “population viability modeling methods that incorporate demographics, limiting factors, threats, future management actions, and uncertainty.” Without this, she argued that recovery criteria “will continue to fall short of the ESA's objective, measurable mandate.” When PVA is not possible because of data limitations, the structure and data requirements of PVA should be used as the organizing framework of the recovery plan, and recovery criteria should be expressed in the interim as an extinction risk threshold, whereby recovery occurs when a PVA model yields a probability of extinction less than X% over Y years (Himes Boor 2014).

We agree with Himes Boor (2014) that objective, measurable criteria should be quantitatively, temporally, spatially, and statistically specific, with explicit scientific justification. However, we see significant limitations with the practical application of a PVA-centered framework for setting recovery criteria that meet the ESA's mandates. First, a PVA-centered framework is prone to limiting the scope of recovery, because PVA does not address key components of recovery required under the act. PVA modeling methods estimate the likelihood that a population or populations will be above some minimum size at a given future time (Morris and Doak 2002). However, the statutory language of the ESA indicates that recovery is broader than populations meeting minimum abundance thresholds to exceed a chosen extinction risk threshold. Rather, it requires recovered populations to be geographically representative, ecologically functional, and evolutionary viable, for which threats are abated so that species can persist without the provisions of the act. The most commonly implemented count-based and structured PVAs are focused on the abundance

and demography of single populations and fail to address species-level recovery. Complex PVA, such as multisite or spatially explicit PVA, have the potential to inform aspects of geographic distribution (e.g., the number and distribution of populations), ecosystem characteristics (e.g., patch size), and threats management (e.g., changes in mortality sources) needed to meet a specified extinction probability. However, these data-intensive models do not address key facets of recovery, such as the conservation and restoration of ecological and evolutionary processes and representation across the historic range. As such, PVA-based criteria run the risk of declaring the species *recovered* when one or a handful of populations meet an extinction-risk threshold but encompass only a small portion of a species' historic range and no longer meaningfully perform their ecosystem function.

Second, data inadequacies for most listed species—including the lack of basic abundance data required by even the simplest count-based PVAs—limit the use and reliability of PVA for setting recovery criteria that meet the ESA's recovery mandates (Beissinger and Westphal 1998, Morris et al. 2002, Crone et al. 2011, Flather et al. 2011, Neel et al. 2012, Zeigler et al. 2013). For example, 67%–98% of 1174 species with recovery plans historically lack data on population size, at listing or at plan writing (Neel et al. 2012). Time series data for abundances and stage- or age-based demographic rates are even scarcer, and when they are available, demographic data typically come from one or a few populations over short time frames (Crone et al. 2011, Zeigler et al. 2013). Because of these data limitations, PVA is not feasible for most listed species. Furthermore, even when PVA is applied, it may not provide sufficiently precise or accurate estimates of the demographic status or the minimum population size needed to stay above a chosen extinction probability for even one population or a few populations (Crone et al. 2011, Zeigler et al. 2013) and would fail to inform range-wide recovery. For example, in a recent review, Zeigler and colleagues (2013) found that of 280 published PVAs for listed and unlisted plant species, most were parameterized with 5 years or less of demographic data and did not address important factors, such as stochasticity, density dependence, seed banks, vegetative reproduction dormancy, threats, or management strategies (Zeigler et al. 2013). Because population growth rates for different populations of the same species or for the same population at different time periods often were significantly different, PVA estimates from limited spatiotemporal data cannot be generalized over a species' range or over long time scales in ways that inform recovery criteria (Johnson et al. 2010, Zeigler et al. 2013).

We are particularly concerned with the recommendation by Himes Boor (2014) to express recovery criteria for species for which PVA is not possible solely in terms of a viability standard, with the instruction that population and threat reduction targets needed to yield that level of risk be specified when sufficient data have been collected. Because of the ubiquity of data limitations, this approach

would leave the majority of listed species without concrete recovery targets for the number, size, or distribution of populations, no specific threat reduction targets, and no clear way to gauge progress toward recovery. This strategy is legally and practically problematic, because it would allow the services to avoid substantial recovery planning by deferring decisionmaking and target-setting based on the promise future PVA analyses in plan revisions that may never be undertaken. Indeed, the ESA, itself, does not require revisions to recovery plans. Combined with minimal annual funding allocation for most species (Schwartz 2008), the likelihood that the services will update recovery plans and revise placeholder viability criteria is low. Overall, only 20% of all recovery plans have been updated (see the supplemental material). Recent status reviews of 15 South Florida plants illustrate that “temporary” placeholder viability-based recovery criteria are typically never refined (see the supplemental material).

Finally, we disagree with Himes Boor (2014) that PVA yields recovery criteria that are more scientifically robust and transparent than “any other approaches used to set recovery thresholds.” PVA modeling results are highly dependent on data quality (i.e., the use of proxy data, age and stage classes, variance estimates, the inclusion of catastrophes, differences in sampling protocol), data set length, modeling assumptions, model structure, and validation of results and assumptions (Beissinger and McCullough 2002). These important methodological caveats are not always acknowledged or transparent to those without technical expertise, including managers implementing recovery plans, although they can lead to different population forecasts and management recommendations.

Moreover, targets for PVA-centered criteria are based on an “acceptable” extinction risk threshold, and do not escape the normative decisionmaking required of other methods of setting thresholds. As was noted by Shaffer (1981) in his original development of PVA, there is no exact value that connotes viability. Although having a lower extinction risk indicates that a species is more secure, there is no scientific basis for claiming that a 6% probability of extinction over 100 years equates to threatened or endangered status, whereas a 5% probability equates to viability and recovery. Moreover, PVA may fundamentally mislead a decisionmaker regarding the true risks to a species. A use of short time horizons in PVA may falsely inflate the perceived security of the species. For example, a 95% chance of persisting for 100 years may suggest a sufficiently low extinction risk to justify delisting. However, that same PVA—if run over a longer time frame—may also demonstrate that a species only has a 20% chance of persistence for 200 years and only 6% chance of persistence over 300 years (Shaffer and Samson 1985).

When the services have specified extinction risk thresholds in recovery criteria, they have used a wide spectrum of normative thresholds. For example, the viability standard for delisting the dwarf lake iris (*Iris lacustris*; less than a 5%

probability of extinction in 20 years) is less precautionary than that for the Hawaiian crow (*Corvus hawaiiensis*; less than a 5% probability of extinction in 100 years), and both are less precautionary than for the Steller's eider (*Polysticta stelleri*; less than a 1% probability of extinction in 100 years; USFWS 2002, 2009, 2012b). Downlisting criteria for 15 South Florida plants (20%–90% probability of persistence for 100 years) are particularly problematic because of the large range of extinction risk deemed acceptable within each species and the low probability of persistence considered acceptable (see the supplemental material). In contrast, Shaffer (1981) “tentatively and arbitrarily” proposed a definition of a viable population as one having a 99% probability of persisting for 1000 years. If PVA-centered criteria are used, we support application of a more uniform, precautionary, and transparent viability standard across listed taxa, in place of the *ad hoc* and relatively nonprecautionary risk thresholds discussed above.

The three Rs approach to recovery

We recommend a more holistic and comprehensive framework for recovery planning based on the conservation biology principles of representation, resiliency, and redundancy (the *three Rs*) proposed by Shaffer and Stein (2000) for lowering extinction risk and maintaining self-sustaining populations. In essence, the three Rs require a recovered species to be present in multiple large, resilient populations arrayed across a range of ecological contexts. Representation requires the protection of populations across the full range of ecological settings of a species' range, meeting the ESA's geographic representation mandate. Resiliency encompasses population-specific attributes that increase long-term persistence in the face of disturbance. Resiliency can also address related issues regarding threats abatement and recovery of ecologically effective populations. Redundancy requires establishing multiple populations in each ecological setting to spread extinction risk and to increase species' viability. The three Rs are rooted in findings from ecological theory and empirical studies (e.g., Diamond 1975, Ellstrand and Elam 1993, Gaston 1994, Frankham 2005) that, all else being equal, larger range, more populations, larger populations, larger habitat areas, sufficient gene flow, and more intact ecosystems all lower extinction risk.

Any successful recovery planning framework must explicitly require that recovery criteria fulfill the ESA's recovery mandates. In order to meet these mandates, a three Rs framework must require that all plans include a standard checklist of recovery criteria under each of the three Rs that are objective and measurable, meaning quantitative and temporally and spatially specific, and that set targets for geographic representation, ecosystem recovery, and threats abatement; provide a range of analytical tools for determining the recovery targets for each criteria which can accommodate data constraints; include an explicit justification for each criterion that explains the scientific rationale and analytical approach for setting that target; and require

future data collection, collection protocols, and analyses to fill important data gaps needed to better inform recovery.

A primary advantage of a three Rs framework is that criteria can be developed using multiple analytical approaches—including PVA—as appropriate, depending on the data constraints for each listed species. By allowing for a range of analytical approaches, recovery planners can set quantitative population and threat reduction criteria based on the best-available information and can require future data collection and analyses when needed. We recognize that setting meaningful recovery targets is challenging in a data-limited environment. However, we argue that provisional quantitative targets, even when they are chosen on the basis of expert opinion (e.g., Martin et al. 2012) or limited historical information on abundance and range, better serve imperiled species in need of immediate conservation action than the placeholder viability standards that would predominate in a PVA-centered approach. Provisional targets can be refined when resources and information are available to update the recovery plan with more data-driven analyses. We also recognize that all recovery targets, regardless of the analytical approach, involve both normative and scientific components. The normative dimension, influenced by societal values and risk tolerance, specifies the “acceptable” extinction risk (i.e., how much is enough to achieve recovery?), whereas the scientific dimension informs the conservation measures that will lower extinction risk and determines whether a species meets that level of risk (Vucetich et al. 2006). We recommend that the rationale for each criterion clearly distinguish between the scientific and normative bases for those targets.

In tables 1 and 2, we recommend recovery criteria under the three Rs framework that should be included in all recovery plans, including examples of quantitative criteria, analytical approaches for setting targets, and the supporting scientific frameworks. Specifically, we recommend that representation criteria include quantitative targets for protecting and restoring populations across the full range of ecoregions or ecological communities of a species' historic range. Representation criteria should specify the percentage of the historic and current range over which recovery will occur and why some portions are considered “significant” and some not. Achieving representation criteria can lower extinction risk by protecting genetic diversity, local adaptations, and ecological interactions across the range (Carroll et al. 2010).

Redundancy criteria should include quantitative targets for establishing multiple populations or habitat areas in each ecological setting. Targets for the number of populations in each ecological setting can be informed by current and historic population distribution and by viability-based approaches such as incidence function models and spatially explicit metapopulation models when data are available. Redundancy criteria can lower extinction risk by buffering populations from environmental variation, reducing the chance of extirpation from catastrophic events and

Table 1. Recommendations for applying the concepts of representation and redundancy to developing objective and measurable recovery criteria.

Principle	Tenet	Recommended measures	Example objective and measurable recovery criteria	Example scientific foundation and analytical tools	References
Representation	Represent historic range	Percentage of historic range over which recovery will occur	Restore species to a range size that represents x% of historic range	Abundance–distribution relationships	Brown (1984), Gaston (1996), Channell and Lomolino (2000)
Representation	Represent full range of ecological settings across historic range	Number or percent of ecological settings across the historic range over which recovery will occur	Protect and restore at least one population in each ecoregion or vegetation type across the historic range; protect and restore x% of the habitat for the species in each ecoregion or vegetation type across the historic range; maintain the elevational range occupied by the species	Risk spreading by reducing covariance among populations in the same habitat; inclusion of genetic diversity in populations locally adapted to different environments	Pressey et al. (1981), Neel and Cummings (2003)
Redundancy	Protect multiple populations	Number of populations, occurrences, or sites in different geographic areas and ecological settings	Maintain x populations in each specified ecological type	Abundance–distribution relationships; metapopulation theory	Brown (1984), Hanski et al. (1995)

Note: The references cited in this table are available in the supplemental material.

Table 2. Recommendations for applying the concept of resiliency to developing objective and measurable recovery criteria.

Principle	Tenet	Recommended measures	Example objective and measurable recovery criteria	Example scientific foundation and analytical tools	References
Resiliency	Population size: Maintain higher abundance	Individual abundance within populations	Maintain a harmonic mean of x individuals in each population for y years	Small population and declining population paradigms	Soulé (1987), Gabriel and Burger (1992), Blackburn and Gaston (2002), Matthies et al. (2004)
Resiliency	Population stability: Maintain stable and increasing populations	Population stability and growth	Maintain populations that are stable or increasing through x years	PVA; occupancy modeling	Shaffer (1981), Noon et al. (2012)
Resiliency	Maintain or restore historic connectivity	Distribution of distances among habitat patches and/or a specified effective connected area or connectivity rate	Ensure the median distance among occupied habitat patches does not increase by more than x%; restore habitat such that the connectivity-corrected habitat amount is increased by x% to reflect historic conditions; restore a connectivity rate of greater than x genetically effective migrants per generation	Effects of fragmentation and reduced gene flow; graph theoretic approaches to quantifying connectivity; incidence function models; PVA	Urban and Keitt (2001), Pascual-Hortal and Saura (2006), Ferrari et al. (2007), Saura and Pascual-Hortal (2007), Saura et al. (2011), Carroll et al. (2013), Neel et al. (2014)
Resiliency	Conserve ecosystems on which listed species depend	Habitats of appropriate type, size, and quality to include necessary ecosystem components to support listed species	Manage x number of habitat patches to maintain or restore canopy size distribution such that y% of tree canopy is in the z size class to provide nest trees	Species-habitat relationships; niche theory	Grinnell (1917), Morrison et al. (2006)
Resiliency	Maintain or restore species' ecological roles	Measures of ecosystem function or species interaction	Ensure listed species is sufficiently abundant to maintain x% of its habitat in a kelp-dominated state	Ecologically effective density	Soulé et al. (2005), Estes et al. (2010)
Resiliency	Eliminate or manage threats	Reduce or eliminate the probability of a threat occurring and/or reduce the magnitude of its effects. Ensure that sufficient management regime is in place to keep threat in abatement for foreseeable future	Reduce probability of conversion of habitat to suburban development by protecting x% of remaining unprotected habitat; reduce magnitude of invasive species effects by removing species x from y acres of habitat annually	Expert knowledge; Bayesian network modeling; multiple competing hypotheses; PVA	Peery et al. (2004), Marcot et al. (2006), Amstrup et al. (2010), Fuentes and Cinner (2010), Martin et al. (2012)

Note: The references cited in this table are available in the supplemental material.

increasing the probability of maintaining natural gene flow and ecological processes.

Resiliency criteria should include targets for population size and population trend. Establishing larger populations helps buffer against genetic, demographic, and environmental stochasticity. Maintaining stable or growing populations over a specified time period is important to understanding whether recovery will continue over the long term. PVA can potentially play a role in guiding the selection of population targets when sufficient data are available. Where data are not sufficient for PVA, historic population sizes can provide guidance for the size of individual populations. Tools for helping assess trends across multiple populations include occupancy modeling based on species presence and absence data (Noon et al. 2012) and multisite PVA. Resiliency criteria should also include criteria for habitat quality (i.e., the extent and distribution) and connectivity (i.e., links to other populations), because larger total amounts and patches of habitat help support larger, genetically diverse populations more able to withstand perturbations. Tools for assessing habitat extent, distribution, and connectivity include graph theoretic models (e.g., Neel et al. 2014), incidence function models, and spatially explicit population models. Ecosystem criteria can also include targets for restoring species' ecological roles by establishing ecologically effective densities (i.e., enough individuals over a sufficiently wide geographic distribution to restore the species' ecological role), particularly for keystone species, foundation species, ecosystem engineers, and top predators (Soulé et al. 2005, Estes et al. 2010).

Criteria for threat abatement derived from a formal threat assessment and prioritization, which is recommended by the recovery planning guidance document (NMFS 2010), can be integrated into a comprehensive three Rs analysis. Such an assessment would require explicit measures of how each threat has been eliminated or will be controlled now and in the foreseeable future and require that a management regime be in place to ensure that the threats do not return. PVA can inform threat reduction targets when sufficient data exist on how threats affect population growth or vital rates, but other methodologies for conducting threat assessment, such as Bayesian network modeling (Marcot et al. 2006) and expert opinion (Martin et al. 2012), can inform targets when demographic data are limited.

The three Rs framework is also well suited to addressing emerging threats to species, such as climate change and ocean acidification, for which scientific data and understanding are rapidly evolving. Many of the conservation actions required by recovery criteria under the three Rs approach—such as increasing a population's size, range, connectivity, and habitat restoration—are important steps for reducing extinction risk and increasing resilience to climate change (Heller and Zavaleta 2009, Pearson et al. 2014). In addition, the flexibility of approaches under the three Rs for setting the threat reduction targets allows planners to set criteria for reducing climate threats, such as

actions to reduce greenhouse gas emissions and increase a species' resilience to climate change (Povilitis and Suckling 2010), even when sufficient data are not available to conduct more data-intensive, climate-based PVA (e.g., Brook et al. 2009).

Conclusions

There are many factors that limit the fulfillment of the ESA's mandate for recovery, including insufficient funding, poor enforceability, and challenges to on-the-ground implementation. In addition, a fundamental limitation continues to be recovery criteria that are vague or too lenient to ensure the long-term persistence of species across the landscape (Neel et al. 2012, 2013). Although PVA may be useful as a component of comprehensive recovery planning when adequate data are available, we recommend against its use as an overarching framework for developing recovery criteria. PVA is particularly suited to ranking the importance of threats or management actions, identifying life stages or demographic rates that may be limiting population growth in order to target management of those stages, and identifying gaps in data and monitoring that can be used to improve data collection and analysis (Beissinger and Westphal 1998, Morris et al. 2002). As a way forward, we urge scientists and conservation practitioners to continue to develop rigorous analytical tools, including PVA, to support recovery criteria under a three Rs framework that provides a practical means of addressing the ESA's comprehensive recovery mandates given the significant data limitations and budget constraints inherent in recovery planning today.

Supplemental material

The supplemental material is available online at <http://bioscience.oxfordjournals.org/lookup/suppl/doi:10.1093/biosci/biu218/-/DC1>.

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Shaye Wolf (swolf@biologicaldiversity.org) is the climate science director, *Brett Hartl* is the endangered species policy director, and *D. Noah Greenwald* is endangered species program director at the Center for Biological Diversity, based in Tucson, Arizona. *Carlos Carroll* is the director of the Klamath Center for Conservation Research, in Orleans, California. *Maile C. Neel* is an associate professor in the Department of Plant Science and Landscape Architecture and the Department of Entomology at the University of Maryland, in College Park.

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Realistic levels of inbreeding depression strongly affect extinction risk in wild populations

Julian J. O'Grady^{a,*}, Barry W. Brook^b, David H. Reed^{a,1}, Jonathan D. Ballou^{a,2},
David W. Tonkyn^c, Richard Frankham^a

^aBiodiversity and Bioresources Group, Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia

^bSchool for Environmental Research, Institute of Advanced Studies, Charles Darwin University, Darwin, Northern Territory 0909, Australia

^cDepartment of Biological Sciences, Clemson University, SC 29634, USA

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ABSTRACT

The role of inbreeding depression in the extinction of wild populations is controversial, largely because there are no quantitative estimates of its impact using realistic levels of inbreeding depression. To address this deficiency, this study (1) provides a comprehensive estimate of the impact of inbreeding depression on wild, mammalian and avian species via a meta-analysis, and (2) determines the impact of this level of inbreeding depression on extinction risk over a broad taxonomic range via stochastic computer projections with and without inbreeding depression for populations with carrying capacities of 100, 500 and 2000 individuals. An average overall effect of 12 diploid lethal equivalents was found across the life-history of the species in the meta-analysis. In the stochastic computer projections, 12 diploid lethal equivalents of inbreeding depression (with purging) decreased median times to extinction by an average of 37%. These decreases were significant and of very similar magnitude, regardless of the carrying capacity modelled. Disregarding the influence of inbreeding depression on extinction risk will lead to serious overestimates of the survival prospects of threatened mammalian and avian taxa. Further, inappropriate recovery plans may be instituted if the causes of extinction risk and their relative contributions are not recognized.

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1. Introduction

Wild species can be driven to extinction by both deterministic factors (habitat loss, overexploitation, pollution and introduced species) and stochastic factors associated with small population size (demographic, genetic and environmental stochasticity, and catastrophes) (World Conservation Monitoring Centre, 1992; Baillie et al., 2004). However, most extinc-

tions seem to result from the combined effects of both deterministic and stochastic processes (Lande et al., 2003; Reed et al., 2003b; O'Grady et al., 2004b).

Genetic stochasticity encompasses inbreeding depression, loss of potentially adaptive genetic diversity, and mutation accumulation (Frankham et al., 2002; Reed, 2005). Inbreeding depression is the most immediate and potentially damaging of these (Frankham et al., 2002; Vilas et al., 2006). Essentially

* Corresponding author. Tel.: +61 2 9850 6950; fax: +61 2 9850 9237.

E-mail addresses: jogrady@bio.mq.edu.au (J.J. O'Grady), barry.brook@cdu.edu.au (B.W. Brook), dreed@olemiss.edu (D.H. Reed), ballouj@nzp.si.edu (J.D. Ballou), tdavid@clemson.edu (D.W. Tonkyn), rfrankha@bio.mq.edu.au (R. Frankham).

¹ Present address: Department of Biology, The University of Mississippi, P.O. Box 1848, University, MS 38677-1848, USA.

² Present address: Department of Conservation Biology, Conservation and Research Center, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA.

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all well studied naturally outbreeding species show depressed reproductive fitness in inbred individuals, termed inbreeding depression (Falconer and Mackay, 1996; Lacy, 1997; Lynch and Walsh, 1998; Hedrick and Kalinowski, 2000). This has been demonstrated for wild (Crnokrak and Roff, 1999), laboratory (Bijlsma et al., 2000; Frankham et al., 2002; Reed et al., 2002, 2003a), and zoo (Ralls et al., 1988) populations. While there has been scepticism about the occurrence of inbreeding depression in wild populations, compelling evidence now exists. Of 157 valid data sets across 34 taxa reviewed by Crnokrak and Roff (1999), 90% showed deleterious effects due to inbreeding (Frankham et al., 2002).

The contribution of inbreeding depression to the extinction risk of wild species has been the subject of a long-running controversy. It has been argued that inbreeding plays little role in extinction as demographic and environmental stochasticity will drive small populations to extinction before genetic factors become important (Lande, 1988; Caro and Laurenson, 1994; Caughley, 1994; Dobson, 1999). An explicit meta-analytical test of this hypothesis based on 170 pair-wise comparisons of genetic diversity in threatened and taxonomic related non-threatened species showed that the majority of threatened species are not driven to extinction before genetic threats affect them adversely (Spielman et al., 2004).

Inbreeding depression has been linked with population declines and/or extinction of wild (Vrijenhoek, 1994; Newman and Pilson, 1997; Saccheri et al., 1998; Westemeier et al., 1998; Madsen et al., 1999, 2004) and captive (Frankham, 1995; Bijlsma et al., 2000) populations. These studies discussed individual cases, but none provided comprehensive evidence covering a wide range of threatened species, or gave a clear perspective on the circumstances under which inbreeding is important, and when it is not.

Studies of the impacts of inbreeding on extinction risk in wild populations are hampered by the difficulties in separating genetic and non-genetic components. Further, constraints on time and resources have forced past studies to concentrate on only a few, high profile species. Stochastic computer simulations are standard heuristic tools in population biology (Levins, 1966) and are widely used for predicting the fate of threatened populations by projecting life-history and environmental information forward in time (Akçakaya and Sjögren-Gulve, 2000; Beissinger and McCullough, 2000; Menges, 2000). Thus, stochastic computer projections offer the only feasible means for comprehensively and promptly investigating the role of inbreeding on extinction risk across a broad range of taxa (Brook et al., 2002). They allow many species to be investigated, can be performed relatively quickly, and allow the inclusion or exclusion of inbreeding in concert with demographic and environmental stochasticity; this is impossible in field experiments.

Computer projections have been used to investigate the effects of inbreeding depression on population growth and/or extinction risk. Some have found that inbreeding depression had strong effects (Vucetich and Waite, 1999; Oostermeijer, 2000), while some reported little impact on population viability (Burgman and Lamont, 1992). Others found that the impact depended on population size (Dobson et al., 1992), and that the impact was strongly affected by the

interaction between inbreeding, population size, and carrying capacity (Thévenon and Couvet, 2002). However, these studies focused on specific or hypothetical cases and were often projected for a few generations only. Whether these specific results hold for a broader taxonomic range requires evaluation.

The sole published study across a broad taxonomic range (birds, mammals, plants and reptiles) found a clear reduction in population viability (Brook et al., 2002) by applying a very conservative level of inbreeding depression; the average level found for juvenile survival in captive mammals (Ralls et al., 1988). Yet inbreeding depression affects all components of the life cycle (Frankel and Soulé, 1981; Keller, 1998; Frankham et al., 2002). Further, inbreeding depression is typically greater in more stressful wild environments than in more benign captive ones (Armbruster and Reed, 2005). For example, Crnokrak and Roff (1999) reported that inbreeding depression was seven times higher in the wild than in captivity. For these reasons, the reduction in population viability reported by Brook et al. (2002) is likely to be a serious underestimate.

The objectives of this work were to (1) undertake a meta-analysis of the literature to determine the full impact of inbreeding depression on the fitness of wild species; and (2) evaluate the effect of this level of inbreeding depression on extinction risk for 30 species using stochastic computer models.

2. Methods

2.1. Meta-analysis for the effects of inbreeding depression

An extensive literature search was undertaken for studies measuring inbreeding depression for natural populations in wild habitats. To match the aims of this study, only data from wild species were analysed (Table 1). The published data allowed resolution of the number of lethal equivalents (LEs) per haploid genome (β) for three vital rates viz. fecundity, first year survival (0–1 year old), and survival to sexual maturity (1 year old to age of sexual maturity). Where possible, β -values were estimated (Morton et al., 1956) for each of these rates from the regression of survival (S) on the inbreeding coefficient (F), as follows:

$$-\ln S = A - \beta F$$

where A is a constant.

Where there were only two categories of data, inbred and non-inbred, the following relationship (Crnokrak and Roff, 1999) was used to estimate β :

$$\beta = (-\ln[X_I/X_o])/F$$

where X_I is the rate for inbred individuals, X_o is the rate for non-inbred individuals, and F is the inbreeding coefficient for the individuals at which the rate was measured. A mean β -value was calculated for each of these rates by weighting each data set by the square root of its sample size. These calculated β -values are reported as diploid lethal equivalents (2β) in Table 1 for comparison to the familiar standard of 3.14 diploid lethal equivalents found by Ralls et al. (1988) for juvenile survival in zoo populations.

Table 1 – The number of diploid lethal equivalents (2β) estimated to affect fecundity, first year survival (0–1 year old), and survival to sexual maturity (1 year old to age of first reproduction)

Species	2β	N	Reference
A: Fecundity			
<i>Cervus elaphus</i>	2.69	209	Slate et al. (2000)
<i>Gambusia holbrooki</i>	8.072	14	Leberg (1990)
<i>Melospiza melodia</i>	2.516	161	Keller (1998)
<i>Mus domesticus</i>	5.65	144	Meagher et al. (2000)
Weighted mean	3.936		
B: First year survival			
<i>Gallinula chloropus</i>	4.342	72	McRae (1996)
<i>Geospiza fortis</i>	4.29	2040	Gibbs and Grant (1989)
<i>Lenontopithecus rosalia</i>	4.65	481	Dietz et al. (2000)
<i>Melospiza melodia</i>	2.88	1091	Keller (1998)
<i>Parus major</i>	1.23	7346	Greenwood et al. (1978)
<i>Parus major</i>	1.474	1297	van Noordwijk and Scharloo (1981)
<i>Picoides borealis</i>	0.74	745	Daniels and Walters (2000)
Weighted mean	2.354		
C: Survival to sexual maturity			
<i>Aphelocoma ultramarina</i>	>13.44	858	Brown and Brown (1998)
<i>Geospiza fortis</i>	8.816	918	Gibbs and Grant (1989)
<i>Melospiza melodia</i>	2.64	1127	Keller (1998)
<i>Parus major</i>	3.73	616	Greenwood et al. (1978)
<i>Picoides borealis</i>	1.08	795	Daniels and Walters (2000)
Weighted mean	5.968		

When determining these mean β -values (see Section 2), the contribution of each data set was weighted by the square root of its sample size (N).

2.2. The species modelled

The stochastic modelling encompassed 18 mammal and 12 bird species of varied ecologies (carnivores, herbivores, omnivores), geographical regions (Africa, America, Asia, Europe, and Oceania), generation lengths (range 2–22 years) and population growth rates (deterministic $r = -0.214$ to $+0.253$), for which robust population models had already been constructed in previous works (Brook et al., 2000; Reed et al., 2003b; O'Grady, 2002; O'Grady et al., 2004a,b). Fifty seven percent (17) of the species modelled are Red Listed by the World Conservation Union (Baillie et al., 2004) as threatened. The species are listed in Table 2. The data sources for the species modelled in this study are published in Brook et al. (2000), Reed et al. (2003b), O'Grady (2002), O'Grady et al. (2004a,b). Similarly, the population models used in this study were drawn from these aforementioned studies, but modified as described in Section 2.3 to account for the full effects of inbreeding depression.

2.3. Stochastic computer modelling

The program VORTEX v8.41 (Miller and Lacy, 1999) was used to model the impacts of inbreeding depression on extinction risk. The models were parameterised using the protocols prescribed for this program (Miller and Lacy, 1999), and as de-

scribed in a previous study which evaluated the predictive capacity of this program (Brook et al., 2000). These models incorporated all published factors known, or suspected by field experts to influence each species' extinction risk. All models incorporated the effects of genetic, environmental, demographic and catastrophic stochasticity, and were parameterised via long-term studies (>10 years data) published for the species. Each species was modelled at the time point where the most comprehensive data set was found to build its stochastic computer model. In some cases, these data sets no longer reflect the species' current circumstances. Thus, the extinction risks generated in this study are for heuristic purposes only and are not intended as prospective viability analyses applicable to conservation decision-making.

Based upon the meta-analysis, a total of 12 LEs per diploid genome were apportioned across the life span. All models incorporated the effects of natural selection (purging) reducing the frequency of deleterious alleles. To approximate observed proportions of inbreeding depression due to highly deleterious alleles and mildly deleterious alleles (Simmons and Crow, 1977), five lethal equivalents were assumed to be due to recessive lethal alleles and seven due to deleterious alleles of small effect. Hence, in the models five LEs were assumed to be due to recessive lethal alleles and therefore subject to purging. VORTEX allows purging of a maximum of 5 LEs through both selection and genetic drift (Miller and Lacy, 1999). The other seven lethal equivalents were modelled to be sub-lethal alleles of smaller effect that are little affected by purging. Four of these were modelled to reduce survival of juveniles until sexual maturity, and the remaining three to reduce fecundity. The term "lethal equivalents" is more commonly related to a reduction in survival in response to inbreeding rather than fecundity. In this study, "lethal equivalents" is related to fecundity as a short-hand for the slope in the log-linear model of inbreeding's effects on fecundity. In species where juveniles attained sexual maturity in one year, all β -values for survival were used to reduce survival of inbred individuals during that year. Where juveniles took more than one year to reach sexual maturity, the effect of the 4 LEs was divided equally across these years. For example, where a species took two years to reach sexual maturity, 2 LEs were applied to first year survival, and 2 to second year survival. The effect of these sub-lethal alleles was modelled by an exponential decline (Morton et al., 1956):

$$S = S_0 e^{-\beta F}$$

where S is the value calculated by the model for the rate affected by inbreeding depression, S_0 is the rate for non-inbred individuals, β is the average number of lethal equivalents per haploid genome, and F is the inbreeding coefficient automatically calculated (Miller and Lacy, 1999) for individuals by VORTEX.

Initial population sizes (N) of 50, 250 and 1000 were used, broadly corresponding to the IUCN Red List categories of Critically Endangered, Endangered and Vulnerable, respectively based on Criterion D of the Red List system (Baillie et al., 2004). Following the protocols of Brook et al. (2002), a ceiling carrying capacity (K) of twice the initial population size was imposed for most species. This is a less assumption-driven means of representing habitat limitation in the absence of

Table 2 – Impacts of inbreeding depression on median times to extinction for 30 species, based upon stochastic computer projections

Species	R ₀	N = 50				N = 250				N = 1000			
		MTE _{noID}	MTE _{3,14}	MTE _{ID}	%Rdn	MTE _{noID}	MTE _{3,14}	MTE _{ID}	%Rdn	MTE _{noID}	MTE _{3,14}	MTE _{ID}	%Rdn
<i>Aphelocoma coerulescens</i>	0.324	16	11	9	44	53	31	21	60	127	63	42	67
<i>Bubalus depressicornis</i>	0.984	2	2	2	0	3	3	3	0	3	3	3	0
<i>Bubo virginianus</i>	1.976	3	3	3	0	5	5	5	0	7	6	6	14
<i>Capra species</i>	0.260	10	8	7	30	19	15	13	32	27	22	19	30
<i>Cervus elaphus</i>	0.723	2	2	2	0	4	4	4	0	6	5	5	17
<i>Cervus eldii eldii</i>	1.451	9	8	6	33	21	16	12	43	34	26	19	44
<i>Charadrius melodus</i>	1.084	8	7	6	25	17	14	12	29	26	23	19	27
<i>Copsychus seychellarum</i>	0.886	6	5	5	17	12	10	9	25	18	16	14	22
<i>Delichon urbica</i>	0.875	49	21	11	78	292	85	33	89	464	273	95	80
<i>Dendroica kirtlandii</i>	0.957	3	3	3	0	5	5	5	0	7	7	6	14
<i>Dendrolagus matschiei</i>	1.107	8	5	4	50	18	11	8	56	28	18	12	57
<i>Gorilla gorilla beringei</i>	1.452	7	6	5	29	13	11	9	31	19	16	14	26
<i>Gymnobelideus leadbeateri</i>	0.673	12	9	7	42	34	22	15	56	64	37	26	59
<i>Leucopsar rothschildi</i>	0.517	5	5	4	20	9	8	7	22	12	11	11	8
<i>Lipotes vexillifer</i>	0.020	2	2	2	0	4	4	4	0	5	5	5	0
<i>Lycan pictus</i>	1.580	5	5	4	20	7	7	6	14	13	11	9	31
<i>Lynx rufus</i>	0.869	14	12	9	36	45	27	17	62	95	51	29	69
<i>Marmota flaviventris</i>	0.885	5	5	4	20	11	9	8	27	14	13	11	21
<i>Meles meles</i>	0.997	7	6	5	29	14	11	10	29	19	17	14	26
<i>Melospiza melodia</i>	0.762	32	18	12	63	169	64	29	83	783	188	71	91
<i>Mirounga leonia</i>	0.921	11	8	6	45	44	21	13	70	84	38	24	71
<i>Neophema chrysogaster</i>	1.000	5	5	4	20	13	11	9	31	24	20	16	33
<i>Oreamnos americanus</i>	0.606	7	6	5	29	15	12	10	33	23	19	15	35
<i>Panthera leo</i>	0.972	11	7	6	45	38	19	12	68	88	36	21	76
<i>Petaurus australis</i>	1.352	14	10	7	50	52	27	18	65	144	63	36	75
<i>Picooides borealis</i>	1.039	10	8	6	40	25	18	13	48	42	30	22	48
<i>Rhinoceros sondaicus</i>	0.900	6	5	4	33	10	9	8	20	13	12	11	15
<i>Streptopelia mayeri</i>	1.662	6	6	5	17	11	10	9	18	15	13	13	13
<i>Trichechus manatus latirostris</i>	1.050	16	10	7	56	41	23	15	63	69	38	25	64
<i>Zosterops lateralis chlorocephalia</i>	0.823	22	12	9	59	98	35	20	80	319	52	42	87
Mean					31				39				41

R₀ is deterministic growth rate per generation. Median time to extinction (MTE) is measured in generations. Nine scenarios were modelled (i.e., three levels of inbreeding depression for each of three initial population sizes N = 50, N = 250, and N = 1000). The levels of inbreeding depression were: no inbreeding depression (noID), the full impact of 12 diploid lethal equivalents (ID), and the effect of 3.14 diploid lethal equivalents applied to juvenile survival only (3.14). The percent reduction in median time to extinction (%Rdn) due to inbreeding depression was also calculated for each of the 30 species.

detailed data required to estimate the additional parameters in density dependent models of vital rates (a common situation for threatened species). It also provides a conservative estimate of extinction risk as other functional forms of density dependence often strongly affect predicted risk (Ginzburg et al., 1990). However, where density dependence of the vital rates was detected for a particular species (Akçakaya, 1996; Miller and Lacy, 1999) it was modelled explicitly via a negative feedback on that vital rate instead of a ceiling carrying capacity.

Three levels of inbreeding depression were modelled for each initial population size. These were: no inbreeding depression, the full impact of 12 diploid lethal equivalents, and a partial effect of 3.14 diploid lethal equivalents applied to juvenile survival only to permit comparison with Brook et al. (2002) and other PVA simulations which commonly use this value as a default input. In this way nine scenarios were modelled. All stochastic simulations were replicated 1000 times. The simulations were projected forward to estimate median time to extinction for each species, median time to extinction being the time taken for half of the simulation replicates become extinct. Extinction was defined as no remaining animals of one sex. Median time to extinction was assessed as it has an unbounded scale, in contrast to the proportion extinct. In addition, this measure is not biased by occasional run times that are extremely long, which is a problem with mean time to extinction (Brook et al., 2002). Further, this variable was used as it is intuitively clear to most wildlife managers.

Median time to extinction was assayed in generations as extinction risk has been shown to scale better to generations than to years (Leigh, 1981; O'Grady, 2002; Frankham and Brook, 2004). Median time to extinction and probability of extinction were obtained and averaged across species for the nine scenarios modelled. Most analyses were performed using the percentage difference in median time to extinction (MTE) between the models for a species with inbreeding depression (MTE_{ID}), and without (MTE_{noID}), computed as $([MTE_{noID} - MTE_{ID}] / MTE_{noID}) * 100$.

2.4. Statistical analysis

Non-parametric analyses were used for most analyses, as percent differences were not normally distributed. Wilcoxon signed rank tests were used to test whether differences for each of the three population sizes across all species, and across mammals and birds as groups, were significantly greater than zero. Variation in median time to extinction between mammals and birds was tested using a Kruskal–Wallis test, while differences among population sizes and among these broad taxonomic groups were compared using Friedman's test (Sokal and Rohlf, 1995). The relationship between the species' growth rates (measured as R_0) and the percentage difference in their median time to extinction was measured using Spearman's rank correlation and by linear regression. As the data were close to a normal distribution in this test, these two parametric tests were used not only as heuristic tools but to cross check the results of the other. As both gave identical conclusions, only the correlation analyses are reported. For all tests $\alpha = 0.05$. All tests were performed using MINITAB v12 statistical software (Ryan and Joiner, 1994).

3. Results

3.1. Meta-analysis of inbreeding depression

The meta-analysis revealed an overall inbreeding depression of 12.3 diploid lethal equivalents, consisting of 3.9 lethal equivalents for fecundity, 2.4 for first year survival (0–1 year old), and 6.0 for survival from 1 year old to sexual maturity (Table 1).

3.2. Stochastic computer projections to determine the impact of inbreeding depression on extinction risk

Inbreeding depression (12 LEs) markedly reduced the median time to extinction across the species (Table 2). All species showed a pattern of lower population size with inbreeding depression that translated into a higher probability of extinction. Representative population trajectories are shown in Fig. 1. Mean reduction in median time to extinction was 31%, 39% and 41% for initial population sizes of 50, 250 and 1000 respectively. These reductions in median times to extinction were all highly significant ($p < 0.001$) for each population size, though not significantly different between the three population sizes ($p = 0.459$). Similarly, these reductions were also highly significant for the three initial population sizes where mammals and birds were assayed separately as broad taxonomic groups (for all three mammalian N , $p < 0.001$; for birds, $p = 0.003$, $p = 0.003$ and $p < 0.001$ respectively).

There was a significant relationship between the species' growth rates and the reduction in their median time to extinction ($r_s = 0.580$, $p = 0.001$; $r_s = 0.594$, $p = 0.001$; and $r_s = 0.696$, $p < 0.001$ for initial N of 50, 250 and 1000, respectively). Species with the highest growth rates generally had the greatest reduction in their median time to extinction (Table 2).

No significant differences in median time to extinction were found between mammals and birds for the three initial population sizes ($p = 0.416$, $p = 0.314$, and $p = 0.309$, respectively) under the impact of 12 LEs. Similarly, the Friedman's test indicated that there were no significant differences in median time to extinction among population sizes and among these broad taxonomic groups ($p = 0.135$).

The effect of 3.14 LEs applied to juvenile survival only also reduced median time to extinction across all the species, though to a lesser degree than the realistic impact of 12 LEs. Average reductions in median times to extinction across all the species for the initial population sizes of 50, 250 and 1000 were 17%, 26%, and 28% respectively.

4. Discussion

This study provides the first realistic estimate of the impact of inbreeding depression on the fitness of wild mammals and birds across a broad taxonomic range. The estimated value of 12.3 diploid lethal equivalents is almost four times greater than that used in previous simulation studies. This modelled level of inbreeding depression markedly and significantly reduced the estimated median times to extinction compared to models lacking inbreeding depression. Unlike most simulation studies on the effects of inbreeding depression, this study encompasses the effects of purging. It extends the evidence on the role of inbreeding depression in extinction from

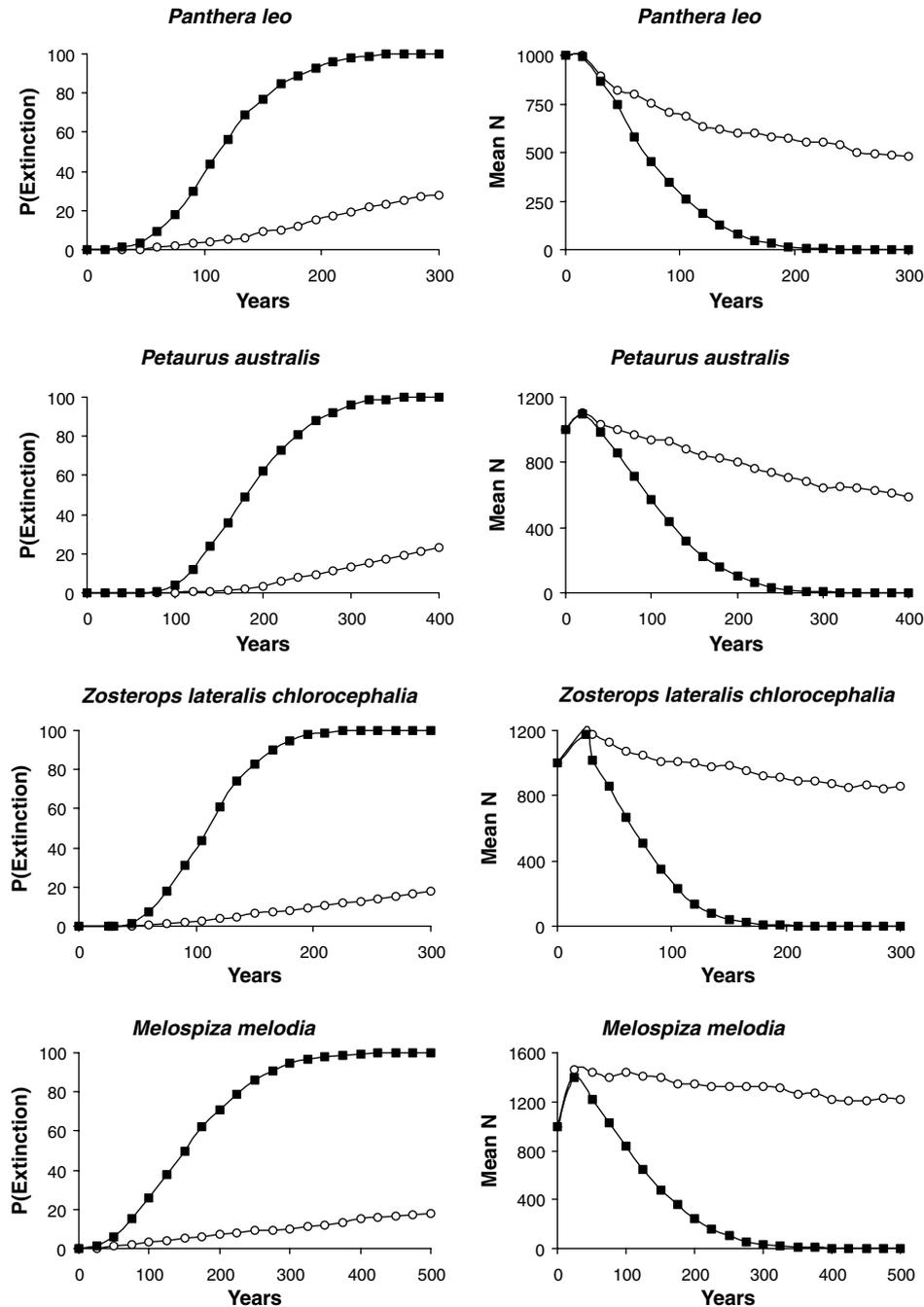


Fig. 1 – Impact of inbreeding depression (square data points) versus none (circles) on the cumulative probability of extinction (P[Extinction]) and mean final size (Mean N) for four representative species. These data were determined by stochastic computer models with all known demographic, environmental and catastrophic threats included and with an initial population size of 1000 (see Section 2).

hypothetical life-histories (Mills and Smouse, 1994) and a few real species (Dobson et al., 1992; Newman and Pilson, 1997; Saccheri et al., 1998; Oostermeijer, 2000) to a wide range of mammalian and avian taxa.

The mean level of inbreeding depression in juvenile survival found in this study is much less than that for the other two components of fitness viz. fecundity and survival to sexual maturity. Hence, the results of this study suggest that, in the absence of specific data, more severe default values for

inbreeding depression (i.e. spanning the entire life cycle) should be applied in population viability analyses for species in the wild, or risk underestimating their probability of extinction. For example, the reductions in median time to extinction found in this study where 3.14 diploid lethal equivalents were applied to juvenile survival only were 17%, 26% and 28%, while 12 lethal equivalents produced reductions of 31%, 39% and 41% for the same suite of species and respective initial population sizes.

The findings in this study are corroborated by other lines of evidence for the role of inbreeding depression in extinction. Most endangered species have less genetic diversity than related non-endangered species (Spielman et al., 2004). If 'non-genetic' factors drove species to extinction before inbreeding was a problem, there would be no such difference. Further, loss of genetic diversity is related to reduced population fitness (Reed and Frankham, 2003).

Even with a comprehensive meta-analysis, these results are still likely to slightly underestimate the impact of inbreeding depression on extinction in wild populations. The total estimate of lethal equivalents for fecundity and survival in mammals and birds obtained from the meta-analysis agrees with the only comparable datum of the full number of lethal equivalents from an individual species (found by summing the individual components reported by Keller, 1998). However, the estimate found in this study may be conservative because it was not possible to resolve any effects of inbreeding depression on adult survival after first reproduction (see Section 2), and these effects may be important for long-lived, iteroparous species. In addition, the models assumed that all adults in each species are equally likely to reproduce. This does not apply in most wild populations (Frankham et al., 2002) leading to a smaller effective population size and potentially greater inbreeding. Consequently, the results of this study are likely to underestimate the impact of inbreeding depression.

In what circumstances is inbreeding depression likely to make important contributions to extinction risk? The level of inbreeding depression applied in this study produced a near ubiquitous decrease in median time to extinction across all the population sizes modelled, for both mammals and birds, and for a wide range of life-histories. Thus, the results in this study indicate that inbreeding depression is a major threat for most mammalian and avian taxa at small to moderate population sizes (i.e., less than a few thousand individuals). However, the meta-analysis showed some variation among species in the severity of inbreeding depression (Table 1). Species with lower genetic loads would likely be relatively more resistant to this threat.

Inbreeding depression may have little time to affect populations declining rapidly due to deterministic pressures, such as habitat loss (Brook et al., 2002). For example, at the time two of the species within this study (viz. *Bubalus depressicornis* and *Lipotes vexillifer*) were assessed by the World Conservation Union, both were experiencing substantial anthropogenic threats (Manansang et al., 1996 and Kaiya et al., 1994 respectively). Both had highly negative growth rates such that inbreeding depression did not foreshorten their estimated median time to extinction at any population size modelled (Table 2). This study, like that of Brook et al. (2002), found a significant positive relationship between population growth rate (r) and reduction in median time to extinction (Table 3). However, compared to the study of Brook et al. (2002), this study found a smaller reduction in median time to extinction due to inbreeding depression as the species' population growth rate (r) decreased (Table 3). The smaller reduction in median time to extinction found in this study is largely a consequence of the greater deterministic pressures faced by some species in this study compared to those faced by the species in Brook et al. (2002). These deterministic pressures

Table 3 – Panel A: the relationship between population growth rate per generation (R_0) and reduction in median time to extinction measured in generations (MTE) due to a modelled inbreeding depression of 12 diploid lethal equivalents [three initial population sizes ($N = 50$, $N = 250$, and $N = 1000$) were modelled]; Panel B: a comparison of the relationship between initial population growth rate (r) and reduction in median time to extinction measured in years (MTEy) at an initial population size of 250 individuals found in this study Ba250 and by Brook et al. (2002) Bb250

N	Regression	r^2 (%)	p
Panel A			
50	Percent reduction MTE = $7.8 + 24.2R_0$	22	0.006
250	Percent reduction MTE = $9.1 + 30.7R_0$	19	0.009
1000	Percent reduction MTE = $7.0 + 35.1R_0$	26	0.003
Panel B			
Ba250	Percent reduction in MTEy = $0.64 + 2.14r$	42	<0.001
Bb250	Percent reduction in MTEy = $0.46 + 4.42r$	72	<0.001

sharply reduced the growth rate of some species in this study (Table 2) and overwhelmed or dominated the influence of inbreeding depression on their median time to extinction.

Inbreeding will have less impact on naturally inbreeding species, as they express lower inbreeding depression on average due to long-term purging of the genetic load (Husband and Schemske, 1996). Populations that have had a very small effective population size for a long period of time, or those that have recovered from population bottlenecks, should be less sensitive to inbreeding depression due to purging of deleterious recessive alleles, though the effects of purging are usually relatively small (Lacy and Ballou, 1998; Byers and Waller, 1999; Miller and Hedrick, 2001; Frankham et al., 2001; Crnokrak and Barrett, 2002; Reed et al., 2003a). Inbreeding impacts will probably be less in species with polyploid ancestry, as they seem to suffer less inbreeding depression than equivalent diploids due to a lower frequency of homozygotes (Husband and Schemske, 1997; Frankham et al., 2002).

Inappropriate recovery programs may be devised if realistic levels of inbreeding depression are not taken into account. There are at least three major reasons for this. First, reproductive fitness is often improved if inbred populations are outcrossed (Vrijenhoek, 1994; Westemeier et al., 1998; Madsen et al., 1999; Ebert et al., 2002; Vilà et al., 2003; Schwartz and Mills, 2005). If this is not done, an inbred population with low fitness may continue to decline, as happened with the Illinois population of the greater prairie chicken (*Tympanuchus cupido pinnatus*; Westemeier et al., 1998). Attempts to recover the population by habitat improvement failed to halt its decline, and it only recovered following outcrossing with another population.

Second, as shown in this study, even moderately large populations (initial $N = 1000$) are susceptible to the deleterious effects of inbreeding, and are unlikely to be viable over the long-term. This result accords with stochastic computer modelling by Reed et al. (2003b) who concluded that populations of similar size to those studied here are unlikely to be viable in the long-term (40 generations). Further, this study shows that the relative impact of inbreeding on median time to extinction is similar over a range of different population sizes

of general relevance to conservation biology (i.e., a few thousand or less, although the absolute value of median time to extinction increases as population size increases). For such moderately small populations, the results of this study indicate that there is often a time lag until inbreeding accumulates and increases extinction risk (Fig. 1). Thus, the management of genetic threats attending small population size should not be postponed in the expectation that if the species has persisted at a certain size for a few decades then inbreeding depression will not substantially affect its viability, or hasten its median time to extinction.

Third, funding priorities for conservation and restoration will be distorted if the impacts of different factors on extinction risk are not correctly understood. The greater prairie chicken mentioned above is a good example. As recounted by Westemeier et al. (1998), management strategies were initially focussed on non-genetic recovery methods to recover a decline in population size and reproductive fitness. However, despite these efforts the population continued to decline until genetically unrelated individuals were introduced. These introductions subsequently restored egg viability.

In conclusion, realistic levels of inbreeding depression markedly elevate extinction risk for most threatened mammalian and avian taxa. These results emphasise the importance of avoiding inbreeding and maintaining genetic diversity in threatened species.

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Review

Pragmatic population viability targets in a rapidly changing world

Lochran W. Traill^{a,*}, Barry W. Brook^a, Richard R. Frankham^b, Corey J.A. Bradshaw^{a,c}^a Environment Institute and School of Earth and Environmental Sciences, University of Adelaide, South Australia 5005, Australia^b Department of Biological Sciences, Macquarie University, NSW 2109, Australia^c South Australian Research and Development Institute, P.O. Box 120, Henley Beach, South Australia 5022, Australia

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ABSTRACT

To ensure both long-term persistence and evolutionary potential, the required number of individuals in a population often greatly exceeds the targets proposed by conservation management. We critically review minimum population size requirements for species based on empirical and theoretical estimates made over the past few decades. This literature collectively shows that thousands (not hundreds) of individuals are required for a population to have an acceptable probability of riding-out environmental fluctuation and catastrophic events, and ensuring the continuation of evolutionary processes. The evidence is clear, yet conservation policy does not appear to reflect these findings, with pragmatic concerns on feasibility over-riding biological risk assessment. As such, we argue that conservation biology faces a dilemma akin to those working on the physical basis of climate change, where scientific recommendations on carbon emission reductions are compromised by policy makers. There is no obvious resolution other than a more explicit acceptance of the trade-offs implied when population viability requirements are ignored. We recommend that conservation planners include demographic and genetic thresholds in their assessments, and recognise implicit triage where these are not met.

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1. Introduction

Extinction is the natural endpoint in the evolutionary process, with most species typically persisting 1–10 million years (Frankham et al., 2002). Evolutionary theory and numerical simulation of population persistence (or demise) has allowed the estimation of thresholds, or key ‘turning points’, after which extinction is more likely. The turning point in the trajectory of a population

is complex, such that simplifications of the process are often used to make conservation decisions in an imperfectly measured world. This is why the concept (and applied use) of population viability and minimum viable population size (MVP) gained momentum in the early years of conservation biology (Beissinger and McCullough, 2002), and why population thresholds remain in use today (Traill et al., 2007), albeit concomitant with extinction correlates such as habitat loss (Mace et al., 2008). Importantly, these thresholds imply the moment at which a *declining* population becomes a *small* population, with increased vulnerability to extinction (Caughley, 1994). Small populations are uniquely vulnerable to demographic stochasticity at this crucial stage (Melbourne and Hastings, 2008). Moreover, the number of individuals

* Corresponding author. Tel.: +61 8 83036070.

E-mail address: lochran.traill@gmail.com (L.W. Traill).

required to maintain the *small* population is generally underestimated.

But are people really listening to the key, inconvenient truths that emerge here? The present-day increase in the rate of extinction is rapid and can be principally attributed to an explosion of modern human activity (IUCN, 2008). In response to the perceived biotic crisis that looms as a result (Ehrlich and Pringle, 2008), multi-lateral conservation organisations such as the World Conservation Union (IUCN) and the scientific community have worked hard to understand and quantify extinction risk, and communicate this knowledge to policy-makers, governments and the general public (Beissinger and McCullough, 2002). Here we review the evolutionary and demographic requirements of populations and argue that evidence-based scientific estimates of what is required to achieve viability are (often considerably) larger than targets outlined by conservation organisations. While we cannot provide an exhaustive review of the practical challenges of conservation biology, we suggest that most vulnerable species are not really being managed for viability (continued existence under trying environmental circumstances); rather, conservation targets in most cases merely aim to maximise short-term persistence and fit with complex political and financial realities (see Duffy, 2008). The problem is similar to the dilemma faced by climate scientists, where national and international policy seems incapable of meeting the emissions reduction implied by the available geophysical and biological evidence to avert severe anthropogenic interference with the climate system, let alone to reverse the damage already done (Chakravarty et al., 2009; Hare, 2009). Numerous socio-political impediments (IPCC, 2007; Working Group III) do not invalidate the science behind climate change and its impacts (Working Group I and II); rather, they capitulate to the reality of what is considered politically possible. Here we argue that preventing species extinctions by applying knowledge derived from the discipline of conservation biology has an analogous problem, admittedly with no immediate resolution.

2. The scientific basis for minimum viable population sizes

Despite a good deal of empirical development of the concept of minimum viable population size (Frankham, 1995; Franklin and Frankham, 1998; Reed et al., 2003; Brook et al., 2006; Traill et al., 2007), there is a disconnect between associated theory and conservation practice. It is irrefutable that population size matters for extinction risk, with small and isolated populations being particularly vulnerable to: (1) demographic fluctuation due to random variation in birth and death rates and sex ratio, (2) environmental fluctuation in resource or habitat availability, predation, competitive interactions and catastrophes, (3) reduction in co-operative interactions and subsequent decline in fertility and survival (Allee effects), (4) inbreeding depression reducing reproductive fitness, and (5) loss of genetic diversity reducing the ability to evolve and cope with environmental change (see Caughley, 1994; Frankham, 1995).

The idea of a MVP has its foundation in efforts to capture, in population viability analyses (PVA), the many and interacting determinants of extinction risk. In this original context, MVP is defined as the smallest number of individuals required for a population to persist in its natural environment (Shaffer, 1981). The likelihood of success is measured on a probability scale (0–1), and projections into the future can be scaled to years or generations (Reed et al., 2003).

Alternatively, evolutionarily determined MVPs are based solely on the maintenance of evolutionary potential, that is, the population size required at equilibrium to balance the loss of

quantitative genetic variation with the gain from mutation (Franklin, 1980; Franklin and Frankham, 1998). Although the arguments are theoretically different, both recommend similar turning points toward extinction, as we demonstrate below.

2.1. Empirical MVP

Estimates of MVP size can be derived by empirical simulation, experiments, or long-term monitoring. An example of long-term census study is that by Berger (1990) who evaluated the persistence of isolated populations of bighorn sheep (*Ovis canadensis*) over 50 years. Populations <50 individuals went locally extinct, while those containing ≥ 100 individuals generally persisted.

Most empirical MVPs are probabilistic estimates of population persistence over a stipulated period: by arbitrary convention at least 90% certainty of persistence for at least 100 years (Shaffer, 1981). Typically, PVAs are stochastic systems models which project changes in population abundance over time and account for demographic and environmental variation, catastrophic events, density dependence and inbreeding depression (Gilpin and Soulé, 1986). PVAs are used to predict population persistence in the short (a few years) to medium term (10s–100s of years) and allow quantitative comparison and qualitative ranking of alternate management strategies. Persistence over generations (from as low as 3 to 40 or more generation spans) is used as an alternate to time steps in years, and is seen as biologically more appropriate when working across taxonomic groups (O'Grady et al., 2008). Simulation models can be individual- or matrix/cohort-based and implemented using generic computer software packages (see Lindenmayer et al., 1995) or tailored models. Most estimates of empirical MVP have been obtained using PVAs; indeed, a recent review of MVP-related literature found that 95% of 141 published articles used PVA as their basis for estimating extinction risk (Traill et al., 2007).

Median estimates of the empirical MVP derived from PVAs range from ~1300 (Brook et al., 2006) to ~5800 individuals (Reed et al., 2003), depending on the method and underlying assumptions. The lower estimate derives from scalar population growth models that do not include demographic stochasticity, fluctuation in age structure or genetic deterioration. The upper estimates of MVP (Reed et al., 2003) accounted for all major deterministic and stochastic threats and some positive feedbacks, including inbreeding depression. Of note, Melbourne and Hastings (2008) find that most population analyses have underestimated viability by not accounting for all major factors contributing toward stochasticity.

A recent review and meta-analysis reported that 60% of published PVAs included genetic effects (Traill et al., 2007). Yet, even PVAs that take genetic factors into account usually underestimate their impacts on extinction risk. First, these only encompass the deleterious genetic impacts of inbreeding on reproduction and survival (inbreeding depression), but do not consider the loss of genetic diversity which effectively reduces a population's ability to evolve and cope with environmental change (Visser, 2008). Second, all studies that include inbreeding depression underestimate its effect on population viability. Many use small impacts of inbreeding depression based on juvenile mortality in captive populations, rather than those for all components of reproduction and survival in wild populations (O'Grady et al., 2006). Further, all assume Poisson-type variation in family size, but variation is typically much greater leading to lower effective population sizes (Box 1), more rapid inbreeding and greater reduction in reproductive fitness (Frankham et al., 2002).

Box 1 Genetically effective population sizes.

The genetically effective population size (N_e) is a measure of a population's genetic behaviour relative to that of an 'ideal' population (Frankham et al., 2002). Technically, it is the size of an idealised population that would result in the same inbreeding or loss of genetic diversity as that in the population under study. An idealised population is a conceptual closed, random-mating population of hermaphrodites that have Poisson variation in family size, constant numbers of breeding individuals in successive, non-overlapping generations, and no mutation or selection (Wright, 1931). Real populations deviate from the idealised population due to fluctuations in population size, unequal sex ratios, family size variation greater than Poisson and overlapping generations. The first three factors reduce N_e to below the census size, while the effects of overlapping generations are not consistent in direction (Frankham, 1995). Genetic impacts depend on N_e , rather than N , with genetic diversity being lost at a rate of $1/(2N_e)$ per generation within closed populations, and inbreeding increasing at this same rate in random-mating populations. The N_e is the 'currency' used to describe the evolutionary MVP.

Critics argue that PVAs are only practically useful for predicting extinction risk where data are extensive and reliable and projection time frames are short (Fieberg and Ellner, 2000). Further, the IUCN Red List does not base the categorisation of any threatened species on PVAs alone (IUCN, 2008). However, Boyce (1992) and Burgman (2006) suggest that PVAs are indispensable when done properly because they cause assumptions regarding the processes leading to decline to be made explicit, and bring together scientists and policy-makers to assess the costs and benefits of alternative approaches to population management.

2.2. Evolutionary MVP

Few conservation programs (for wild-living populations) explicitly incorporate genetic goals or attempt to maintain wild populations large enough to retain a substantial fraction of genetic diversity (Frankham et al., 2002). Genetically viable populations are those large enough to avoid inbreeding depression, prevent the accumulation of deleterious mutations, and maintain evolutionary potential. Small populations can persist in the wild for some time, but the reproductive fitness of these, and especially the ability to adapt to change (evolutionary potential) is compromised and extirpation is likely (Spielman et al., 2004; Kristensen et al., 2008). So what population sizes are required to ensure genetic viability, and how do these compare to empirical MVPs?

The MVP to retain evolutionary potential in perpetuity is the equilibrium population size where loss of quantitative genetic variation due to small population size (genetic drift) is matched by gains through mutation. Franklin (1980) estimated this to be a genetically effective population size (N_e) of ~500 individuals (50 to avoid inbreeding). Critically though, the mean ratio of the N_e to the census population size (N) is ~0.1 (Frankham, 1995) and therefore a census population of ~5000 adults. The concept of N_e is described in Box 1, but we note here that the estimation of the census N allowed biologists to move on from the 50/500 rule (after Franklin, 1980). Other estimates of the evolutionary MVP have attained a N_e of ~5000, corresponding to an adult population size of 50,000 (Lande, 1988; Franklin and Frankham, 1998).

Unfortunately, the population sizes of many threatened species are likely to fall below this range (perhaps >2000 species, given the total number of *Critically Endangered* populations in the Red List; IUCN, 2008). The loss of genetic variation within these populations can be regenerated through mutation, but this will typically take hundreds to thousands of generations (Frankham et al., 2002). Small populations have therefore reached a point-of-departure: away from the ability to adapt to changing environmental circumstances and toward inflexible vulnerability to these same changes (Frankham and Ralls, 1998).

3. Generalities

The bottom line is that both the evolutionary and demographic constraints on populations require sizes to be at least 5000 adult individuals. These seem to be large requirements, but a number of studies across taxonomic groups have made similar findings: the median MVP derived from PVA of 102 vertebrate species was 5816 individuals (Reed et al., 2003), and 4169 individuals from a meta-analysis of 212 species (Traill et al., 2007). The census-based MVP of 5500 reported by Thomas (1990) is also remarkably congruent; all similar to the recommended census N of 5000 individuals (Frankham, 1995). We note though that similarities are not strictly equivalent, and are a result of evaluation of some non-overlapping factors, meaning minimum viable population size in many circumstances will be larger still.

4. Conservation in the long term

The science of more than 30 years of empirical and genetic research on the viability of wild-living populations thus implies that the number of individuals (required to avoid a turning point toward extinction) is greater than generally appreciated or implemented within conservation management. Although our contention that conservationists often manage below a biologically reasonable extinction threshold is not new (see Tear et al., 1993; Reed et al., 2003), debate persists. Disagreement hinges on two main issues: (i) the accuracy of predictions and (ii) their real-world applicability to conservation action (Beissinger and Westphal, 1998; Coulson et al., 2001).

Regarding accuracy, criticism centres on the general low quality of available population data and the high sensitivity of predictions to assumptions made. A response to this is that the rapidity with which the extinction crisis is unfolding means that biologists and managers cannot afford to wait for the collection of the necessary high-quality data before making decisions (Lee and Jetz, 2008) – and that given their relative simplicity, most biases are likely to underestimate rather than over-estimate risk (though see Brook, 2000 for a counter-example). Many conservationists also question the real-world relevance of MVP estimates given their high associated uncertainty bounds and the wide cross-species range. For example, some published PVAs have specified MVP sizes as low as 20 individuals (Sæther et al., 1998) and others as high as 100,000 (Reed, 2005). However, variation arises in part from the complexity, biological reality and type of PVA used, and median confidence intervals from meta-analysis of standardised MVPs still provide reasonable guidance on the most likely targets that will be required (e.g., 3577–5129, 95% CI; Traill et al., 2007). Further, conservationists working within developing nations will rarely have the resources available to collect the demographic and other data necessary to model viability for specific species or taxa; there is thus a compelling argument to develop rules of thumb for population size extinction-risk thresholds. Moreover, related species tend to have similar characteristics and response.

Differences between published MVP estimates, even for the same species, can also be explained by the different survival probabilities and timescales used. For example, median MVP values estimated from time series models fitted to 1198 species (Brook et al., 2006) differed substantially (by up to 10,000 individuals) depending on whether the risk criteria specified a >50% or >90% probability of survival (Fig. 1). The first is a 'coin toss' level of risk acceptance, the latter is equivalent to being listed as *Threatened* by the IUCN (Criterion E). Further, median MVP values increase by many thousands of individuals as the projection interval increases from 10 to 1000 years (Fig. 2). The implication here (of selecting a particular frame of reference) is that conservation decision-makers must explicitly choose a period over which they are managing for persistence, and with a specified certainty of success. Beyond that chosen frame of reference, nothing useful can be said about the long-term persistence of a given species.

The science of integrated population biology is now clear enough that we can state that if conservation practitioners purportedly manage for population viability with a few hundred individuals or less, then they effectively manage at a 50:50 odds of success on a century time scale (see Fig. 1). Clearly, any conservation project that is serious about the long-term survival (and continued ability to evolve) of a species must aim for a meta-population of thousands of individuals (Figs. 1 and 2), or else re-evaluate their stated position. Practitioners can validly take issue with high population targets, because of the impracticality of preserving adequate contiguous habitat, especially for large-bodied species (e.g., Armbruster and Lande, 1993). In reality, most populations presently exist as fragmented sub-populations within a larger meta-population (Akçakaya et al., 2004), with their successful conservation depending on genetic exchange among units to maintain high genetic diversity (Hoegh-Guldberg et al., 2008).

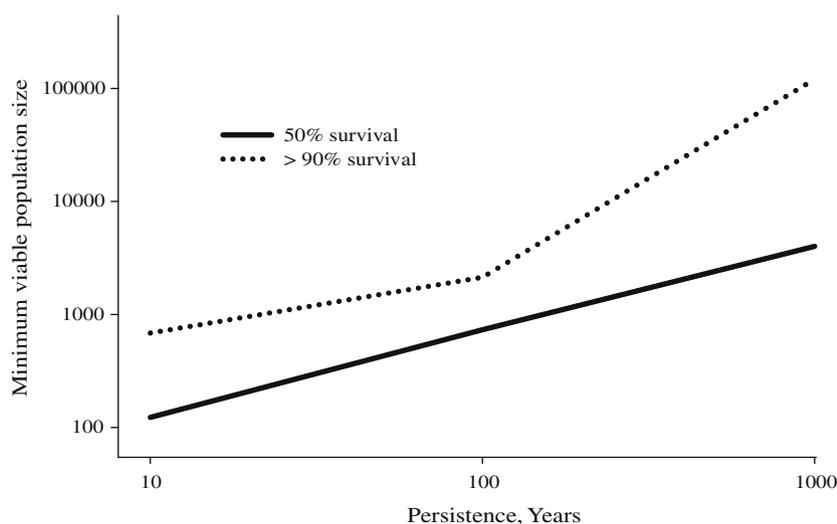


Fig. 1. Line plot of median minimum viable population estimates (scaled to \log_{10}) for 1198 species derived from time series analyses (see Brook et al., 2006) along a logged timescale (10–1000 years). The full line represents median MVP size at 50% probability of persistence. The dotted line is the median MVP at greater than 90% probability of persistence.

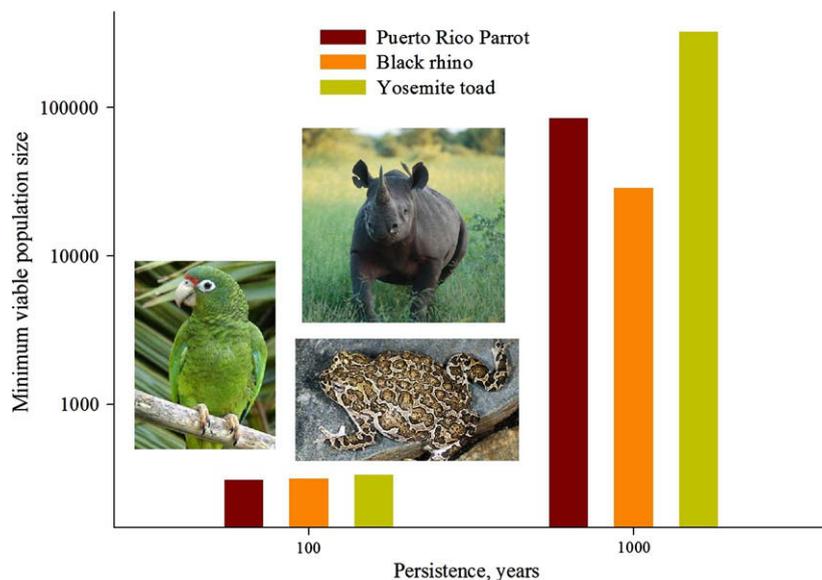


Fig. 2. Bar chart of (\log_{10}) MVP estimates for three threatened vertebrate species from time series population viability analyses (Brook et al., 2006). Selected species are the Yosemite toad (*Bufo canorus*), black rhinoceros (*Diceros bicornis*) and the Puerto Rico parrot (*Amazona vittata*). Data are model-averaged MVP values for 100 years (90% probability of survival, as used by the IUCN, 2008) and 1000 years (99% probability of survival). Images, PR parrot (<http://kevinschafer.com>), black rhino (<http://wildcast.net>) and Yosemite toad (<http://calacademy.org>).

5. Conclusions

We maintain that given demographic, genetic and phenomenological consensus, the concept of the minimum viable population is a useful benchmark, and highly relevant in today's biodiversity crisis. The poor implementation of empirically derived MVP targets is not the fault of the available data or theory arising; rather, we argue it is more constrained by political and logistic challenges. In other words, MVP estimates bring scientific frankness to the socio-political arena. Geophysical scientists use climate models to advise decision makers on the risks posed by global warming associated with different scenarios of carbon emission reductions (IPCC, 2007). Similarly, conservation biologists have a critical role to play in providing a scientific reality check on whether, and to what degree, decisions made in the interests of threatened species management or under the motivation of avoiding extinctions, will be effective. This can be done openly, thereby avoiding the tag of stealth policy (see Lackey, 2007; Wilhere, 2008). By explicit presentation of threshold data at alternate probabilities of success (Fig. 1), biologists leave the ultimate decision to the political process.

Current evidence from integrated work on population dynamics shows that setting conservation thresholds at a few hundred individuals only is a subjective and non-scientific decision, not an evidence-based biological one which properly accounts for the synergistic impacts of deterministic threats (Brook et al., 2008; Visser, 2008). Many existing conservation programs might therefore be managing inadvertently or implicitly for extinction – a clearly illogical and counter-intuitive aspiration. If practitioners cannot justify using conservation triage to alleviate problems associated with unrealistic targets (see Box 1), where small, inbred populations are neglected in preference to more viable options, then they must manage for biologically relevant MVPs at least 5000 adult individuals (or 500 simply to prevent inbreeding) whilst addressing the concomitant mechanisms of decline (Balmford et al., 2009).

Box 2 Ecological triage.

Ecological (or conservation) triage is a concept enveloped in an evolving, but unfortunately acrimonious, debate at the centre of conservation biology. Polarity centres on two fundamentally different approaches toward conservation, viz. 'no species extinction, at any cost' and 'extinction is inevitable for some species, let's manage the process rationally' (e.g., Jachowski and Kesler, 2009).

The debate has a long history. Walker (1992) advocated the prioritisation of species (conservation status) according to the necessary functions that species or populations provided to ecosystem function; and the abandonment of functionally redundant, or highly diminished species. While few conservationists explicitly advocate extinction of no-hopers, triage is implicit through recognition that current threats to biodiversity outweigh the resources available to mitigate these (Bottrill et al., 2008). Thus, a number of approaches can be taken to optimise conservation effort, albeit acknowledging that preventing extinction altogether is at the very least daunting. For example, Hobbs and Kristjanson (2003) advocate adaptive management strategies ranging from *no immediate management action* (say, for non-threatened species) to *urgent protection or restoration*, without stating that populations should be abandoned. Carefully thought-out resource allocation thus allows more efficient conservation effort, and hopefully, better outcomes.

Recent advances in the science include 'prioritisation protocols' that optimise (conservation) resource allocation through cost-benefit analyses (Murdoch et al., 2007) and the likelihood of management success (Joseph et al., 2009). The authors build on the *Noah's Ark* framework (Weitzman, 1998) through consideration of conservation costs and benefits, species utility and value; but take these a step further by accounting for the probability of management success. Wilson et al. (2007) developed a conservation prioritisation framework that addressed geographic priorities, fund allocation and area-specific threats. By applying this framework across Mediterranean ecoregions, they found that more species could be conserved through targeted conservation actions than through sole reliance on acquisition of appropriate habitat.

Conservation planning uses many criteria to guide decisions on conservation action, principally based on (biodiversity) representation and persistence (see Sarkar et al., 2006). Among the principles relevant to biodiversity persistence are population viability and evolutionary potential. Nonetheless, the point we make is that even (conservation) planners practice an implicit form of triage through recognition that entire conservation networks are not feasible. Conservation is one form of land use among many, and planners optimise conservation outcomes given the constraints.

Criticism of triage basically comes down to 'defeatism'. Pimm (2000) argues that triage is inappropriately seductive because "it combines the semblance of tough decision-making style with the substance of doing nothing." The argument to let species X go will be repeated years later for species Y. Further, triage inhibits science; saving the very rarest pushes the technical frontiers of conservation biology. To quote Pimm (2000) again, "nothing concentrates the mind like impending extinction, nor so openly tests whether our knowledge of ecology, genetics and is up to task."

More recent critics point out that a shift in philosophical stance by conservation biologists will have ramifications far beyond the current debate. If conservation biologists, the very people dedicated to prevent extinction via scientific investigation and restorative problem solving, sanction this, then what is there to stop others with no sympathy for conservation from justifying extinction (Jachowski and Kesler, 2009)? Others highlight conservation success stories such as the whooping crane (*Grus americana*), or indicate new funding possibilities for conservation through carbon financing (Pimm, 2000; Parr et al., 2009).

The debate is not likely to go away. In the interim, and on a positive note, the explicit nature of triage-based analyses will likely prompt funding from Government and donor sources that may not otherwise have been freed.

One partial remedy is for prioritisation of conservation funds to be based on indices of the distance of species population sizes from MVP. So for example, a small population of 50 individuals will score 0.01 (percent of 5000), and the inverse of this can be used as a modifier for fund allocation. A simple scoring system such as this can be the basis of a decision-framework for threatened species within a particular management region, and conservationists can factor in other considerations such as likelihood of success and economic value (see Joseph et al., 2009). Indeed, both

demographic and evolutionary MVPs have been, and continue to be influential to real-world conservation planning (Sarkar et al., 2006). As with the use of biodiversity surrogates in conservation planning (Pressey, 2004), rules of thumb on species' demographic and genetic requirements are often the only option when dealing with the current crisis under conditions of great uncertainty and severe resource constraints.

Further, minimum viable population sizes are legitimate and concrete targets that policy-makers can digest and implement. While scientists debate MVP variance, the extinction crisis deepens. Thresholds at 500/5000 are communicated more effectively to policy-makers who do not have the time to read the extensive literature surrounding viability. Indeed, the lack of communication between science and conservation policy can be improved through dissemination of generalities (such as thresholds) that can be formulated as policy (see Gibbins et al., 2008).

If, on the other hand, scientists regard MVP thresholds to be too high to implement practically, then what are the alternatives? Is managing for hundreds of individuals over short time-frames sensible? If biologists believe that meta-populations numbering less than a few thousand individuals are capable of survival in a globally changing world, then this needs to be argued with relevant empirical and genetic data as support. Other than that, a more explicit and honest acceptance of the biological trade-offs implied in ignoring MVPs on logistical grounds is needed, for credibility's sake.

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In My Opinion

Can Restoring Wolves Aid in Lynx Recovery?

WILLIAM J. RIPPLE,¹ *Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA*

AARON J. WIRSING, *School of Forest Resources, Box 352100, University of Washington, Seattle, WA 98195, USA*

ROBERT L. BESCHTA, *Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA*

STEVEN W. BUSKIRK, *Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA*

ABSTRACT Herein, we examine the hypothesis that relatively low densities of snowshoe hares (*Lepus americanus*) and the imperiled status of lynx (*Lynx canadensis*) may be partially due to an ecological cascade caused by the extirpation of gray wolves (*Canis lupus*) in most of the conterminous United States decades ago. This hypothesis focuses on 2 plausible mechanisms, one involving “mesopredator release” of the coyote (*C. latrans*), which expanded its distribution and abundance continentally following the ecological extinction of wolves over the temperate portion of their geographic range. In the absence of wolves, coyotes may have affected lynx via increased predation on snowshoe hares, on which the lynx specializes, and/or by direct killing of lynx. The second mechanism involves increased browsing pressure by native and domestic ungulates following the declines in wolves. A recovery of long-absent wolf populations could potentially set off a chain of events triggering a long-term decrease in coyotes and ungulates, improved plant communities, and eventually an increase in hares and lynx. This prediction, and others that we make, are testable. Ecological implications for the lynx may be dependent upon whether wolves are allowed to achieve ecologically effective populations where they recolonize or are reintroduced in lynx habitat. We emphasize the importance of little-considered trophic and competitive interactions when attempting to recover an endangered carnivore such as the lynx. © 2011 The Wildlife Society.

KEY WORDS Canada lynx, *Canis lupus*, competition, coyote, endangered species, gray wolf, *Lynx canadensis*, mesopredator release, *Lepus americanus*, white-tailed jackrabbit.

Under the auspices of the Endangered Species Act of 1973, the Canada lynx (*Lynx canadensis*) was listed in 2000 as a threatened species across the conterminous United States (US; U.S. Fish and Wildlife Service 2000). This listing was in part a response to sharp declines in distribution and abundance of lynx in several states for which reliable historical trapping records were available during the latter stages of the 20th century (e.g., MN, MT, NH, and WA; Federal Register 2000). These “southern lynx” are poorly understood relative to conspecifics occupying the boreal forests of northern Canada and Alaska, USA; therefore, their conservation remains a subject of debate (Koehler et al. 2008, Murray et al. 2008). Lynx face numerous factors that could limit their distribution and abundance, including competition with other mid-sized carnivores and habitat alteration by stand-replacing fires, timber harvest, and insect outbreaks (Buskirk et al. 2000, Koehler et al. 2008, Murray et al. 2008). Even so, there is broad agreement that the fate of the lynx at lower latitudes is closely linked to the distribution and abundance of its obligate primary prey, the snowshoe hare (*Lepus americanus*; Kolbe et al. 2007, Murray et al. 2008). That is not to say that widespread abundance of snowshoe

hares is the only condition for lynx recovery; however, it is a necessary one (Aubry et al. 2000, Murray et al. 2008).

Snowshoe hare populations occupying the forests of northern Canada and Alaska typically exhibit dramatic stable limit cycles with periods of 9–11 yr (Keith 1963, Krebs et al. 2001). The mechanism underlying the hare cycle across this region is debated vigorously, with 2 mechanistic pathways contending for priority. The first, a tri-trophic-level interaction among quantity of winter browse, hare population density, and densities of hare predators involves time-lagged density dependence. Under this mechanism, declining winter browse availability helps to slow the growth of hare populations during the increase phase of the cycle, but predation, especially by lynx, initiates the decline and represents the primary driver of changes in hare abundance (Wolff 1980, Hodges 2000a, Krebs et al. 2001). The second, a hare–winter–browse hypothesis, invokes plant secondary chemical responses to herbivory as the chief factor eliciting the hare cycle (Bryant et al. 2009). Decadal-scale climate fluctuation (Stenseth et al. 2002) is invoked with both mechanisms to account for broad geographic synchrony. Yet, neither primary mechanism is alleged to operate strongly in the temperate, or southern, portion of the distribution of the hare; rather, southern hare populations in the conterminous US appear to exhibit attenuated dynamics and exhibit only relatively low densities at population peaks (Murray 2000, Murray et al. 2008, but see Hodges 2000b).

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¹E-mail: bill.ripple@oregonstate.edu

Dampened dynamics in southern hare populations have been attributed to a combination of forest fragmentation and predation by prey-generalists (Wolff 1980, Wirsing et al. 2002, Griffin and Mills 2009). Specifically, both limited suitable habitat offering enough protective cover and persistent pressure from facultative predators (e.g., coyotes [*Canis latrans*]) appear to not only increase predation mortality to hares but also prevent the recruitment necessary to generate a cycle. By implication, forces that reduce forest fragmentation or suppress facultative hare predators could enhance the size of southern hare populations and perhaps promote cyclic dynamics. Increased hare abundance in this region can plausibly be expected to improve the viability of southern lynx populations.

The objective of this article is to briefly examine a hypothesis that chronically low densities of southern snowshoe hares and the imperiled status of lynx may be partially the result of an ecological cascade caused decades ago by the extirpation of the gray wolf (*Canis lupus*) over most of its conterminous US range. This hypothesis focuses on the subsequent ecological release of coyotes and of the ungulate prey of wolves and leads us to the prediction that wolf restoration could help facilitate lynx recovery in the conterminous US.

THE CONCEPTUAL MODEL

During the 1800s and early 1900s, gray wolves were extirpated throughout much of the conterminous US, and ungulate and coyote irruptions often followed the loss of wolves (Leopold et al. 1947, Presnall 1948, Prugh et al. 2009, Ripple et al. 2010). Herein, we describe a series of trophic and competitive interactions connecting wolves to lynx (Fig. 1). With wolves present, we hypothesize that coyotes would be maintained at low densities, resulting in little competition—either exploitative or interference—between coyotes and lynx. Interspecific interactions helped shape

the evolution, structure, and function of carnivore communities, with exploitative competition occurring when one species limits populations of another by using a common resource. Interference competition involves harassment, kleptoparasitism, or outright killing of one species by another (Van Valkenburgh 1991, Merkle et al. 2009).

In the absence of wolves, coyote densities and distributions generally expanded in the US—into the Midwest (Bekoff 1977), to the northeast as far as Newfoundland (Parker 1995), and as far northwest as Alaska (MacDonald and Cook 2009). And, because coyotes are known to be effective predators of hares (Wirsing et al. 2002), increased coyote populations can cause exploitative competition with lynx via higher predation pressure on hares (Buskirk et al. 2000, Bunnell et al. 2006). Further, interference competition between coyotes and lynx could limit densities of the latter, since examples of coyotes killing lynx have been reported (O'Donoghue et al. 1995). Interestingly, researchers have attributed declines in bobcat (*Lynx rufus*) populations to exploitation competition for prey caused by increasing coyote populations (Litvaitis and Harrison 1989), as well as increases in bobcats due to decreases in coyotes (Henke and Bryant 1999). Also, in the absence of wolves, population densities of wild cervids typically increase (Leopold et al. 1947), creating prey and winter-killed carrion subsidies to coyotes (Weaver 1979). These carrion subsidies have the potential to increase densities of facultative hare predators, and thereby predation on hares, during periods when lynx are ordinarily uncommon and predation on hares is low (Kolbe et al. 2007, Gompper and Vanak 2008). Note that coyotes may also benefit from the provision of wolf-killed carrion in wolf-dominated landscapes (Merkle et al. 2009), but we hold that interference competition with wolves would likely have a stronger negative effect on coyotes than any positive effects from this type of carrion subsidy. Additionally, we

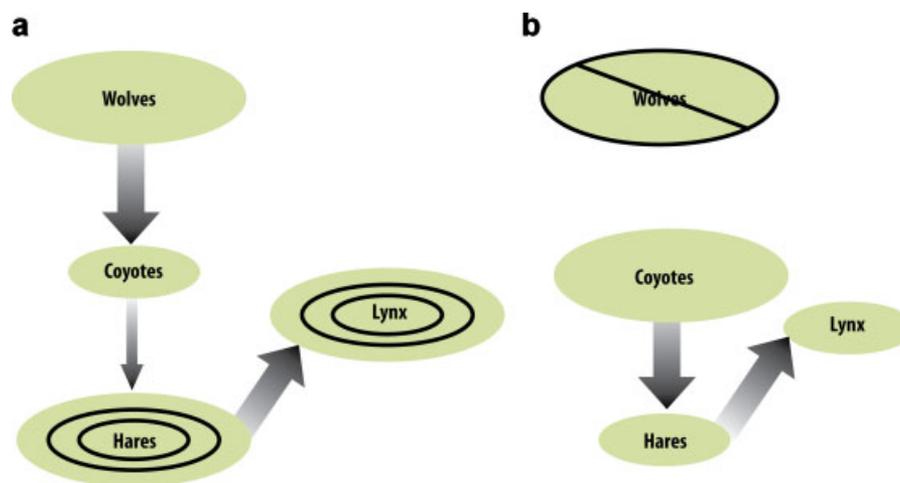


Figure 1. Conceptual diagram showing how the (a) presence or (b) absence of wolves may affect lynx across multiple trophic levels. We hypothesize (a) that in the presence of wolves, this apex predator maintains coyote populations at low densities, thus minimizing the potential influence of coyotes on hares. However, (b) in the absence of wolves, coyote densities increase, causing exploitative competition with lynx through high predation pressure on hares. Furthermore, in the absence of wolves, wild ungulates may not only provide a prey and carrion subsidy to coyotes but can also increase herbivory levels upon forest understory plants that satisfy important habitat needs for hares. Changes in ungulate herbivory and interference competition are not shown on the above diagrams. Note: Wide arrows denote strong effects; thin arrows, weak effects; large ellipses denote high densities; small ellipses, low densities; concentric lines in ellipses, variable and/or cycling densities.

hypothesize that elevated coyote predation pressure can, in addition to that from other hare predators, contribute to trait-mediated effects on hare populations via behavioral and physiological pathways. For example, in the Yukon Territory of Canada, increased stress from high levels of predation risk has been documented to cause marked deterioration in hare reproductive rates (Boonstra et al. 1998) and quality of offspring (Sheriff et al. 2009). Such trait-mediated effects could result in sustained, rather than decadal periodic, reductions in hare reproduction.

In northern latitudes (e.g., Yukon) where wolves are present and where hares are the main prey for coyotes, populations of coyotes generally occur at relatively low densities, as well as cycle up and down in concert with hares and lynx (O'Donoghue et al. 1998, Sheriff et al. 2009). This is in contrast to wolf-free southern latitudes (e.g., the conterminous US) where coyote densities are commonly an order of magnitude higher than those in the north (O'Donoghue et al. 1998, Bekoff and Gese 2003). Coyote diets are also more varied in the south. For example, in Wisconsin, coyote numbers did not decrease with declining snowshoe hare densities, because of attendant buffering from alternative food sources (Niebauer and Rongstad 1977). Thus, coyotes in the south have the potential to suppress hares through both habitat switching and prey switching, while subsisting, as opportunists, on other food resources: ungulate prey and carrion, fruits, smaller mammals, insects, birds, and human-generated garbage. Further, domestic cattle and sheep occur more broadly and at higher densities in the south than the north, which, for the southern portion of the range of the hare, could 1) increase the prey and carrion subsidy to coyotes, and 2) affect hare habitat through decreased forest understory vegetation due to herbivory. Most spatial overlap between lynx and livestock would occur at lower elevations of the lynx range because livestock are not generally distributed as high as lynx. Murie (1951) suggested that high densities of cattle in Arizona resulted in a lack of vegetative cover causing low densities of leporids.

To further explore hare dynamics in the south, we contrast hare populations in 2 unfragmented National Park settings: Isle Royale and Yellowstone. Neither park features livestock grazing. On Isle Royale, wolves colonized the park in 1949, and soon thereafter eliminated all coyotes from the island. With wolves and no coyotes, hares on Isle Royale cycled to high levels (Hodges 2000*b*). In Yellowstone National Park, Bailey (1930:125) described the distribution and abundance of snowshoe hares at around the time of the final eradication of wolves in this way: "Snowshoe rabbits are fairly common throughout the Canadian Zone timbered area." Similarly, Murie (1940:124) reported snowshoe hares in Yellowstone to be abundant in the early 1900s, writing that, "... at Sylvan Pass in 1903, 15 or 20 hares were frequently reported seen in a day so that hares at that time must have been quite plentiful." In contrast, a recent survey spanning the years 2002–2007 documented snowshoe hares as rare in Yellowstone (Hodges et al. 2009). This putative hare decline generally coincides with the absence of wolves and the consequent abundance of coyotes and high levels of herbivory

from elk (*cervus elaphus*; Murie 1940, Berger and Gese 2007, Beschta and Ripple 2009). Ungulates can compete with small mammals for forage and reduced ungulate densities can cause increases in small mammals (Keesing 2000). Lynx were common in Yellowstone at the turn of the 20th century but, similar to hare trends, have since declined (Buskirk 1999). Thus, we hypothesize that even with a lack of human fragmentation of landscapes or livestock grazing—as observed in Yellowstone National Park—disrupted trophic and competitive interactions alone may have been enough to chronically depress hare and lynx populations. With wolves now reestablished in Yellowstone (as of 1995), a test of this hypothesis is possible because it appears that coyote densities significantly declined in parts of the Greater Yellowstone Area following wolf reintroduction (Berger and Gese 2007). Moreover, early evidence tentatively suggests that a hare recovery may be taking place; namely, the 6 hare sampling sites for which the aforementioned Yellowstone survey had the longest time series all showed an upward trend in hare abundance in the final year (2007; Hodges et al. 2009). More hare sampling in Yellowstone in the future will be required to determine whether this initial trend continues.

An alternative explanation for the low snowshoe hare densities observed in Yellowstone invokes human-caused fire suppression in altering the spatial patterning of various successional stages important to this species. Fortunately, this mechanism can be tested by examining patterns of hare abundance in relation to changes to lodgepole pine (*Pinus contorta*) communities brought about by the wildfires of 1988, and subsequent successional changes. Under the fire suppression hypothesis, for example, we would expect increases in hare abundance to coincide primarily with the regeneration of high sapling density in stands burned in 1988 (Bryant et al. 2009, Hodges et al. 2009) rather than depression of coyote numbers caused by the presence of wolves.

We considered whether other leporids might have been affected by the processes hypothesized above for snowshoe hares. Again, early on Bailey (1930:127) described white-tailed jackrabbits (*Lepus townsendii*) as common in northern Yellowstone National Park "... and along the open valley of Lamar River." However, white-tailed jackrabbits in the Yellowstone–Grand Teton region apparently declined over the course of the mid- to late 20th century in the absence of wolves, and were recently reported as rare (Gunther et al. 2009) or completely absent (Berger 2008*a, b*) from the shrub–steppe-dominated Lamar Valley. Could this decline of white-tailed jackrabbits have been caused by increased coyote populations and elk herbivory following wolf extirpation? We believe that this scenario is both plausible and consistent with our hypothesis. During the 7-decade wolf-free period in Yellowstone, the Lamar Valley had both high densities of coyotes (high predation pressure on leporids) and intensive herbivory (reduced shrub and understory cover) from elk (Murie 1940, Beschta and Ripple 2009). Also, during the wolf-free period and consistent with the food-subsidy portion of our hypothesis, Gese et al. (1996) found that coyote densities and litter sizes in the Lamar Valley were

directly correlated with the amount of elk carcass biomass (carriion).

The hypothesized negative effect of wolf removal on snowshoe hares and lynx that we describe here could be exacerbated by climate change. In the high-elevation regions of the conterminous US where lynx still occur, deep winter snow provides these boreal specialists with a competitive advantage over coyotes, which have a higher foot-load (Murray and Boutin 1991, Crête and Lariviere 2003). Accordingly, while both species can overlap where snow is deep (Kolbe et al. 2007), coyotes have been shown to be more abundant during winter than lynx at lower elevations where snow is shallow and the energetic cost of movement is affected by snow (Murray and Boutin 1991). Thus, if climate change results in warmer and less severe winters, declining snow pack could allow coyotes (released by the absence of wolves) to exploit hares at higher elevations. Accordingly, additional studies, with and without wolves, that elucidate the winter sympatry among southern coyotes, lynx, and hares at high elevations are required to test for this scenario. Results of this research could be important for lynx conservation efforts in the conterminous US (Kolbe et al. 2007).

CONCLUSIONS

If the removal of wolves initiated trophic and competitive adjustments resulting in the decline of hare and lynx populations in southern latitudes, would the restoration of this apex predator help their recovery? Based on the above considerations, we hypothesize that the answer is yes, but we are unsure as to what extent and how long it might take. A recovery of long-absent wolf populations could potentially set off a chain of events triggering a long-term decrease in coyotes and ungulates, recovery of previously degraded native plant communities, and eventually an increase in hares and possibly other leporids as well. Furthermore, we think the answer is at least partially dependent upon whether wolves are allowed to achieve ecologically effective populations (Soulé et al. 2003) where they recolonize or are reintroduced. For example, aggressive wolf harvesting may have important negative effects on hares and lynx as described above, as well as on other species of concern. We encourage managers and policy makers to consider the potential for the types of ecological cascades hypothesized herein when 1) significantly altering or fragmenting habitat via intensive herbivory from high numbers of livestock or wild ungulates, as well as from other management practices (e.g., logging), and 2) designing and implementing wolf and lynx management plans. Perhaps more importantly, we encourage ecologists to test—experimentally or with observational data—our hypotheses regarding community interactions originating with wolves, but manifested in the distribution and abundance of snowshoe hares and lynx. We have proposed mechanisms that involve food of hares (via ungulate densities), predation on hares (coyote populations released from top-down control by wolves and food subsidized by ungulates and humans), and behavioral–physiological effects on hares via temporally sustained predation and harassment by facultative hare predators. We propose exacerbation of these effects by

an abiotic factor: altered patterns of snow depth and hardness in the face of climate change. All of these hypotheses are testable, at least indirectly.

Where wolf restoration is the objective, we believe that it is especially important to consider the ecological roles of these top predators in the ecosystem, rather than focusing solely on their demography (Estes et al. 2009). Accordingly, wolf recovery criteria in regions where hares and lynx occur can and should include measures of coyote densities, to index predation on hares, and the recruitment of woody browse species, which provide food and cover for hares.

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Wolves–coyotes–foxes: a cascade among carnivores

TAAL LEVI¹ AND CHRISTOPHER C. WILMERS

Environmental Studies Department, 1156 High Street, University of California, Santa Cruz, California 95064 USA

Abstract. Due to the widespread eradication of large canids and felids, top predators in many terrestrial ecosystems are now medium-sized carnivores such as coyotes. Coyotes have been shown to increase songbird and rodent abundance and diversity by suppressing populations of small carnivores such as domestic cats and foxes. The restoration of gray wolves to many parts of North America, however, could alter this interaction chain. Here we use a 30-year time series of wolf, coyote, and fox relative abundance from the state of Minnesota, USA, to show that wolves suppress coyote populations, which in turn releases foxes from top-down control by coyotes. In contrast to mesopredator release theory, which has often considered the consequence of top predator removal in a three-species interaction chain (e.g., coyote–fox–prey), the presence of the top predator releases the smaller predator in a four-species interaction chain. Thus, heavy predation by abundant small predators might be more similar to the historical ecosystem before top-predator extirpation. The restructuring of predator communities due to the loss or restoration of top predators is likely to alter the size spectrum of heavily consumed prey with important implications for biodiversity and human health.

Key words: coyote; fox; indirect effects; intraguild predation; mesopredator release; Minnesota, USA; predator interference; wolf.

INTRODUCTION

Cascading species interactions are critical to structuring ecological communities (Pace et al. 1999). Cascades are a type of indirect effect in which linear chains of direct effects propagate for three or more nodes (species or groups of species). Among trophic-level cascades are now well documented in both aquatic and terrestrial ecosystems (Terborgh and Estes 2010). Little attention, however, has been given to among-guild cascades. Such among-guild cascades whereby the largest or competitively dominant species directly suppresses a mid-sized guild member thus releasing the smallest guild member might importantly influence the composition of species guilds as well as the trophic levels above and below them.

As large predators are extirpated in certain parts of the world and recolonize in others, knowledge of such among-guild, or more specifically among-predator, cascades will be crucial to understanding and predicting changes in community composition. Among trophic-level cascades involving an apex predator that suppresses a smaller or mesopredator with consequent impacts on the mesopredator's prey have been well studied in recent years. Mesopredator releases have been documented in over 60 systems worldwide (Ritchie and Johnson 2009) in species complexes as varied as African lions (*Panthera leo*) and wild dogs (*Lycaon pictus*) (Creel

and Creel 1996, Creel 2001) to black-backed gulls (*Larus marinus*) and crabs (Ellis et al. 2007). In North America, coyotes (*Canis latrans*) have been shown to suppress numerous smaller predators ranging from domestic cats (*Felis catus*) to opossum (*Didelphis virginiana*) (Crooks and Soule 1999, Ritchie and Johnson 2009), though their impacts on suppressing fox populations (*Vulpes vulpes*, *Urocyon cinereoargenteus*, and *Vulpes velox*) are the most well documented (Harrison et al. 1989, Ralls and White 1995, Henke and Bryant 1999, Fedriani et al. 2000, Kamler et al. 2003, Mezquida et al. 2006, Karki et al. 2007, Moehrenschrager et al. 2007, Thompson and Gese 2007). The influence of coyotes in suppressing mesopredators has been shown to increase rodent and songbird diversity and boost duck nesting success (Sovada et al. 1995, Crooks and Soule 1999, Henke and Bryant 1999). Separately, wolves (*Canis lupus*) in Yellowstone have been shown to suppress coyote populations (Berger and Gese 2007) leading to higher pronghorn (*Antilocapra americana*) calf survival (Berger and Conner 2008, Berger et al. 2008). An among-predator cascade by which wolves suppress coyotes thus releasing foxes has not yet been demonstrated but is plausible because niche overlap between wolves and coyotes, and between coyotes and foxes, is high relative to niche overlap between wolves and foxes. A high degree of niche overlap is expected to lead to higher rates of interference competition, including spatiotemporal avoidance, kleptoparasitism, and direct killing. We hypothesize that this will cause wolves to suppress coyotes, and coyotes to suppress foxes, more than wolves suppress foxes.

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¹ E-mail: thetaaltree@gmail.com

In Minnesota, gray wolves were extirpated from nearly all of the state by the early 1970s (Mech 1970). Since the passage of the endangered species act in 1973, wolves have recolonized much of the northern-forested part of the state, and are now present, but at low abundance in the center of the state, which is transitional between farmland and forest. In the southern part of the state, which is largely farmland, wolves are not present. Here we make use of an over 30-year time series of wolf, coyote, and fox relative abundance to test the hypothesis that wolves suppress coyote populations, resulting in a cascading release of fox populations.

METHODS

The data

The Minnesota Department of Natural Resources began monitoring terrestrial carnivore populations in 1975 using scent station surveys (Sargeant et al. 1998). The scent station survey technique has been used to monitor foxes, coyotes, wolves, bobcats, and bears (Conner et al. 1983), which are difficult to survey using traditional methods (e.g., distance sampling, mark-recapture). Each scent station uses a fatty-acid tab to attract carnivores and sifted soil to record their tracks the following morning. Ten stations are placed on each 4.3 km long survey route for one night between late August and mid-October, which avoids pseudoreplication due to temporal correlation in visitation. All routes are separated by at least 5 km to avoid recording animals on multiple routes. The survey routes cover three geographically and ecologically distinct habitat zones of Minnesota (southern farmland, middle transition, and northern forest; Fig. 1A). In each habitat zone, we use the percentage of scent stations visited by foxes, coyotes, and wolves as an index of abundance for each species.

There are both red and gray foxes in Minnesota, but red foxes are historically much more abundant. Red fox harvests were 20–40 times higher than gray fox harvests until red fox entered a protracted decline in the mid-1990s from which they have not recovered (Fig. 1). The fox indices that we report are intended to be for red fox alone, but gray fox may represent a relatively stable background rate unlikely to influence our results. Gray fox tracks are differentiated from red fox by size and the presence of prominent nail prints and ridge on the interdigital foot pad.

Tests of this survey technique against independent estimates of population abundance have verified its use as a proxy of both seasonal and annual relative abundance (Conner et al. 1983). While scent station surveys reflect real changes in populations over time, their statistical power to detect changes in abundance is positively related to visitation rate (Sargeant et al. 2003). Thus, as visitation rate declines, more stations are needed to detect changes in abundance. When visitation rates are very low (1–5%), many hundreds of scent stations might be required to detect moderate changes in

visitation rate (Sargeant et al. 2003). In order to meet statistical power requirements, therefore, the Minnesota Department of Natural Resources operates between 2500 and 4000 scent stations, divided among the three habitat zones.

The three habitat zones have qualitatively distinct canid communities allowing us to test hypotheses about the interactions among wolves, coyotes, and foxes. Wolves are absent in farmland, scarce in the transition zone, and relatively abundant in the northern forests. Foxes and coyotes are present in all three zones. Each species is cosmopolitan in their habitat requirements, with high densities occurring in both open and forested habitats in certain areas throughout their North American range. We analyze the canid time series in these three zones to test the among-predator cascade hypothesis: wolves suppress coyotes, which releases the fox population.

Statistical analysis

To test the among-predator cascade hypothesis, we analyze the 30-year time series of fox, coyote, and wolf relative abundance using two complementary statistical approaches. First, we examine how the abundance of each species changes with the abundance of other species in each habitat zone over the 30-year time series. Second, we examine how the year-to-year changes in population growth rate of foxes and coyotes are influenced by density dependence and the presence of the other species.

In the first approach, we examine the long-term population trends of each species (Fig. 1B–D) with respect to one another in each habitat zone using linear and quadratic regression analysis. In particular, we test whether there has been a significantly greater decline of foxes where coyotes are more abundant, and a significantly smaller coyote population increase where wolves are more abundant. We also explore interspecific correlations between the three species by regressing the relative abundance of each species against each other in each zone. Note that temporal autocorrelation can increase Type I errors, so we include a separate P value, P_{ac} , that accounts for temporal autocorrelation in the residuals by including a one-year lag term in the regression as indicated by the partial autocorrelation function.

In the second approach, we analyze fox, coyote, and wolf population time series with autoregressive linear models that test how the population growth rate of foxes and coyotes depends on intraspecific density dependence and interspecific competition. To demonstrate the biological relevance of our statistical models, we begin by modifying the discrete logistic growth equation for species n , where n can equal f or c for foxes and coyotes, respectively. The model is then given by

$$n_{t+1} = n_t \times \exp \left[a_n \left(1 - \frac{n_t}{K_n} \right) \right] = n_t \times \exp [\beta_{n0} + \beta_{n1} n_t] \quad (1)$$

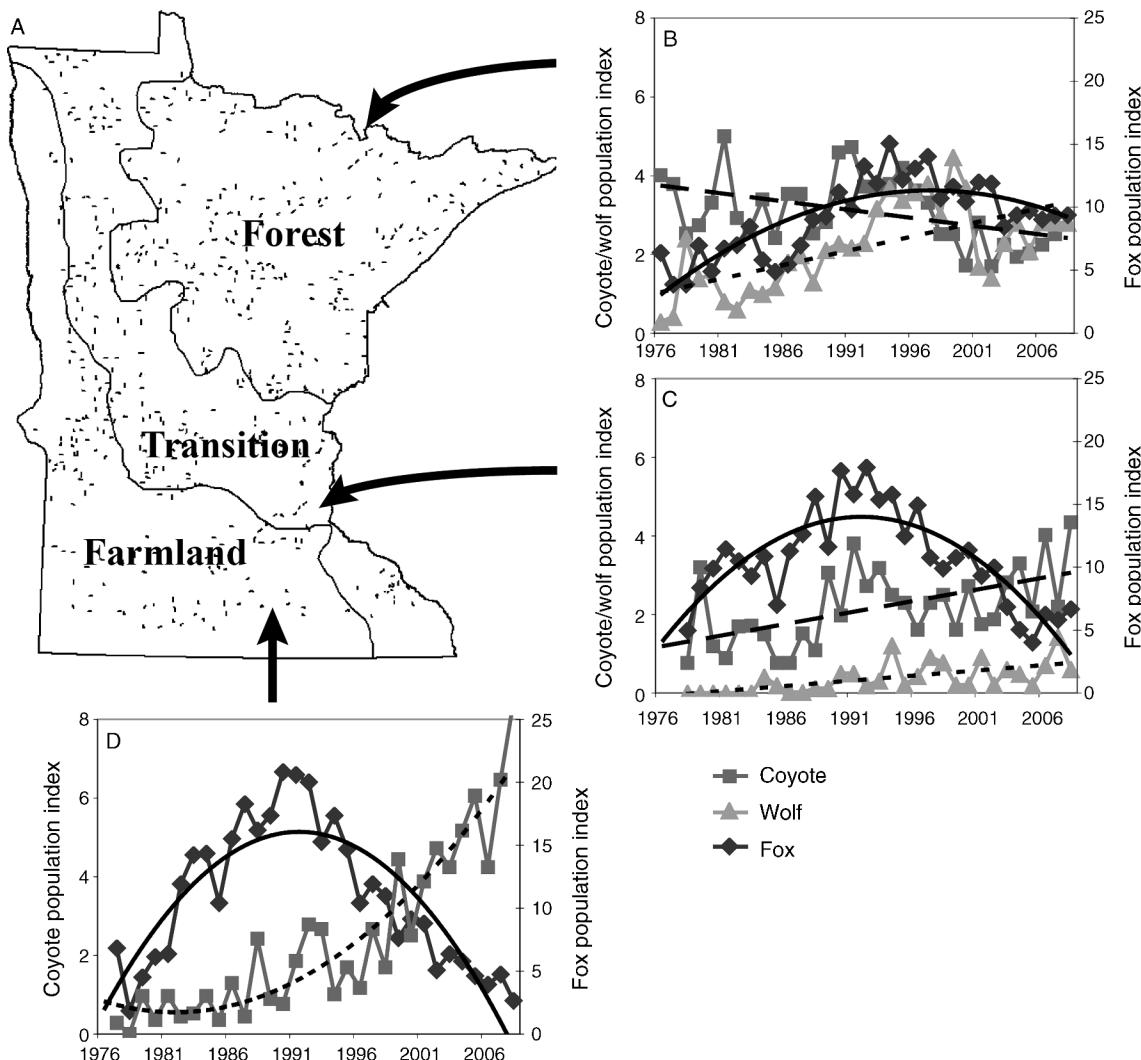


FIG. 1. (A) Map of carnivore scent station survey routes (black dashes) organized by habitat zones in Minnesota, USA, and (B–D) the corresponding time series of relative canid abundances, shown as unitless indexes. Best-fit lines shown are: solid, fox; dashed, coyote; dotted, wolf.

where n_t is the population index of foxes or coyotes at time t . The carrying capacity, K_n , and the maximum intrinsic population growth rate, a_n , are transformed into the regression coefficients β_{n0} and β_{n1} . Rearranging terms and taking the natural logarithm yields the log-difference equation

$$\ln \frac{n_{t+1}}{n_t} = \ln n_{t+1} - \ln n_t = \beta_{n0} + \beta_{n1}n_t. \tag{2}$$

Replacing the log-difference with $r_n(t) = \ln n_{t+1} - \ln n_t$ and using a Gaussian error structure, we derive the following regression model:

$$r_n(t) = \beta_{n0} + \beta_{n1}n_t + \varepsilon_{nt} \tag{3}$$

$$\varepsilon_{nt} \sim \mathcal{N}(0, \sigma_n^2).$$

The parameter β_{n1} can now be interpreted as the strength of density dependence of species n on itself.

To provide a biologically meaningful method for including as covariates the time series of species other than focal species n , we modify the discrete Lotka-Volterra competition equation for species n , with two competing populations, p_{1t} and p_{2t} . The model is given by

$$n_{t+1} = n_t \times \exp \left[a_n \left(1 - \frac{n_t + \alpha_{n1}p_{1t} + \alpha_{n2}p_{2t}}{K_n} \right) \right] \tag{4}$$

$$= n_t \times \exp[\beta_{n0} + \beta_{n1}n_t + \beta_{n2}p_{1t} + \beta_{n3}p_{2t}]$$

where α_{n1} and α_{n2} are the competitive effects of species p_1 and p_2 on species n , and β_{ni} ($i=0, \dots, 3$) are regression coefficients. This equation leads to the more complete statistical model that can explore the strength of competition between foxes, coyotes, and wolves given by

$$r_n(t) = \beta_{n0} + \beta_{n1}n_t + \beta_{n2}p_{1t} + \beta_{n3}p_{2t} + \varepsilon_{nt}$$

$$\varepsilon_{nt} \sim \mathcal{N}(0, \sigma_n^2) \quad (5)$$

where β_{n2} and β_{n3} can be interpreted as the strength of the negative or positive impact of species p_1 and p_2 , respectively, on the population growth rate of species n . We additionally include interaction terms in our final statistical model because nonlinearities in the population dynamics and/or the time series may exist.

We make inferences using corrected Akaike Information Criterion (AIC_c ; Hurvich and Tsai 1989, Burnham and Anderson 2002). Specifically, we calculate the AIC_c of models with all possible combinations of wolves, coyotes, foxes, and pairwise interaction terms in each zone. We use AIC weights to indicate our degree of confidence in each model relative to other potential models and we report the results for models with >15% AIC weight.

Using proportions as predictor variables can sometimes lead to violations of model assumptions such as normality of the residuals and constant variance. In these cases, proportions might need to be logit-transformed to map them to the whole real line. As such, we assessed residual and q-q plots using both proportions and logit-transformed proportions. Using proportions generally met model assumptions and using logit-transformed proportions did not change this. As such, we use raw visitation proportions as our index of population abundance throughout.

Hypotheses

Changes in the relative abundance of canids might be due to bottom-up changes in resources, interspecific interactions, or a combination of the two. Here, we consider three possible mechanisms: (1) a simple bottom-up model whereby populations of all three species in each zone increase when conditions are good and decrease when conditions are poor, (2) a habitat specific bottom-up model whereby bottom-up processes have primacy, but these vary according to habitat zone, and (3) an interactive model whereby changes in one or more canid populations directly or indirectly impact changes in another.

To provide support for the simple bottom-up model, we would expect populations of each species to be positively correlated throughout the state of Minnesota. To provide support for the habitat-specific model, we would expect populations of each species to be positively correlated within each habitat zone, but not necessarily across the entire state. To investigate whether certain species in one or more zones might be bottom-up regulated, we also used the winter North Atlantic Oscillation (NAO; Hurrell 1995) as a proxy for resource availability in our statistical analyses. While the NAO is not a direct measure of productivity, previous work has shown a strong correlation between the NAO and population dynamics of canid prey species in nearby

areas such as snowshoe hare (*Lepus americanus*; Stenseth et al. 2004) and moose (*Alces alces*; Wilmers et al. 2006). As well, the NAO often predicts population dynamics data better than locally collected weather data (Stenseth et al. 2003). Finally, to provide support for the interactive model, we would expect some combination of negative and positive correlations among canid species. To support the among-predator cascade hypothesis in particular, we would expect wolves to have a negative and positive impact on coyote and fox populations, respectively, and for coyotes to have a negative impact on fox populations.

RESULTS

Changes in the relative abundance of wolves, coyotes, and foxes were best supported by the among-predator model over the bottom-up models. In the farmland zone, the fox population shows a strong decline as the coyote population grows (Fig. 2C). Conversely, in the forest zone where wolves are present, coyote and fox populations show no relationship (Fig. 2D), while wolves and foxes exhibit a strong positive relationship (Fig. 2E). The winter NAO was not a significant predictor in any of our regression models.

Population trends

The fox population trends in each zone were best explained by quadratic regression models with positive linear terms and negative quadratic terms (Fig. 1). The linear term in a quadratic model controls the slope of the initial population growth at population size zero. As the population size increases, the quadratic term begins to dominate the expression causing the population curve to bend over and decline. The more negative the coefficient on the quadratic term, the stronger the decline. Comparisons of the coefficients of the quadratic terms in the fox population trend in each zone indicate that the rate of decline is significantly higher in farmland over transition ($P < 0.01$), and in transition over forest ($P < 0.01$; Fig. 1). Corresponding to these fox trends, the coyote population increase was quadratic in the farm zone where wolves are absent ($P < 10^{-11}$, $r^2 = 0.86$), linear in the transition zone where wolves are slowly recovering ($P = 0.001$, $r^2 = 0.30$), and the coyote population decreased linearly in the forest zone where wolves have recovered strongly ($P = 0.02$, $r^2 = 0.17$), indicating top-down control of coyotes by wolves.

Population fluctuations

Examination of year-to-year changes in fox population growth rate revealed that the strength of fox density dependence in the single-species fox model (Eq. 1, $n=f$) was not significant and weakest in farmland, stronger in transition, and strongest in the forest, (Fig. 2A). The increase in the magnitude of the density-dependent coefficient, and variance of the model explained by density dependence (r^2) from farmland, where coyotes are abundant, to forest, where coyotes are relatively

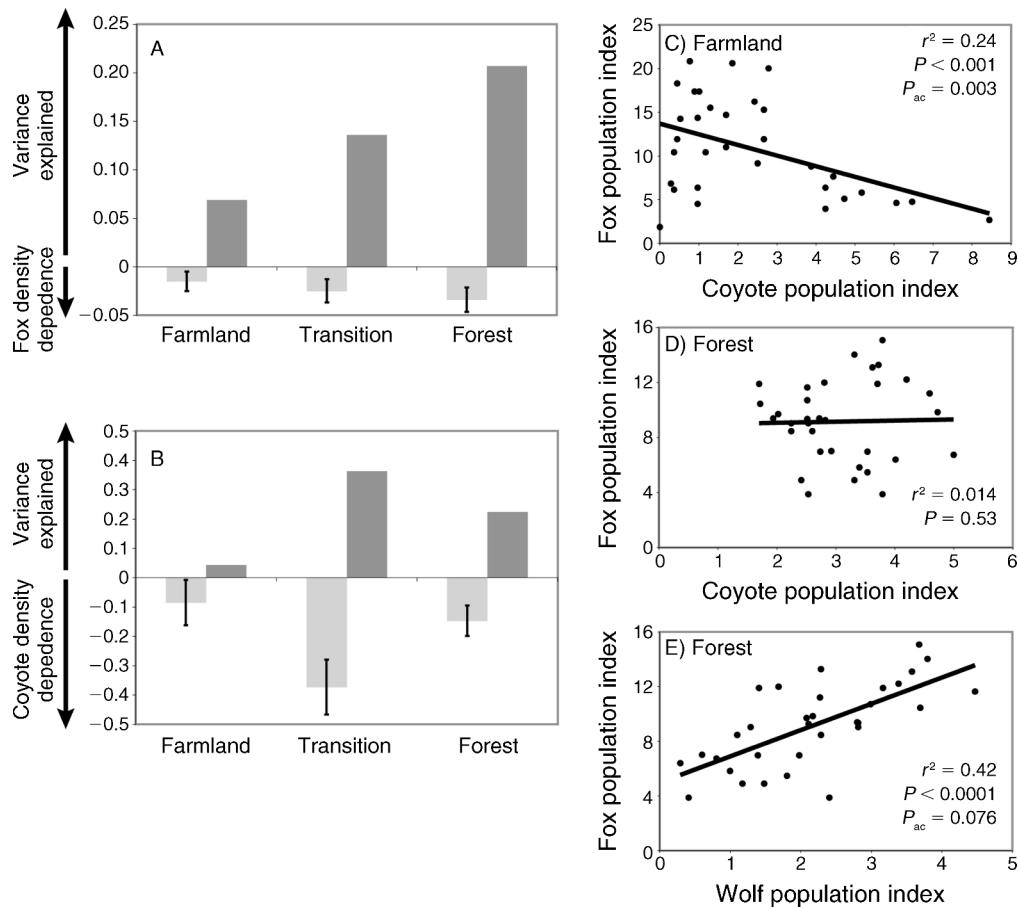


FIG. 2. Density-dependent effects of (A) fox and (B) coyote in the single-species models (Eq. 1), showing density dependence for fox (b_{f1}) and coyote (b_{c1}) and the proportion of variance explained (r^2) by the density dependence. (C–E) Linear regressions predicting fox populations across habitat zones in the presence of coyotes or wolves. P values corrected for autocorrelation in the residuals by including a lag term in the regressions are labeled P_{ac} . Error bars represent \pm SE.

scarce, is suggestive of a release from interspecific competition with coyotes to self-regulation by foxes (Fig. 2A).

The strength of coyote density dependence in the single-species coyote model (Eq. 1, $n = c$) was weakest in forest, strongest in transition, and weak in farmland (Fig. 2B). This is generally consistent with the idea of bottom-up control in the farmland giving way to increasing among-predator control in the transition and the greatest among-predator control in the forest zone, with the exception that we would expect negative density dependence to be stronger in the farmland than in the transition. However, inspection of the coyote time series in the farmland reveals that the coyote population is still growing nearly exponentially so that this population has not yet experienced competition for food resources. Therefore it is not surprising that strong density dependence has not been achieved thus far in that zone.

The multispecies model predicting fox population growth rate (Eq. 2, $n = f$) revealed a strong negative effect of coyotes on fox population growth in the

farmland zone, where wolves are absent (Table 1). This suggests that in the absence of wolves, coyotes strongly limit fox populations. In the forest zone with relatively abundant wolves, fox are released from top-down control by coyotes and show only a small positive correlation with coyotes (Table 1). This positive correlation is likely to come about when populations fluctuate in response to a shared food resource, a lower trophic level that we cannot explicitly account for in our model. In the transition zone, the best model explaining fox population growth rate included fox, coyote, and an interaction between the two (Table 1). This interaction reveals that when the coyote population is low, fox density-dependent effects dominate, but as the coyote population increases the fox population is regulated more by competition with coyotes than by density dependence.

Wolves did not have an important effect on fox population growth rate in the transition zone where they occur at low abundance. Wolves were not included in the best model ($\Delta AIC_c = 0$, AIC weight = 0.56) and while they revealed a small negative effect on foxes in the

TABLE 1. The best model or group of models explaining fox population growth in the farmland, transition, and forest zones of Minnesota, USA, by Akaike weight (w).

Covariate	Farmland ($w = 0.73, R^2 = 0.43$)		Farmland ($w = 0.25, R^2 = 0.45$)		Transition ($w = 0.56, R^2 = 0.43$)	
	Estimate (SE)	P	Estimate (SE)	P	Estimate (SE)	P
b_0	0.613 (0.146)	0.0003	0.683 (0.170)	0.0004	1.27 (0.299)	0.0002
f_t	-0.031 (\pm 0.009)	0.002	-0.040 (\pm 0.014)	0.01	-0.116 (\pm 0.028)	0.0003
c_t	-0.114 (\pm 0.028)	0.0003	-0.152 (\pm 0.054)	0.009	-0.429 (\pm 0.120)	0.001
w_t						
$f_t \times c_t$			0.006 (\pm 0.007)	0.42	0.039 (\pm 0.011)	0.002

Note: The covariate b_0 is the intercept parameter, f_t , c_t , and w_t , are the time-dependent fox, coyote, and wolf indices.

second best model ($\Delta AIC_c = 0.82$, AIC weight = 0.37), the effect was not significant ($P = 0.17$). Wolves, however, had a strong positive effect on fox population growth rate in the forest zone. In fact, the effect size is the strongest of any that we observe in any zone. The best model in the forest zone also includes a negative cross term for wolves and foxes, implying that as wolves increase, foxes are increasingly regulated by density dependence, which is evidence that wolves are allowing foxes to approach their carrying capacity. Direct inclusion of wolves into the multispecies coyote model (Eq. 2, $n = c$) did not reveal a significant negative effect of wolves on coyotes in either the transition or forest zones.

DISCUSSION

Taken as a whole, our analysis supports an among-predator cascade from wolves through coyotes to foxes. While the evidence we present is correlational, it is based on a plausible mechanism of increased interference competition between more closely sized canids. Wolves are more likely to kill coyotes than foxes because they might perceive coyotes as more direct competitors because of the coyote’s larger size and more similar diet preferences. This mechanism is supported by data demonstrating wolf suppression of coyotes (Berger and Conner 2008, Berger et al. 2008) and separately, coyote suppression of foxes (Harrison et al. 1989, Ralls and White 1995, Henke and Bryant 1999, Fedriani et al. 2000, Kamler et al. 2003, Mezquida et al. 2006, Karki et al. 2007, Moehrensclager et al. 2007, Thompson and Gese 2007). While bottom-up forces surely play a role in this system, neither the simple nor habitat specific bottom-up models were sufficient to explain the pattern of alternating negative and positive effects among these three canid species. Other alternative hypotheses explaining these data might include land use change as a driver of change in canid populations over time, and underlying habitat differences among the three zones. Neither of these alternative hypotheses stands up when confronted with all the available data. Land use change occurs too slowly to account for interannual variations in population growth, while habitat differences are unlikely to explain the patterns we report here as both foxes and coyotes have achieved high densities in both

forested and farm habitats here and elsewhere (Kays et al. 2008). Finally, disease, particularly mange, likely impacts populations of canids in Minnesota, but without data we could not include this in our analysis. Qualitatively, it does not appear that shared disease drives the among-predator interactions because the canid populations do not exhibit a temporally correlated decline. The uniformity of the coyote increase and fox decrease is more consistent with direct killing of foxes by coyotes rather than interspecies pathogen transmission.

Size asymmetric among-guild effects whereby larger competitors suppress smaller ones have been shown in various taxa including plants (Schwinning and Weiner 1998), insects (Rosenheim 1998), and fish (Munoz and Ojeda 1998). Research in these systems has focused on pairwise interactions and their effects on lower trophic levels (e.g., Polis and Strong 1996, Sih et al. 1998). While the mechanisms driving among-guild interactions can vary from resource competition to interference competition or direct killing, our results indicate that indirect effects can cascade through a guild to impact the abundance of tertiary guild members. As a general rule, we would expect Fretwell’s (1977) idea that the parity of a food chain determines the alternate suppression and release of plant biomass to apply to chains of among-guild interactions as well. Namely that among-guild interaction chains with even numbers of species will result in the smallest competitor being suppressed while among-guild interaction chains with odd numbers of species will result in the smallest competitor being released.

Our results indicate that the restoration of wolves to areas across the northern hemisphere might lengthen species interaction chains. This is likely to result in an increase in smaller predators (or those that like foxes are suppressed by coyotes but not wolves) in wolf occupied habitat, and consequent changes in prey community composition. As coyotes have expanded their range in the absence of wolves, the resulting exclusion of foxes is expected to lead to much lower predation rates on small mammals because fox densities are on the order of 5–10 fox families (2 adults and 4–6 kits per family) per 10 km² (Trehwella et al. 1988), but Eastern coyote densities are an order of magnitude lower at around 0.5 individuals per 10 km² in forested landscapes and around 1

TABLE 1. Extended.

Transition ($w = 0.37, R^2 = 0.47$)		Forest ($w = 0.51, R^2 = 0.52$)	
Estimate (SE)	<i>P</i>	Estimate (SE)	<i>P</i>
1.27 (0.293)	0.0002	-0.560 (0.345)	0.12
-0.115 (\pm 0.027)	0.0003	0.002 (\pm 0.032)	0.961
-0.398 (\pm 0.120)	0.003	0.093 (\pm 0.038)	0.021
-0.151 (\pm 0.106)	0.168	0.440 (\pm 0.143)	0.005
0.038 (\pm 0.011)	0.002	-0.031 (\pm 0.013)	0.031

individual per 10 km² in rural landscapes (Tremblay et al. 1998, Patterson and Messier 2001, Way et al. 2002). Small mammals are present in only 2–13% of Eastern coyote scats, but in 11–50% of red fox scats (Major and Sherburne 1987). The numerical and dietary difference between foxes and coyotes can combine to result in a markedly lower predation rate on small mammals when coyotes exclude foxes.

While the three canid species have some dietary overlap, wolves are most efficient at killing large prey

such as ungulates; coyotes are most efficient at killing intermediate-sized prey such as lagomorphs, squirrels, and ungulate neonates; and foxes are most efficient at killing small prey such as small rodents, invertebrates, and birds, but also lagomorphs (Major and Sherburne 1987, Gompper 2002). As such the size spectrum of canid prey communities are likely to vary depending on whether wolves are present or not. In ecosystems with wolves, large and small prey will experience higher rates of predation than intermediate-sized prey, whereas in ecosystems lacking wolves, intermediate-sized prey are likely to experience higher predation rates (Fig. 3).

Consistent with this idea, there is evidence that the increasing coyote population (in the absence of wolves) has caused the decline of white-tailed jackrabbits over the past 40 years in the farmland and transition zones of Minnesota (Haroldson 2008). This decline may reflect a loss of preferred habitat, but a resurgence in jackrabbit populations during the peak of pelt prices in the late 1970s and early 1980s (when furbearers were heavily trapped) is suggestive of a temporary release from predation.

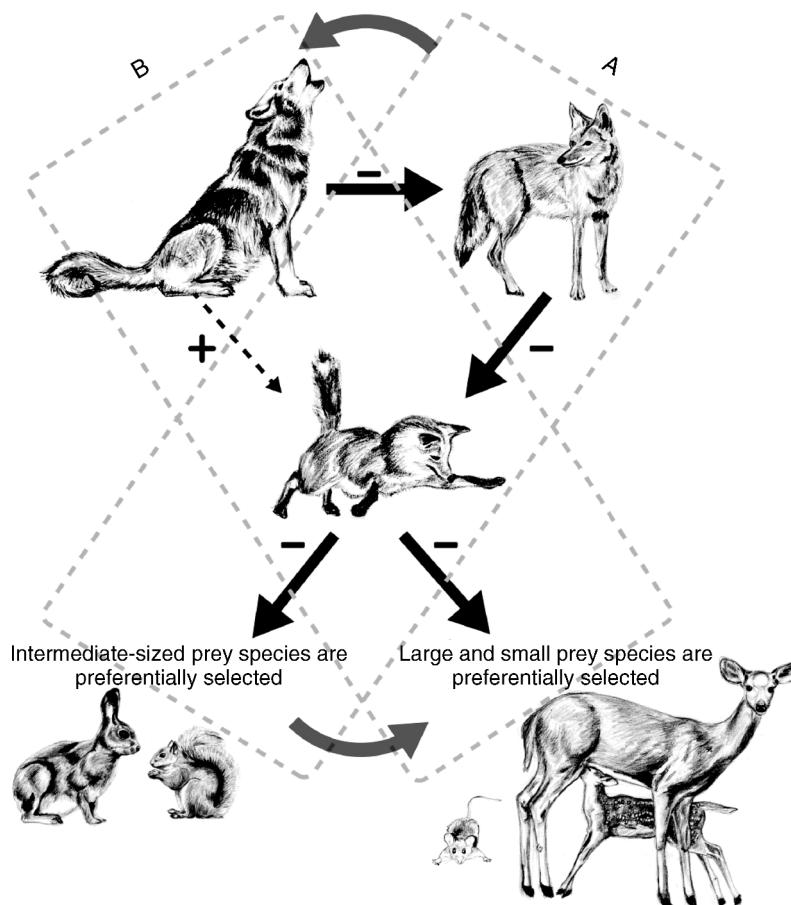


FIG. 3. Hypothesized impact of the among-predator cascade on food-web dynamics (with interactions indicated by black arrows). (A) Without wolves, coyotes suppress fox populations such that preferred coyote prey items are preferentially consumed by the canid guild. (B) With wolves, the interaction web transitions from A to B (indicated by the curved gray arrows). Coyotes are suppressed, releasing foxes and leading to dominant fox and wolf prey items being preferentially consumed.

The discovery of this among-predator cascade opens the door to search for similar types of cascades and to explore their implications. For example, the change in the size spectrum of preferred prey might importantly impact human–ecosystem interactions. Common prey species are often responsible for the emergence of zoonotic infectious diseases, including hantavirus and Lyme disease (Ostfeld and Holt 2004). For instance, the dominant reservoir hosts for Lyme disease in North America are small mammals (LoGiudice et al. 2003, Brisson et al. 2007), and deer are an important reproductive host for adult ticks. A wolf and fox dominated predator community is expected to preferentially prey on these important hosts, and prey less on the medium-sized hosts that are incompetent Lyme disease reservoirs (LoGiudice et al. 2003).

This among-predator cascade also informs our understanding of mesopredator release in terrestrial systems. Mesopredator release theory has often considered the consequence of top predator removal in a three species interaction chain (i.e., coyote–fox–prey) where the coyote was considered the top predator (Ritchie and Johnson 2009). However, the historical interaction chain before the extirpation of wolves had four links. In a four-link system, the top predator releases the smaller predator. The implication is that a world where prey species are heavily predated by abundant small predators (mesopredator release) may be similar to the historical ecosystem. As top predators recolonize their former ranges, ecological communities may be predictably restructured with consequences that are important to explore in future research.

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Aquilegia

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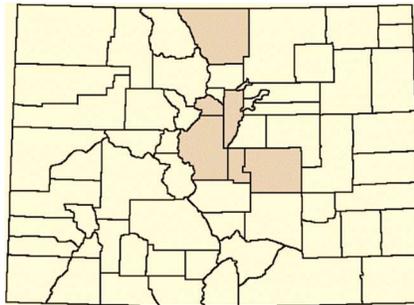




James's alumroot, *Telesonix jamesii* (Saxifragaceae). James's alumroot—or brookfoam—was first collected by Edwin James on Pikes Peak in 1820. It is usually found in the cracks of granite outcrops at 8,700-13,050 ft elevation, although the plant can also be found on scree slopes. While it seems to prefer east and north exposures on these surfaces, it can be found growing in full sun to full shade and in variable levels of moisture. Most populations are found on Pikes Peak granite, but there are a few outlier populations in Rocky Mountain National Park, which are on Precambrian gneiss and schist. *T. jamesii* is 60-180 mm tall, with glandular-pubescent stems and leaves.

Alumroots (*Heuchera spp.*) are often found nearby *Telesonix*. Be careful not to confuse the plants when they are not in bloom. *Telesonix heucheriformis*, which has a wider distribution, was once considered a variety of *T. jamesii*, but is now categorized as its own species.

The name *Telesonix* is derived from Greek; “tele” translates as “perfect” and “onyx” translates as “claws.” *T. jamesii* has been reported to be used medicinally by the Cheyenne. KA



Map adapted from Ackerfield, J. *Flora of Colorado*, (2018), p. 757.

Botanicum absurdum by Rob Pudim



© Rob Pudim

PHOTO CREDITS: James's alumroot, *Telesonix jamesii*; FRONT COVER © Mike Kintgen; PAGE 2 © Kelly Ambler, Pikes Peak region, July 13, 2020. BACK COVER: © Mo Ewing, *Aquilegia coerulea*, Crested Butte, and *Oenothera sp.* and others, Pawnee Buttes.

Aquilegia uses Jennifer Ackerfield's *Flora of Colorado* (2018, second printing) as its preferred guide to plant naming conventions. Readers may also want to familiarize themselves with other guides such as *Colorado Flora*, Eastern and Western editions, by William A. Weber and Ronald C. Wittmann (2012), as well as The Biota of North America Program online guide to North American Vascular Flora (<http://www.bonap.org/>), and other resources.

Aquilegia: Magazine of the Colorado Native Plant Society

Dedicated to furthering the knowledge, appreciation, and conservation of native plants and habitats of Colorado through education, stewardship, and advocacy

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Managing Editor: Mary Menz, mary.t.menz@gmail.com
Associate/Design Editor: Kelly Ambler, alpineflowerchild@gmail.com
Assistant Editor: Nan Daniels
Cartoonist: Rob Pudim
Proofreaders: Suzanne Dingwell, Cathi Schramm, Linda Smith, John Vickery

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OTHER CONTACTS

COMMITTEE CHAIRS: Conservation: Mo Ewing, bayardewing@gmail.com; **Education & Outreach:** David Julie, bldrjardin@live.com; **Field Studies:** Steve Olson, sdolsonoslods@aol.com, Lara Duran, ld.ecowise@gmail.com; **Finance:** Mo Ewing; **Horticulture:** Ann Grant, odygrant@gmail.com; **Media:** Deryn Davidson, ddavidson@bouldercounty.org, Lenore Mitchell, zap979sar@icloud.com, Steve Olson, sdolsonoslods@aol.com; **Research Grants:** Stephen Stern, stern.r.stephen@gmail.com; **Restoration:** Haley Stratton, hbstratton94@gmail.com; **Scholarships:** Cecily Mui, chmui@hotmail.com

SOCIAL MEDIA: E-News Editor: Linda Smith, conpsoffice@gmail.com; **Facebook:** Denise Wilson, conpspromote@gmail.com; Jen Boussetol, Jennifer.Boussetol@colostate.edu; Deryn Davidson, ddavidson@bouldercounty.org; Carol English, daleanana@gmail.com; Anna Wilson, annabwilson@gmail.com; Denise Wilson; Tom Zeiner, tzeiner303@gmail.com. **Twitter and Instagram:** Jen Boussetol, Denise Wilson; **Webmaster:** Mo Ewing, bayardewing@gmail.com

CoNPS PAID STAFF: Linda Smith, administrative coordinator, conpsoffice@gmail.com, 970-663-4085; Denise Wilson, marketing & events coordinator, conpspromote@gmail.com; Kathleen Okon, workshop coordinator, CoNPSworkshops@outlook.com

17TH Annual Colorado Rare Plant Symposium

“Globally Imperiled Plants Found on the Front Range and Central Rockies”

Friday September 18

8:30 AM to 2:30 PM

Registration is \$10 per person at <https://conps.org>. The Colorado Rare Plant Symposium is held each fall in conjunction with the Colorado Native Plant Society's annual conference. Hosted by the Colorado Natural Heritage Program, the symposium is an annual meeting to address current status and conservation needs of rare plants in Colorado.

This year the symposium is going virtual and consists of three short sessions. Topics will include:

- A progress report on the conservation actions needed for the Tier 1 and Tier 2 plant species included in the 2015 State Wildlife Action Plan;
- An update to the Floristic Quality Assessment revision; and
- A photo review of the rare plants found on the Front Range and Central Rockies in Colorado.

The 2015 State Wildlife Action Plan included plants for the first time and identifies conservation needs and actions for 120 of Colorado's rarest plant species including federally-listed species such as *Astragalus*



osterhoutii, and endemic species like *Aliciella sedifolia*. Jessica Smith of CNHP will provide a review of the conservation needs of these species and the actions that have been taken to date to meet those needs. The Rare Plant Addendum to the SWAP can be viewed at

[https://cpw.state.co.us/](https://cpw.state.co.us/aboutus/Pages/StateWildlifeActionPlan.aspx)

[aboutus/Pages/StateWildlifeActionPlan.aspx](https://cpw.state.co.us/aboutus/Pages/StateWildlifeActionPlan.aspx)

In 2007, CNHP published Colorado's initial FQA report that included Coefficient of Conservation or C-values for the Colorado flora for 80% of the known taxa at that time (Rocchio et al. 2007 <https://cnhp.colostate.edu/download/documents/2007/FQAFinalReport.pdf>).

However, since 2007 there have been numerous taxonomic changes to the Colorado flora—with new species added to the flora, and other species dropped. During 2019 and 2020, CNHP updated the C-values for the 20% of the flora that did not yet have values. C-values are assigned to each plant taxa based on its affinity for natural habitats (for example, those not affected by human disturbance). Land managers can apply the C-values to a plant list for an area, which can then be used to help quantify the quality of that

site. Many of the new C-values were evaluated using habitat quality information collected from nearly 3,000 wetland and riparian plot locations (see <https://cnhp.colostate.edu/cwic/tools/plot-database/>).



At the 2020 meeting, Pamela Smith of CNHP will present the new C-values calculated for the 20% of the flora that did not have values. She will also discuss how the new online calculator works and how the values were evaluated for the update. In addition, through this effort a

number of sources of taxonomic information have been cross-walked for the entire Colorado plant list including the USDA PLANTS database, Weber and Wittmann (2012), and Ackerfield (2015).

In the afternoon session, CNHP botanists will provide a photo review of the rare plants of the Front Range and Central Rockies in Colorado. This will include several species of *Aletes*, *Potentilla*, *Penstemon*, and local favorite *Physaria bellii*.

CNHP tracks the location and condition of over 500 imperiled plants. Tracking and monitoring efforts guide effective management and protection of those species and thereby prevent extinctions or statewide extirpations of Colorado's native plant species. ►



Aliciella sedifolia (stonecrop gilia). © Connie Colter



Physaria bellii (Front Range or Bell's twinpod).
© Georgia Doyle

◀ CNHP conducts field surveys for rare native plants; designs and implements monitoring studies; produces models, best management practices, and conservation strategies; and develops detailed maps

for rare plants as well as noxious weeds. The CNHP team has active members on the Colorado Rare Plant Technical Committee, the Colorado Weed Advisory Committee, the Colorado Native Plant Society, and NatureServe. Colorado Natural Heritage Program staff works closely with botanists and land managers across Colorado to develop the state's most comprehensive and accurate dataset of Colorado's rare flora.

Annual presentations and species-specific meeting notes are available for past years at <https://cnhp.colostate.edu/projects/colorado-rare-plant-symposia/>

View the Colorado rare plant guide at <https://chnp.colostate.edu/library/field-guides>

Past presentations and species-specific meeting notes are available on the CNHP website for 2004-2019. View or download copies of past symposia presentations at <https://cnhp.colostate.edu/projects/colorado-rare-plant-symposia/> or view the Colorado rare plant guide here: <https://cnhp.colostate.edu/library/field-guides/>

Contact Jill Handwerk for more information at (970)491-5857 or jill.handwerk@colostate.edu 

Member Input Needed for Virtual Social

Peaks to Prairies: CoNPS Members in the Field!

By Lenore Mitchell

Share your summer activities!

Eventually we'll be able to hike together again, attend in-person meetings and workshops, and give and receive hugs. In the meantime, we can still share.

Whatever you're doing this summer, wherever you are from—Durango to Denver, La Junta to Grand Junction—if it involves native plants, tell us about it! Whether you're doing research, sleuthing around for rare plants, taking fun hikes to worship the blooms, or working away at native plant garden projects, please snap a few pics and jot down a few notes.

Send a few photos and your brief notes to Tom Schweich (tomas@schweich.com) no later than August 25 so we can include you and your activities in the member slide show during the virtual social at this year's annual conference. Include a photo of yourself, preferably in the midst of your activity.

Here is the agenda for the Virtual Social on Friday, September 18. The following is included with your paid conference registration:

- Welcome to the conference and announcements;
- Narrated presentation about a July 2020 hike to Pikes Peak to commemorate the 1820 Long's Expedition to Colorado;
- Narrated presentation of slide show with photo contest finalists and first place winners;
- Narrated presentation of slide show depicting state-wide member projects, including research, hikes, and gardening related to native plants; and
- Brief narrated slide show to introduce key people who keep CoNPS running, including board members, chapter presidents, and others.

44TH Annual CoNPS Conference

“Peaks to Prairies—Plants in the Land of Extremes”

Friday through Sunday, September 18-20
Four sessions over three days

Welcome to the 2020 Annual CoNPS Conference, hosted by the Metro-Denver Chapter, which now has 420 members. State-wide membership totals more than 1100 members in six chapters and includes everyone from professional botanists to beginning plant enthusiasts.

This year’s Annual Conference is brought to you in a webinar format that allows participants to join in from the comfort of their own homes.

We look forward to having as many as 500 native plant lovers gathering virtually to hear and watch this year’s expert speakers.

Daily schedules contain ample break times and a lengthy lunch time on Saturday. In addition, recordings of select presentations may be available for viewing by registered participants for a limited time after the conference—in case you either missed a presentation or wish to repeat it. Audience questions may be submitted via chat boxes during live presentations.

Thanks not only to all conference committee members whose efforts have made this year’s conference possible, but also to the many other volunteers who’ve offered their assistance. Kudos to everyone for

making the best of a challenging situation. We give a very special note of gratitude to each of our speakers.

State-wide leadership of CoNPS begins with the operating committee comprised of: Ginger Baer, Deryn Davidson, Mo Ewing, Ann Grant, Irene Weber and Amy Yarger. In addition to the OC, CoNPS board of directors includes chapter presidents and members-at-large. CoNPS staff includes Linda Smith, who keeps us all organized as administrative coordinator, Denise Wilson as marketing and events coordinator, and Kathy Okon as the workshop coordinator. Volunteers from all over the state contribute in various ways to CoNPS success and new volunteers are always welcome.

Thanks to this year’s conference committee members: Kelly Ambler, Courtney Cowgill, Sue Dingwell, Mo Ewing, Lenore Mitchell, Tom Schweich, Bruce Tohill, John Vickery, and Denise Wilson. Special thanks to *Aquilegia* managing editor Mary Menz and associate/design editor Kelly Ambler.

Registration

See page 11 for registration information. Registrants will receive an email with details for accessing all webinar events. There will be instructional materials available for those unfamiliar with webinar formats.

Speakers and Presentations

(arranged in order of presentations)



Heidi Steltzer **“The richness of plants in the mountains benefits people”**

Mountain regions are home to 25% of the earth’s biodiversity, provide water to billions of people, and sustain us by providing refuge. The Colorado mountains are a unique

place for plant life, and one that is changing quickly due to the warming of our planet and a changing snow pack. Heidi will provide insights into the benefits that

mountain plants provide for people to inform why we should conserve these incredible species. They are resilient, and this contributes resilience to each of us.

Heidi Steltzer, PhD, is professor of environment and sustainability at Fort Lewis College in Durango. Heidi is a mountain scientist, explorer, and science storyteller. She has spent 25 years conducting field studies in remote regions of Colorado, Alaska, Greenland, and China to understand how mountain ecosystems are unique and valued regions of our world. She is a lead author on high mountain areas in a recent intergovernmental panel on climate change report and has testified before US Congress on climate change. Find her on social media @heidimountains ►



Mike Kintgen “Circumboreal Alpine Plants and Biogeography”

Mike will share some of the circumboreal element in our flora—species found both in Colorado and in places as diverse as Newfoundland, Kamchatka, Norway,

Iceland, and Switzerland. Colorado's flora share diverse links with mountainous and high latitude regions around the world. Mike will show how Colorado flora is linked to that of Eurasia and South America. He will also dip into the Asiatic element in Colorado's high elevation flora, as well as links with the flora in places as far away as Patagonia. Lastly, he will brush on the rich, endemic North American influence on our flora which includes genera such as *Penstemon*, *Eriogonum*, *Calochortus*, and *Heuchera*.

Mike Kintgen is the curator of alpine collections at Denver Botanic Gardens, where he has been a formal part of the staff since 2004. He has played an informal role since 1992, having started as a volunteer at age eleven. His botanical travels have taken him to most of Western Europe, European Russia, Morocco, and Argentine Patagonia.



Jennifer Boussetot “Colorado Native Plant Availability in the Green Industry”

Native plant aficionados often struggle to find Colorado native plants available in the green industry. Often that is due to two things: lack of demand

so most producers do not grow them, and the fact that many Colorado native plants are not as attractive in containers so most gardeners don't buy them. Because of this, Jen and others have acquired USDA funding and have begun plant finishing protocol research on several of the species in Plant Select® that are native to Colorado. Jen will talk about one of her greatest passions—how to ensure that our beloved Colorado native plants become more available in the green industry.

Jennifer Boussetot, PhD, is an assistant professor in the department of horticulture and landscape

Architecture at Colorado State University. Jen completed her doctorate research studying green roof species selection, including Colorado native plants, at Colorado State University in 2010. Jen also does research ensuring that Colorado native plants are marketable in the green industry. She is a previous marketing and events coordinator for CoNPS and is co-author of the CoNPS-published 3rd edition of Common Southwestern Native Plants.



Jennifer Ackerfield “Thistle Be Fun: Untangling Taxonomy and New Species Discoveries”

Have you ever wondered what defines a species, or how new species are discovered and named?

Well, wonder no longer! Join Jennifer as she talks about the process of defining a species and all the lines of evidence that scientists use to inform this decision. After laying the groundwork for how species are named, she will discuss an exciting development in her alpine thistle research.

Many members of CoNPS participated in Team Thistle, a citizen science initiative in which Jennifer asked members to “get high on alpine thistles” with her. Through this initiative, approximately 50 collections of alpine thistle were made and observations recorded on iNaturalist. She used several of these collections and observations in her research and discovered an unnamed species hidden right under our noses! This exciting discovery also highlights the need and importance of field studies, iNaturalist observations, archives, and natural history collections.

*Jennifer Ackerfield, PhD, is the head curator of natural history collections at Denver Botanic Gardens. She was previously a curator at the Colorado State University herbarium and also taught plant identification at CSU. Most notably, she is the author of the Flora of Colorado. She has been studying the flora of Colorado for 25 years and has traveled extensively across the state documenting its rich floristic diversity. She received her master's in botany with a concentration in taxonomy and systematics in 2001 and is currently working on her PhD in botany, studying the taxonomy and evolution of the genus *Cirsium* (thistles) in North America. Jennifer has worked with the Colorado Native Plant Society, Colorado Natural Heritage Program, US Forest Service, Colorado BLM, Rocky Mountain National Park, and Mesa Verde National Park. ►*



◀ **Shannon Murphy**
“Light Pollution Affects Invasive & Native Plant Traits Important to Plant Competition & Herbivorous Insects”

Many exotic invasive species have traits that allow them to outcompete native species when there

have been changes in the environment relative to conditions under which the native plants have evolved. However, invasions in urban settings have been insufficiently studied, including exploring the impacts of the uniquely urban stressors of streetlights.

Plant physiology and phenology are impacted by Artificial-Light-at-Night, but no studies have yet examined if light pollution differentially affects native versus invasive plant species. We tested the hypothesis that ALAN affects plant traits important to plant fitness and susceptibility to herbivory and found that these effects differ between some invasive and native grass species. As urbanization increases, its role in understanding invasion biology becomes more important, especially when an urban disturbance such as ALAN benefits the growth of invasive species.

Dr. Shannon Murphy is associate professor of biology at the University of Denver. She graduated from the University of Colorado at Boulder in ecology and evolutionary biology. She received her PhD in ecology and evolutionary biology from Cornell University and completed two post doctorates, one at the University of Maryland in entomology and the other at the George Washington University in biology.



Steve Yarbrough
“Fen Ecosystems of Colorado”

Finns? Or Fins? No....Fens! Fens are groundwater-fed, peat-forming wetland ecosystems. Where exactly are they hiding out and why the heck are they so

interesting for native plant enthusiasts?

Fens contain a great number of Colorado rare plant species and even a few globally rare species. They occur in a variety of landscapes and boast some interesting chemistries. What conservation measures and strategies are being used with fens and what difference will it make in the long run? Steve will

provide some insights from 22 years of balancing through, plunging in, and rescuing himself from a variety of fens across Colorado.

Steve Yarbrough is a senior ecologist and professional wetland scientist working for Tetra Tech, Inc., in Golden, Colorado. He has enjoyed a 36-year career in the environmental consulting field. His job assignments typically involve siting studies for renewable energy projects, assessing impacts, obtaining required permits, and monitoring the recovery of damaged resources, including wetlands and native prairie. He has previously served on the CoNPS board of directors and served stints as field trip coordinator and workshop coordinator for the society. He is currently a member of the conservation committee.



Tim Seastedt
“Climate Change Effects on Herbaceous Plant Community Composition in the Colorado Front Range”

The high elevation ecosystems of the Colorado Front Range have been under study by CU

researchers for 70 years, a time period sufficient to study impacts of climatic changes. Climate and atmospheric inputs are the dominant change factors of high elevation ecosystems, but these drivers influence a complex terrain that is impacted unevenly by local growing season length, moisture, and nutrient limitations. These differential outcomes produce differential changes in vegetation composition across the landscape that benefit components of the alpine flora while penalizing others. Willow invasions into herbaceous areas have, perhaps, been the most dominant change to date. The entire alpine zone is undergoing elevation changes, but these changes are controlled by the interaction of climate changes with local abiotic and biotic factors.

Tim Seastedt is professor emeritus for the department of ecology and evolutionary biology and is a fellow, Institute of Arctic and Alpine Research, University of Colorado, Boulder. Seastedt spent a decade studying grasslands in Kansas prior to coming to Colorado in 1990. He became the principal investigator of the Niwot Ridge long-term ecological research program in 1992 and has continued studies to date on plant and soil interactions in herbaceous ecosystems on both at the top and bottom of the Front Range. ☯

“Peaks to Prairies—Plants in the Land of Extremes”

Conference Schedule

Friday Virtual Social Event: Highlights from 2020

Time	Description	Speaker
6:30 PM to 9:00 PM	Introduction	Denise Wilson
	Pikes Peak commemorative hike	Kelly Ambler
	Photo contest winners	Bruce Tohill
	Break	
	Peaks to Prairies: CoNPS Members in the Field	Tom Schweich
	Break	
	Key people who keep CoNPS running	Moderator(s)

Session 1: Saturday morning

Time	Description	Speaker
8:30 AM	Join webinar	Moderator(s)
9:00 AM	Introductions, instructions	Moderator(s)
9:10 AM	The Richness of Plants in the Mountains Benefits People	Heidi Steltzer
9:55 AM	Break	
10:05 AM	Circumboreal Alpine Plants and Biogeography	Mike Kintgen
10:55 AM	Break	
11:10 AM	Colorado Native Plant Availability in the Green Industry	Jennifer Boussetot
11:55 AM	Session closing and midday break	Moderator(s)

Session 2: Saturday afternoon

Time	Description	Speaker
1:30 PM	Announcements, other	Moderator(s)
1:40 PM	Thistle be Fun: New Species Discoveries	Jennifer Ackerfield
2:25 PM	Break	
2:35 PM	Light Pollution Affects Invasive & Native Plant Traits Important to Plant Competition & Herbivorous Insects	Shannon Murphy
3:20 PM	Session closing and day-end	Moderator(s)

Session 3: Sunday afternoon

Time	Description	Speaker
1:00 PM	Getting started, other	Moderator(s)
1:10 PM	Fen Ecosystems of Colorado	Steve Yarbrough
1:55 PM	Break	
2:10 PM	Climate Change Effects on Herbaceous Plant Community Composition in the Colorado Front Range	Tim Seastedt
2:55 PM	Conference closing	Moderator(s)

Annual Silent Auction Benefits CoNPS Activities

The Annual Conference traditionally hosts a silent auction to benefit CoNPS. This year the auction will be online! Proceeds support the Colorado Native Plant Society's wide-ranging projects including education, conservation, native plant gardening, botanical and horticultural publications, and activities.

Denise Wilson and the silent auction committee seek your donations for this event. We are accepting only small, mailable, above \$20 value items, and requesting that donors hang on to them and mail them to the winning bidder afterward. Donors can bill CoNPS for shipping cost reimbursement, but we also appreciate the donation of your time and shipping cost, if you are able to do so. To submit an item, email to Denise 1) 2-3 good quality photos showing different angles (one picture of the cover is good for a book), 2) a short description, 3) and the value.

Denise suggests donations might include—

- Sample of a member artist's painting, photography, or other work; flat, small and no glass;
- Greeting cards, stationary, markers, pens, stickers, and so on;
- Tee shirts, hats, gloves, raingear, gaiters, UPF clothing, technical fabric clothing;

- Water bottles;
- Lightly used backpacks, items to fill a backpack, other outdoor gear;
- An unopened bottle of wine or liquor;
- SMALL garden tools, art, statuary, wind chimes in "like new" condition;
- Gift cards;
- Any unused SMALL gift you've received and would like to find a home for; and
- Money that the committee can use to make a great gift basket.

We are asking donors who are able to donate their time and shipping cost to hang onto the item until the end of the auction, and then mail it to the winning bidder for us.

If you or your business are interested in donating an item for the silent auction, please contact Denise at deniseclairewilson@gmail.com Likewise, if you'd like to volunteer to help with the silent auction, contact Denise.

Auction preview will be available September 1-11. Bidding on silent auction items will be open September 12-20.

Annual Photo Contest—Call for Entries

Have you taken some spectacular photos of native plants this summer or in years past? If so, consider entering the CoNPS annual photo contest. Photos may only be submitted electronically with a completed entry form. You must be a CoNPS member to participate.

Entries can be made in any of five categories including:

- Colorado Native Plant **Landscapes**;
- Colorado Native **Plants**;
- **Artistic** (of Colorado Native Plants or Native Plant Landscaping);
- Colorado Native Plants & **Wildlife** (including native insects/pollinators); and
- Native Plant **Gardens**.

Contest rules and agreements are posted on the CoNPS website. Photos may be submitted to the

contest August 1-31. Photos will be displayed on the CoNPS website and judged by CoNPS members prior to the conference (September 1-15). Winners will be announced on September 18 during the Friday night social.

Entries must be a single work of original material taken by the contest entrant. No more than one photo per category may be submitted. Photos may be from previous years (for example, you may submit a photo that you took in 2013). A \$50 prize will be awarded to the first place winner of each category.

Contest is open to CoNPS members only. Please see the CoNPS website for entry forms:

<https://conps.org/xyyzz-2020-photo-contest/>

Questions? Contact Bruce Tohill at tohillb@msn.com

Reporters Needed for the Annual Conference

Are you willing to write a summary of one or two of the presentations from the Annual Conference? If so,

please contact Mary Menz (Mary.T.Menz@gmail.com) or Kelly Ambler (alpineflowerchild@gmail.com)

Registration

Registration is available online at <http://conps.org>. Please log in if you are a CoNPS member, then proceed to the Calendar of Events to register. Online registration ends September 15.

If registering by mail, please complete the following registration form for each person attending and submit by September 7.

Mail registration form and payment to: CoNPS, c/o Linda Smith, 4057 Cottonwood Dr., Loveland, CO 80538

Name (first, last) _____

Phone _____ **Email** _____

Mailing address _____

The registration fees include attendance to the Annual Conference webinars on Saturday and Sunday, September 19 and 20 plus the Friday night virtual social on September 18. A separate fee is charged for attending the Rare Plant Symposium. An optional practice webinar is also included in the registration.

Member registration*

The 17 TH Annual Rare Plant Symposium @ \$10	_____
The 44 TH Annual Conference @ \$30	_____
Optional donation	_____
Membership Renewal (if necessary)	_____
Individual @ \$25	_____
Family/Dual @ \$35	_____
Senior or Student @ \$17	_____
Plant Lover @ \$50	_____
Supporting @ \$100	_____
Patron @ \$250	_____
Benefactor @ \$500	_____
Lifetime @ \$800	_____
<i>Aquilegia</i> print subscription @ \$20/year	_____
Total enclosed \$	_____

Non-member registration. Consider becoming a member! See page 26.

The 17 TH Annual Rare Plant Symposium @ \$10	_____
The 44 TH Annual Conference @ \$35	_____
Optional donation	_____
Total enclosed \$	_____

* A limited number of scholarships are available. See CoNPS.org for details.

The Annual Conference Committee appreciates all businesses and individuals who have contributed gift certificates or merchandise to our online auction to help defray cost of this year’s event. Business logos are displayed online at CoNPS.org and will be displayed in *Aquilegia* beginning Fall, 2020.

Featured Story

Celebrating the Bicentennial of Stephen H. Long's Expedition Part 3 of 4: The Ascent of Pikes Peak and Noteworthy Species Found

By Mike Kintgen and Jen Toews

This is the third in a series of four articles about the Long Expedition to the Rocky Mountains.

On July 14, 1820, Edwin James and two other men trudged slowly up what would later be named Pikes Peak. Without today's well-maintained trail system, the ascent would have been especially grueling. It would first have been a long bushwhack through the forest and then a tedious scramble through the talus. It is also easy to imagine that James, the first American botanist of European descent to see the alpine tundra of Colorado, would have been distracted by the flora. Indeed, in his diary and *Account* James lists many species he encountered on this excursion, from the charismatic sky-blue alpine forget-me-not (*Eritrichium nanum* var. *elongatum*) to the circumboreal mountain dryad (*Dryas octopetala*) to the narrowly endemic James's alumroot (*Telesonix jamesii*), which Torrey would later describe.

By 2:00 PM that day, the trio was so exhausted that they stopped for food and rest at a stream about one mile above tree line. That is when they realized that if they continued, it would be impossible to return to camp by nightfall where they had stashed their food and shelter. However, the prospect of summiting the mountain was irresistible and they hiked on.

Just one day prior, James and four others had begun their ascent of the Pikes Peak massif. The plan was

for two men to accompany James to the summit, while the other two would attend to the horses at a camp on Boiling Springs near present-day Manitou Springs. Initially, Lieutenant Swift and his guide Bijou were also in the party. Their duty was to obtain observations for measuring the height of the peak. Having completed this task, they returned to base camp on Fountain Creek where Stephen Long and the rest of the party waited.

Twenty-five miles from Long's base camp and higher up Fountain Creek, James and the others set up a horse camp. Around 3:00 PM and after breaking for lunch and a quick rest, James, Verplank, and Wilson left the horsemen behind and pressed toward the summit. They traveled all of two miles before setting up a precarious camp for the night (their camp would have been on Ruxton Creek). Apparently, "[b]ecause of the steep sides of the ravine, the men placed a pole on the ground between two trees; by laying their beds on the uphill side, they were thus prevented from rolling down into the creek during the night." Before falling asleep, James wrote a somewhat discouraged note in his diary: "[W]e laid down to rest for the night, having found few plants or anything else to reward us for our toils."

On the 14th, James and his two companions hung their camping supplies and food in a tree near their campsite. They planned to return before nightfall. By daybreak they were once again bound for the ►



Eritrichium nanum var. *elongatum* (alpine forget-me-not). © Jen Toews



Dryas octopetala (mountain dryad).
© Mike Kintgen

◀ summit. Interestingly, the route they took up the mountain is nearly the same as the route of the Pikes Peak Cog Railway today.

At around 4:00 PM, James, Verplank, and Wilson reached the 14,115 ft. summit of what would become known as America's mountain. They were the first Americans of European descent to have done so. It must be mentioned that Native Americans had undoubtedly already climbed the mountain during their long history in the region.

Meanwhile, back at base camp, Major Long and Lieutenant Swift had mathematically calculated the height of the mountain to be 11,507.5 feet. They arrived at this figure because they had calculated the elevation at their base camp along Fountain Creek to be 3,000 feet. They surmised that Pikes Peak was another 8,507.5 feet above them. In reality, their base camp was closer to 5,600 feet in elevation.

The short time James and the others spent on the summit was both literally and metaphorically the high point of the Long Expedition of 1820. The alpine was not what James expected. Instead of a barren wasteland, the men were greeted by a multitude of dwarf alpine plant species with showy, colorful flowers in full bloom. A quote from James sums up his surprise and delight with this biome:

"...a region of astonishing beauty, and of great interest on account of its productions; the intervals of soil are sometimes extensive, and are covered with a carpet

of low but brilliantly flowering alpine plants. Most of these have either matted procumbent stems, or such as including the flower, rarely rise more than an inch in height. In many of them, the flower is the most conspicuous and the largest part of the plant, and in all, the coloring is astonishingly brilliant....

...We met, as we proceeded, such numbers of unknown and interesting plants, as to occasion much delay in collecting, and were under the disagreeable necessity of passing by numbers which we saw in situations difficult of access. As we approached the summit, these became less frequent and at length ceased entirely."

It was late in the day, and after spending less than an hour on the summit, James and his party began their long descent. By sunset they reached timber line. Realizing they had lost the route back to their camp, they had no other choice but to spend the night with just a campfire and no food.

At first light on July 15, James and his companions were on the move back to the previous camp of July 13. Three hours later, as they neared their camp, they were greeted with a dense column of smoke. Apparently, they had failed to completely extinguish their campfire. The fire had burned their blankets, clothes, and most of their provisions. They were able, however, to salvage some fragments of charred buffalo meat for breakfast. Curiously, no other mention was made of what became of the fire. (Ironically, the Pikes Peak region has been the site of two of the ▶



Clockwise, from upper left. All photos © Mike Kintgen unless otherwise noted. *Minuartia obtusiloba* Rydb. (alpine sandwort) © Jen Toews, *Pinus flexilis* E. James (limber pine), *Trifolium nanum* Torr. (dwarf clover) © Mike Bone, *Trifolium dasyphyllum* Torr. & A. Gray (alpine clover) © Jen Toews, *Tonestus pygmaeus* Torr. & A. Gray (pygmy goldenweed), *Androsace chamaejasme* Wulfen subsp. *carinata* Torr. Hultén (boreal rockjasmine).



Clockwise, from upper left. All photos © Mike Kintgen unless otherwise noted. *Castilleja occidentalis* Torr. (Western Indian paintbrush), *Mertensia ciliata* E. James ex Torr. (alpine bluebells), *Chionophila jamesii* Benth (Rocky Mountain snowlover) © Jen Toews, *Penstemon glaber* var. *alpinus* Torr. A. Gray (alpine sawsepal penstemon), *Cymopterus humilis* (Pikes Peak alpine parsley © Panayoti Kelaidis; *Paronychia pulvinata* A. Gray (Rocky Mountain nailwort), *Mertensia alpina* Torr. G. Don (alpine bluebells), *Primula angustifolia* Torr. Raf., (alpine primrose).

◀ most costly forest fires in Colorado’s history, both since 2012.)

James, Verplank, and Wilson reached the horse camp shortly after NOON where the other two men awaited with a meal of fresh venison. At 7:00 PM, they arrived on horseback at their base camp farther down Fountain Creek, where the entire Long’s party was once again reunited.

During this three-day trip into the alpine world of the Southern Rockies, Edwin James observed and documented many alpine species, some of which had already been described from other regions of the country and world. Examples of previously described species include: alpine avens (*Geum rossii* var. *turbinatum*), alpine lily (*Lloydia serotina*), shrubby cinquefoil, (*Dasiphora fruticosa* ssp. *floribunda*, now *Potentilla fruticosa*), mountain sorrel (*Oxyria digyna*), mountain death camas (*Anticlea elegans*, now *Zigadenus elegans*), elephant’s head (*Pedicularis groenlandica*), alpine bistort (*Bistorta vivipara*), Whipple’s penstemon (*Penstemon whippleanus*), snowball or diamondleaf saxifrage (*Micranthes*

rhomboidea), moss campion (*Silene acaulis*), and marsh marigold (*Caltha leptosepala*, now *Caltha chionophila*).

In addition, James collected fifteen new species during these three days, which would be described from the Long Expedition of 1820:

- Boreal rockjasmine (*Androsace chamaejasme* Wulfen subsp. *carinata* (Torr.) Hultén);
- Western Indian paintbrush (*Castilleja occidentalis* Torr.);
- Rocky Mountain snowlover (*Chionophila jamesii* Benth), described July 14 in James’s diary as “a small plant somewhat resembling *Penstemon* with erect flowers;”
- Pikes Peak alpine parsley (*Cymopterus humilis* (Raf.) Tidestr.), a rare plant endemic to Pikes Peak;
- Alpine bluebells (*Mertensia alpina* (Torr.) G. Don);
- Streamside bluebells (*Mertensia ciliata* (E. James ex Torr.) G. Don.), though there is no record of this species in James’ diary or the *Account* and it was likely collected between Denver and Cañon ▶

◀ City in July, but certainly could have been collected during their Pikes Peak excursion;

- Alpine sandwort (*Minuartia obtusiloba* (Rydb.) House);
- Rocky Mountain nailwort (*Paronychia pulvinata* A.Gray);
- Alpine sawsepal penstemon (*Penstemon glaber* var. *alpinus* (Torr.) A. Gray.);
- Limber pine (*Pinus flexilis* E. James,) that James described in his diary as having “leaves 5 in a fascicle, branches remarkably flexible;”
- Alpine primrose (*Primula angustifolia* Torr.);
- James’s telesonix (*Telesonix jamesii* (Torr.) Raf.) of which the type collection was made on Pikes Peak probably near Windy Point;
- Pygmy goldenweed (*Tonestus pygmaeus* (Torr. & A. Gray) A. Nelson);
- Alpine clover (*Trifolium dasyphyllum* Torr. & A. Gray); and
- Dwarf clover (*Trifolium nanum* Torr.).

Thus, as the first scientifically-trained botanist to venture to the alpine biome of Colorado and the southern Rocky Mountains, Edwin James left an indelible mark on the alpine flora of this region.

A few days after James’s ascent of Pikes Peak, Long commemorated the accomplishment by naming the mountain James Peak. Later the peak would be christened Pikes Peak after Zebulon Pike. Pike had spotted the mountain fourteen years earlier in November 1806, had attempted to climb it (wrong time of year), and had declared it to be unclimbable. However, the name Pikes Peak would stick. James’s name would be moved to a somewhat prominent mountain and the high point of the 17,015-acre James Peak Wilderness, which is nestled between Rollinsville, Central City, and Winter Park.

Since James climbed Pikes Peak in 1820, much has changed. Instead of bushwhacking their way up, hikers can now choose between a well-maintained 28-mile, class-1 hike up the mountain or a slightly more technical, but shorter, 14-mile hike. However, more people opt for the Pikes Peak Cog Railway (currently closed for improvements, but reopening in 2021). Still more opt to drive their vehicles up a curvy paved road followed by a shuttle ride to the summit. At the top, hikers, tourists, and their dogs, are greeted with a visitor center and gift shop serving warm food and beverages. James probably would have appreciated this. Also available for purchase is an assortment of tchotchkes and souvenirs stating the height of the peak.

On a clear day, visitors to America’s mountain are rewarded with a 360-degree view. To the east, the plains with their “amber waves of grain” gradually decrease in elevation until they are swallowed up by the horizon; the prominent Spanish Peaks tower to the south; and to the north and west the “purple mountains majesty” of the Rockies extend as far as the eye can see. In the foreground, colorful alpine plants abound and a small herd of bighorn sheep can be seen grazing. Two-hundred years later, it is safe to say that the Pikes Peak area remains “a region of astonishing beauty.”

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Mike Kintgen is the curator of Alpine Collection at Denver Botanic Gardens. His work has taken him across the globe to biomes similar to the Rocky Mountains and steppes of Western North America. He greatly enjoys working with regionally native flora and learning the botanical history of Colorado. Jen Toews works in the Plant Records department at the Denver Botanic Gardens and is a Colorado Native Plant Master® who advocates for native plants at every opportunity. In her free time, she enjoys expanding her native plants garden, hiking to see native flora, photographing native flora, and writing. ☺

Recent & Relevant Reading

Mushrooms are Healing the Earth, Starting with Colorado’s Forests

Read about a mushroom project

<https://theknow.denverpost.com/2020/06/11/fungal-degradation-colorado/240382/#:~:text=Native%20mushrooms%20may%20also%20help,to%201%2C000%20years%20to%20regenerate>

Stamp Out Neatness, Save (native pollinators and) the Planet

Blaze a new kind of road. A pathway for pollinators
<https://www.northcountrypublicradio.org/news/story/41669/20200613/stamp-out-neatness-save-native-pollinators-and-the-planet>

Why Plant Blindness Matters—and What You Can Do About It

Appreciate the flora around us

<https://www.bbc.com/future/article/20190425-plant-blindness-what-we-lose-with-nature-deficit-disorder>

Featured Story

Diné Bih Naniseh Bah Haneeh: Navajo Ethnobotanical Teachings

By Arnold Clifford

Editor's note: Approximately half of Colorado's Western Slope is part of the Colorado Plateau, an area that encompasses large portions of Colorado, Utah, Arizona, and Nevada. The 130,000 square-mile region is rich in geologic and floristic diversity. Arnold Clifford is a botanist, a geologist, and a Navajo elder. He is co-author of The Flora of the Four Corners (2013) and is working on a complete Diné Bih Naniseh: Flora of the Navajo Indian Reservation. The following article is reprinted with permission from the Winter 2016 issue of The Plant Press, (Vol 38, No 2), the newsletter of the Arizona Native Plant Society.

Navajo Culturally Significant Plant Species

Navajo people have lived within the physiographic boundaries of the Colorado Plateau for thousands of years before the arrival of the first European settlers. Their extensive understanding of plants and uses of plants were derived from knowledge passed down through Divine intervention of the Navajo Holy People into the lives of the early predecessors of the Navajo people. As a result of species range expansion, travel during herbal pilgrimages, experimentation, and trial and error, other new plant species were incorporated within the vast knowledge of plants.

Various Navajo deities have also instructed the people on uses of native plants and the importance of plants for the well being of the Navajo people.

Navajo Ethnobotany

Plants are sacred, alive, and dynamic, and Navajos refer to them as "Holy Plant People." Knowledge of plant use is interwoven with traditional religious

contexts, cultural oral tales, and history. Navajos have their own plant classification systems, just as the Europeans have in the sciences of systematic botany and plant taxonomy. Navajo herbalists recognize different individual plant species, including grouping together closely related species by generic Navajo names, similar in some respects to Western botanical scientists' use of Latin binomial scientific names for individual plant species. Navajo plant names are very descriptive names that may refer to the morphology of the plant, leaf color, medicinal connotations, ceremonial associations, tobacco types, and animals based on their morphological similarities.

Navajo Philosophy Relating to Plants

In the process of developing intimate relations with all plants, Navajo have drawn similarities between plants and their own bodies. Teachings include how plants are decorations, garlands, and jewelry of Changing Woman, Mother Earth. Plants also play roles in the human anatomy where our blood, arteries, and veins were modeled after the divaricated, branching nature of plant roots. That is how our arteries and veins began branching out of our hearts, the center of the body. The arteries branching into smaller vessels cover the whole human body, providing warmth, oxygen, and other life-giving minerals and elements to the body.

Plant roots function in a similar way as they break down and draw up vital nutrients, minerals, elements from the soil to distribute into all parts of the plant, to give plants life. The Navajo "Holy Plant and People" ►



Golden Mariposa (*Calochortus aureus*).
© Arnold Clifford



Navajo Tea (*Thelesperma megapotamicum*).
© Arnold Clifford

◀ are treated with respect, holiness, and reverence. When plants are collected for ceremonial use, or for medicinal healing properties, Navajo herbalists talk to the plants they need. Herbalists introduce themselves, stating their reason for collecting each plant, describing the ailment of patients in need of treatment, and calling out sacred plant names. Herbalists offer prayers and make offerings to plants that are gathered for use. This ensures the curative healing powers of the plant will be invoked, as well as extends protection to the herbalist gathering the plants.

Navajo Classification of Plants

Navajos have several classifications of plants. One simple Navajo classification is based on its intended use in ceremonies: as tobaccos, as food items, or in everyday utilitarian usage. Navajo plant classification is a primitive, systematic approach to better understanding the flora of the diversified habitats the Navajo people occupied. Classifying also provided a better understanding of different groups of closely related plant species. Navajo plant classification was partly based on floral morphology or physical similarities of natural families. One basic Navajo botanical classification of plants includes the identification of Life Way, Evil Way, and Beauty Way plant groups. These plants are associated with various ceremonial rites. Most plants have more than one use and can be classified under several different categories. A basic way Navajos classify plants is based on its uses: edible plants, medicinal plants, ceremonial plants, tobacco plants, utilitarian plants, dye plants, and plants for protection and for talismans.

Edible Plants

Native plant species are utilized for supplemental food, food additives, seasoning, spices, and sweet treats from flower nectars. Hundreds of plants are

used as foods, with different plant parts such as roots, stems, leaves, flower petals, flower nectar, assorted berries, tasty fruits, and grass seeds all providing valuable nutrients and sustenance for surviving in the desert wilderness environment.

Medicinal Plants

Medicinal plants constitute a large group known as the Life Way medicines. These plants are intended to sustain good health and mental wellness and they help to improve the lives of Navajos who reverently partake of them. Life Way plant knowledge was acquired from supernatural events involving the Holy People. The body of Rainboy was dismembered by lightning bolts sent by Big Thunder as punishment for being promiscuous. A restoration rite was held for Rainboy by the Holy People. Before the rite, insects, animals, and other holy deities were instructed to gather Rainboy's dismembered remains. Rainboy's remains were collected with different herbs that grew nearby. The dismembered remains and sacred herbs were brought back together, so a restoration rite could be performed. The holy plants were applied to different body parts of Rainboy, and each body part began to heal and was restored to health. From that time forward, these plants were considered plants that would heal that particular part of the human anatomy. The healing powers of these holy plants were "life-giving" and therefore, considered Life Way medicinal plants. There are about three hundred Life Way medicines available for Navajos to rely on for curative remedies.

Ceremonial Plants

Numerous species of trees, shrubs, grasses, and flowering, herbaceous forbs are used for different aspects and rites of Navajo ceremonials and chants. Plants designated for ceremonial use are employed to make ceremonial implements and paraphernalia, ▶



Morning Lily (*Oenothera caespitosa* var. *navajoensis*). © Arnold Clifford



Broadleaf Cattail (*Typha latifolia*). © Arnold Clifford

◀ such as prayer sticks, prayer wands, cigarettes for offerings to various deities, ceremonial masks, dry paint material, incense for fumigants, and for medicines administered during ceremonial rituals. Plants used in ceremonial rites are considered sacred and are therefore reserved for chanters and medicine people who perform these chants.

Tobacco Plants

Tobacco plants are utilized reverently. Most tobacco plants are used as offerings for various deities and sacred ceremonial animals. Tobaccos are smoked during ceremonial rites, ritual baths, sweat lodge cleansing rites, as well as during personal meditation and prayer sessions. Tobacco smoke helps to clear the mind and blesses the body and soul. Tobacco smoke also carries a person's prayer to the holy deities. Tobaccos utilized in Navajo society are not for pleasure, relaxation, or for recreational smoking.

Utilitarian Plants

Many plants are used to make every day utensils and household objects for domestic use, such as hair brushes for grooming, floor brooms, kitchen utensils, digging tools, farming tools, weaving looms, weaving tools, carding combs and spinning spindles, weaving dowels, bows, and arrows. Trees and larger shrubs are prepared for hogans, shade houses, sheep corrals, sweat lodges, fencing material, and firewood for heating, cooking, and ceremonial fires.

Native Dye Plants

Numerous plants are used by Navajo weavers for dyeing wool. Various plant parts provide natural dyes. The bark of some shrubs and trees yield red, reddish brown, and brown dye hues. The roots of canaigre dock can produce yellow, yellow-orange, and yellow-brown colors. Flowers and seeds of many plant species are also used to create many unique dye hues. Plants are normally boiled in an acidic solution containing a mordant, which helps the dye color to turn a richer hue and also helps the dye to fix to the wool fibers.

These are specialized plants used to ward off evil influences and also for the protection of individuals. Most of these plants are known by very few people, some are known only by certain clan or family groups. These plants are carried on a person for protection while they are at public functions where many people are in attendance, such as fairs, ceremonial gatherings, rodeos, and other public gatherings.

The Navajo Gods of Botany: The Humpback Gods

The Navajo Gods of Botany, Ah Ghaah Dah Hiskid Dih (Humpback Gods) are fertility gods and are of the utmost importance to the Navajo ceremonial system. Humpback Gods are sacred, powerful deities of native plants and seasonal weather, especially precipitation. They include the god of harvest, of abundance, and the fruits of the fields as well as a polymorphic deity incorporating desert bighorn and Rocky Mountain bighorn sheep, holy people, and humans. They are responsible for revegetation and reseeding the Earth with grasses, flowering plants, shrubs, and trees. In the process of reseeding, they bless each seed with all kinds of precipitation. During the coldest part of winter, when extensive stands of fog cover the desert

southwest, the Humpback Gods come out in large numbers, walking amid fog, each burdened and hunched over, carrying bags of seeds and precipitation. The humpbacks utter "Ah Woo" as they walk about in the cold, frosty fog, often stopping to shake their backs to release native plant seeds and precipitation. They do circular dances to poke each seed into the ground with their planting stick-cane. During the spring and early summer, the whole southwest is blessed with new flowering plants, grasses, and shrubs.

The Humpback God wears an inverted Navajo basket with an opening at the top to help secure it to the top of the impersonator's head.

The basket is painted black with a white zigzag all the way around the rim. The black represents night time clouds, with the white zigzag depicting lightning strikes between adjacent dark clouds. Standing upright around the rim of the baskets are numerous red flicker or red woodpecker feathers. The feathers represent sunbeams shining through clouds immediately after rain. On top of the basket are two bluish horns that represent dark clouds before and during rain. The whole head piece is a crown of thunder, lightning, and rain. Along the back is a rainbow with feathers attached along the crest. The feathers indicate sun rays radiating from the eastern horizon, and the rainbow indicates the presence of holy people and the blessings of rain. Under the rainbow is a dark, black sack with white bars. The hump contains mist, dew, frost, female rain, male rain, snow, all aspects of precipitation, and vegetation seeds of all types. Humpback Gods carry and walk with planting-stick canes. ▶



Navajo Humpback God weaving. © Zonnie Gilmore

◀ A Note from the Author

These stories of the Navajo Gods of Botany, Ah Ghaah Dah Hiskid Dih (Humpback God), the Navajo classification system, the sacred Navajo plant names and its uses were bestowed and blessed upon me from my late maternal grandmother Sarah Charley of Beclahbito, New Mexico.

This ancient, sacred, Navajo ethnobotanical knowledge comes from seven or more generations of my family. Sarah was taught by her late mother Irene Bennallie of Beclahbito, New Mexico. Both Sarah and Irene were instructed by Sarah's paternal grandfather, and both collected ceremonial herbs, medicines, and tobaccos for him. Sarah's grandfather was Tsao Lao Alth Tsosii (police slender, or slim police), who practiced the Shooting Way, Wind Way, and Beauty Way chants. He also specialized in Navajo herbal and medicinal application. I possess a small portion of this once vast family knowledge, and I feel it is time to pass this knowledge onto other Navajos.

This knowledge, I believe, belongs to the collective Navajo people as a whole. It belongs to Navajos willing to learn, willing to keeping an open heart to our traditions, our culture and our religious beliefs. If we horde such sacred knowledge, no one benefits; it all becomes lost. We have already lost over half of our ceremonial systems, including unmatched plant knowledge acquired by our ancestors who never had a chance to pass on their infinite knowledge of all aspects of Navajo teachings.

Examples of Navajo Plant Uses

Navajos still utilize 1,500 or more native plant species; however, plant knowledge is declining. In the past Navajos had a vault of plant knowledge that included more than 3,000 to 4,000 plant species occurring within the Colorado Plateau. Six different plants are

presented here to give an example of the diversity of plants available.

Aliciella cliffordii (Clifford's Diné Star, or Clifford's Gilia), a member of Beauty Way tobacco. They are essential plants required in a mixture of several plants that constitute the Beauty Way tobacco mixture. Different species of the genus *Aliciella* are called by the generic Navajo name Hozho Nah Toh (beauty tobacco) and are classified as Beauty Way plants. Clifford's Diné Star is a rare plant known only from the north foothills of the Lukachukai Mountains and the foothills of Mexican Cry Mesa, Arizona. Named after Arnold Clifford of Beclahbito, New Mexico.

Calochortus aureus (golden mariposa), a showy yellow-flowered member of the Lily family. The white bulbs below the ground are edible when fresh. They taste similar to peanuts. Its Navajo name is Alth Chiin Daah, which means children's food. Found south of Sheep Springs to Window Rock regions.

Oenothera caespitosa var. *navajoensis* (morning lily), a plant called **Kleeh Yih Ghaii**, meaning night time turning white, or night bloomer. A plant used as a medicine to heal body sores. Found on weathered, grayish white-colored Mancos Shale surrounding the Shiprock region to the Four Corners. The papery, white flowers are very large.

Rumex hymenosepalus (canaigre dock), a plant of sandy places. Easily identified by its large wavy leaves and light reddish flowering stems. Its large, bunched root tubers are about a foot and half or more below the basal leaves. The tuber roots are boiled to produce various colors of orange-brown to brownish dyes. Thick lower stems are used to make Navajo pie fillings. In some species, the seeds are used medicinally. Its Navajo name, Chaa Ha Tiin Ni, refers to darkness dweller.

Thelesperma megapotamicum (Navajo tea, greenthread), a plant gathered to make Navajo tea, a mild stimulant, served as a beverage during meals or during social greetings. During illness, Navajo tea helps to reduce fevers, helping the body feel better. Boiling the plant produces different shades of a yellowish-orange dye that is used by weavers to dye sheep wool. Chiil Ah Whee (plant coffee) is the Navajo name. Found throughout the desert southwest.

Typha latifolia (broadleaf cattail), a multi-use plant growing in wetlands. Its fleshy roots are edible. Its long, broad leaves were used to make mats, skirts, and other useful items. Flower stalks provided ceremonial pollen and the fresh green flower stalks are also edible. When the flower stalks matured and were dry, they were collected for stuffing in pillows, pads, kid's toys and other items. The Navajo name, Ethel Nigh Teel, means cattail wide. The generic Navajo name is Ethel. ☯



Clifford's Diné Star (*Aliciella cliffordii*).
© Arnold Clifford

Basic Botany

All Life Depends on Plants

By Deryn Davidson

Do you know that a large percentage of our population is afflicted with something known as plant blindness? It's true. By definition, these poor people have "the inability to see or notice the plants in one's own environment," which leads to "the inability to recognize the importance of plants in the biosphere and in human affairs." It seems that most people favor animals over plants. Sure, animals might seem more charismatic and dynamic, but come on now! We would be nothing without plants.

All joking aside, plant blindness actually has some pretty big implications. The term was coined in 1998 by botanists James Wandersee of Louisiana State University and Elisabeth Schussler of the Ruth Patrick Science Education Center. It's quite a fascinating topic. The average person truly just doesn't process that there are plants in their view. Because plants grow close to one another, are a similar color, and don't move (much), humans tend to clump them together as "non-threatening things" and filter them out of the many, many other bits of visual data the eyes receive.

"There is a kaleidoscopic array of visual information bombarding our retinas every waking second, and plants are so easy to ignore unless they are in bloom," Wandersee says. "Plant blindness is the human default condition."

If people don't pay attention to plants, they won't place much importance on them and the role they play in our daily lives. They are, of course, not only food—they are medicine, they are fiber, they are fuel, they are beauty, and so on.

What can we do about this?? We can be plant advocates! I have no doubt that most, if not all, of the people reading this are already in that camp. Anytime there is an opportunity to tell our family, friends, neighbors, and even complete strangers about the wonders of plants, we should seize that moment. Stimulate their imagination with stories of your favorite plants and gardening moments.

Exposing younger generations to plants is key, too. I'm realizing that my 2.5-year-old has tons of books about animals, but very few about plants. Okay, he does have "Botany for Babies," but he also has two parents who are plant nerds. Perhaps if there were board books with photos of different plants, he would be able to identify penstemons and prickly pears just as quickly as he identifies horses and pigs.

Wandersee recommends having a plant mentor in your life, or you can be the mentor. I am encouraged by the huge uptick in interest around houseplants.

Apparently, houseplants are super hip right now. Surely that will have an impact on combating plant blindness and will extend beyond the walls of their homes as people come to appreciate the positive affects those plants have on them.

The work of volunteer programs like CoNPS, CSU Extension Native Plant Master® and Master Gardener programs, along with public botanic gardens, are doing a lot to help educate the public about the importance of plants.

So, if you're going on hikes with friends who don't understand why you stop every 10 feet to point out a plant, or if you have been putting a lot of time and effort into your garden and people aren't knocking down your door to compliment you, it's

probably because they just don't see the plants. Keep up the good work and little by little we'll help combat plant blindness together!

I do have to share that my 2.5-year-old is doing pretty well with his plant ID skills. So far on his list are yucca, ponderosa, pinon, juniper, cactus, oak, mint, dandelion, and daffodil.

Deryn has been a native plant enthusiast since her time as a horticulturist at the Lady Bird Johnson Wildflower Center. She is now the CSU horticulture extension agent in Boulder County and co-runs the Native Plant Master® program there. She is passionate about helping people understand the importance of native plants in our open spaces and natural areas and also in incorporating them into our urban landscapes. ☺



Field crescent butterfly (*Phyciodes pulchella*) on a ragwort (*Packera* sp.).
© Deryn Davidson

Reintroduction of Wolves to Colorado: Could This Affect Our Native Plant Communities?

By John Emerick

This November, Coloradans will have the opportunity to vote on Proposition 114 to restore gray wolves to Colorado. Wolves were deliberately extirpated from Colorado during the first half of the 20th century. The last wolf in the state was killed in 1945. Since then a few wolves have wandered into Colorado, but most have been killed; there is no sustained population. If Proposition 114 passes, it would mandate the development of a scientifically-based wolf management plan; after which, wolves would be reintroduced to a small number of public land sites on Colorado's western slope.

Those of us who are passionate about our native plant species and plant communities, and who have spent considerable time on our public lands, have undoubtedly observed widespread degradation to these communities by wildlife and livestock grazing. In places of heavy grazing, plant community diversity is low and the composition of introduced plant species is often high. Add the potential impacts of climate



Forty-one gray wolves were introduced to Yellowstone National Park from 1995 to 1997. Their numbers tripled during the first few years, then settled to about 100 wolves in the park since 2009. If Proposition 114 is passed, wolves would be reintroduced to Colorado by the end of 2023 with the numbers of introduced wolves yet to be determined. Photo © National Park Service, Yellowstone National Park.

change and the degradation is liable to worsen. A fair question, then, is whether wolf reintroduction might have a beneficial effect on our native plant communities.

Wolves are a keystone species. Their activities as ungulate predators produce trophic cascades affecting plant survival, pollinators, birds, mesopredators such as foxes and weasels, and smaller herbivores such as rabbits and various rodents. It is difficult to estimate what the disappearance of wolves has meant to the structure of our native ecosystems.

Elk are the primary prey of Rocky Mountain populations of wolves. In Colorado, there are more than 280,000 elk, the largest population of any state. There are also 430,000 mule deer, mostly concentrated in western Colorado. However, there are also approximately 500,000 cattle and 175,000 sheep that also are grazed on public lands west of Interstate 25. Grazing by these ungulates has a significant impact on our plant communities.

To assess the potential impact of wolves on Colorado's landscapes, it is useful to examine the effect that wolves have had on Yellowstone National Park. Wolves were reintroduced to Yellowstone National Park beginning in 1995. That was also about the time when elk populations were at all-time highs in both Yellowstone and Rocky Mountain National Parks, and much has been written about resource damage due to elk in both places.

The most noticeable damage occurs in aspen groves and in riparian willow carrs, particularly if elk use these resources year-round or if unmanaged livestock overgraze these systems. When healthy, both ecosystems support a high diversity of sub-dominant plant species, as well as diverse animal communities including mammals, birds, and invertebrates. This diversity declines with prolonged heavy grazing. In Rocky Mountain National Park, excessive grazing of alpine tundra plants by elk may have contributed to the decline of ptarmigan numbers.

Aspen groves—typically clones in which the trees are interconnected by a common root system—produce shoots, or suckers, from the root system to expand the grove or to replace ageing or diseased trees. When the shoots are heavily browsed by elk and other herbivores, the groves fail to mature. Elk also gnaw the bark of aspen trees during late winter and early spring, and that can lead to infection of the tree by various diseases. Both situations in concert can eventually lead to the death of the entire clone. ►



Elk can severely damage aspen bark, which can lead to disease and death of the grove, as well as the loss of the other plant and animal species that depend on the ecosystem. The inset shows severely browsed aspen suckers. © John Emerick

◀ Riparian willow carrs, particularly those in broader valley bottoms, are commonly occupied by beavers. Willows and beavers are codependent. Beavers use the willows for food and building materials for their dams and lodges. Beaver dams raise the water table, providing shallow ground water that willows need. When willows are browsed heavily by elk, beavers leave due to over-competition with elk, beaver dams are no longer maintained, and streams and rivers begin to run straight and fast. This results in downcutting of the channel, a drop in the water table, and further demise of the willows. Sedges, grasses, and smaller shrubs that help to maintain channel stability are also affected by heavy elk browsing.

Many observations from Yellowstone National Park show that the presence of wolves keep elk moving, preventing them from yarding in riparian willows and aspen groves for long periods of time. There are both diurnal and seasonal movements of elk to avoid areas where wolves are active.

Despite many articles claiming that elk decline and ecosystem recovery in Yellowstone is due to wolf reintroduction, more recent investigations have shown that the situation is much more complicated. For example, cougar and grizzly numbers were also on the increase, which increased elk mortality, and there was a high volume of elk hunting outside of the Park. Beaver populations began to rise in some areas due to declining elk populations, benefitting riparian plant and animal diversity. While there certainly has been ecosystem recovery in some areas, there is little improvement in others. It is worth noting that as elk populations declined, bison numbers increased. Also, since wolves suppress coyote numbers, this could

have led to increases in the numbers of mule deer and pronghorn, two important prey species for coyotes.

The reintroduction of wolves to Colorado is likely to have mostly subtle and indirect effects on our native plant communities. Those effects will probably not occur until wolf populations increase to ecologically effective numbers and stabilize. Some over-browsed ecosystems might benefit through a wolf-elk-plant trophic cascade as has been documented in Yellowstone and Banff National Parks. However, Colorado has experienced almost a century without a full suite of large predators at a time when elk and livestock populations have been climbing on our public lands. The effects of overgrazing on some of these lands will not be reversed by wolves alone; that will also require a public commitment for effective wildlife and grazing management.

Certainly, there is concern from the ranching community about potential livestock depredation by wolves. This is to be expected. However, studies have shown that when wild prey is abundant, wolves will select those animals even when livestock are abundant. In the five states of the northern Rocky Mountains (Washington, Oregon, Idaho, Montana, Wyoming) there were in 2015 a total of 1,980,600 cattle in counties that also had wolves. In those same counties of that year, confirmed depredation of cattle by wolves was 148, or 0.007 percent. It has also been shown that the use of range riders and other predator coexistence strategies can drastically reduce livestock losses to wolves.

Proposition 114 is likely to succeed. According to a recent statewide survey completed earlier this year, ▶



A view of what was once a lush riparian willow carr in Rocky Mountain National Park. Heavy competition between elk and beaver forced the beaver to leave, resulting in a drop in the water table and ultimate death of the willows. What was once home to a thriving community of neotropical migrant birds such as the MacGillivray's and Wilson's Warblers, and plants such as the wood lily, is no longer existent. © John Emerick

◀ 84% of respondents favored the measure. While most ranching and hunting organizations oppose wolf reintroduction, there is high consistency between urban and rural communities, as well as between eastern slope and western slope communities in support of the proposition. Some have suggested that the popularity of the measure is driven by younger Coloradans who are more interested in ecology and conservation than their parents or grandparents.

Perhaps the reintroduction of wolves to Colorado—if it occurs—will be a catalyst for a larger, more holistic movement in which Coloradans will press for:

- Better scientifically-based wildlife management, including non-lethal predator control;
- More responsible livestock management, including predator coexistence strategies and cessation of over-grazing on public lands; and
- Programs to restore plant and animal diversity to our public lands where it has been lost due to excessive livestock grazing and large elk populations.

Native plants matter for ecological sustainability, for aesthetics, and for maintaining the integrity of the natural world. In the face of changing climate, we need to do as much as possible to conserve and restore our native plant communities. Wolves may be part of that equation.

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John Emerick, PhD, is on the emeritus faculty of the department of environmental science and engineering, Colorado School of Mines. In addition to his academic career, John has taught numerous field seminars on various aspects of Colorado's ecology for over 40 years, mostly in Rocky Mountain National Park. He has hiked extensively throughout the state. Nowadays, he spends part of his summers conducting field surveys for the Colorado Natural Heritage Program. 🌀

Basal Rosettes By Arthur Clifford

I have lust
For the living
The Holy Earth
And its giving

Many are such
Common things
The petals on sunflowers
Rayed sparrow's wings

In rapture joined
I am with these
We upward gaze
From our knees



Alpine spring beauty (*Claytonia megarhiza*) on Pikes Peak. © Kelly Ambler

Aspen for the Landscape

By Jim Borland

Not very long ago, every aspen planted in the landscape was collected from the wild. Collected with it was the possibility that any of a myriad of diseases and damage from over 300 insects, if not the insects themselves, came with it, too. These factors, combined with the difficulty of collecting sufficient supporting roots from a species whose root system meanders, non-tapered through the soil, result in losses commonly exceeding 50 percent.

The better aspen for your landscape is the one grown from seed. Yes, seed. Regardless of what you may have heard, aspen do, indeed, produce great quantities of viable seed. Small and losing viability rapidly after shed from the tree, aspen seed rarely finds natural conditions conducive to germination and early seedling success. Instead, the vast quantity of annually-shed seed is simply lost. A successful germination event in the wild is cause for celebration and papers written.

Yet when collected, cleaned, and sown under ideal nursery conditions, aspen seed quickly produces fast-growing, healthy, and strong trees free of diseases and insects. The seed-grown aspen is a tree far superior to those collected from the wild. When

properly grown, pruned, and dug, a much higher percentage of the root system goes with the seed grown tree to the landscape and local establishment is virtually 100% assured.

Through progressive nursery techniques that involve greenhouse sowings, specialized soils, carbon dioxide enrichment of the atmosphere, fertilization with every watering, and 24-hour-per day lighting, 12 feet of growth during the first season is common.

Why, then, are aspens still being dug and sold? They are cheap. And, as one coffee magnate once said, "You get what you pay for." Insist on seed-grown aspen for your landscape. Only when enough of us do this will the nursery industry make the appropriate changes and give us what we insist upon.

Jim has been fooling around with native plants for more than 40 years in private, commercial, and public venues. His home garden contains 1000s of native plants, most grown from seed at home and now not supplementally watered for 20 years. Jim has written hundreds of articles, given talks too numerous to count, and continues to grow and plant the two or three native plants not yet in his garden. ☺



Aspen (*Populus tremuloides*) tree (© Kelly Ambler); female catkins and male catkins (© Bryan Kochis).

Tips from the Pros

Still Seeing Mulch Years Later? Plant More

By Benjamin Vogt

If you planted a garden two to three years ago and you still see lots of wood mulch, then you need more plants. You're probably still seeing a decent number of weeds at this point, too (mulch isn't a magic weed bullet and, if too thick, often creates an ideal seed bed). So, you know, more plants. More layers. More density.

And if you are planting a garden today think about where you do *and* don't want to be in two- to three years:

1. Only put down 1" of mulch if you are using it. More mulch = less plant sowing while generally inhibiting forb and grass growth.
2. Put plants on 12" centers (12" apart) and no more.
3. Consider mixing potted plants and seeds to increase coverage. In spring, sow grasses and annuals among what you planted. In mid-to-late fall, consider a dormant seeding of perennial forbs among what you planted. (Maybe what you plant is the highly designed part, or plants that need a head start because they work on roots first like baptisia and amorpha and silphium, [or other plants suited to Colorado]).

What do you do if you are on a constrained budget?

1. See #3 above. The best advice is to plant the architectural plants—trees, shrubs, and perennial flowers—that take longer to establish and serve as the backbone for the design. You may also want to plant aggressive species and let them start to self-sow or run asap.
2. Get plugs. Most landscapers and nurseries get their plant material from wholesalers, and that requires a business license. But you can also get them (if you're east of the Great Plains) via Izel Native Plants (<https://www.izelplants.com/>), which works as a middleman for wholesalers to sell to the public. That means if you need plants in quantities of 32 and 50 you can get them for a much better per-plant cost.

My new book will attempt to better align these two perspectives, as both are critical for the success of urban gardens that both appeal to and involve people and wildlife together. It is critical that people find nature-inspired gardens beautiful, while it is just as critical that wildlife find them beautiful as well. Just



because one has host plants does not mean the garden is beautiful to wildlife, and just because one has a diversity of flowers doesn't mean the garden is beautiful to wildlife.

Benjamin Vogt is the author of A New Garden Ethic: Cultivating Defiant Compassion for an Uncertain Future. His prairie-inspired design firm, Monarch Gardens, is based out of Nebraska.

<https://www.monarchgard.com/thedeepmiddle/still-seeing-mulch-years-later-plant-more>

News, Events, and Announcements

Please check the **Calendar of Events** online at <https://conps.org/mfm-event-calendar/#!calendar> for chapter meetings, garden tours, and other events. With the evolving COVID-19 situation, CoNPS is not hosting any in-person events. The status of future CoNPS events might also change.

CoNPS may offer some chapter meetings, workshops, and lectures as webinars or other online meetings. Others might be postponed or canceled. Field trips are also being scheduled, but may be canceled or postponed. These will be posted online and will be promoted via the CoNPS E-News.

CoNPS Chapter Events

Plateau Chapter

Help with Native Vegetation Efforts Grand Junction Wednesday mornings

Colorado West Land Trust and the City of Grand Junction are looking for volunteers for the ecological restoration of the Three Sisters/Lunch Loops recreational area south of Grand Junction. Legacy land uses and last year's development of a recreational pathway through the area have impacted soils and native vegetation.

The revegetation project aims to restore native vegetation and establish sustainable community stewardship of the resources. The properties are owned by City of Grand Junction and protected by conservation easements held by the land trust. A grant awarded from Colorado Youth Corps Association (and GoCO) enabled the project to use crews from Western Colorado Conservation Corps to do some of the heavy lifting, but the rest is being done by land trust employees and volunteers.

Volunteers meet Wednesday mornings to do the work. With the pandemic, it has been tricky to engage many volunteers, but social distancing and mask wearing practices are in use. The public, and especially CoNPS members, are invited to participate in the revegetation effort. For more information, contact monument.stewards@gmail.com

Learn About Plants Used by the Utes on the Western Slope Montrose

Thursday, August 20 10:00–11:00 AM

The Plateau Chapter invites CoNPS members on the Western Slope to learn more about Ute ethnobotany at the Ute Indian Museum in Montrose. After the short program, attendees may want to stay for an hour to help weed a section of the garden in preparation for fall mulching. Bring gloves and a trowel.

The Ute Indian Museum is in the final stages of a complete restoration of its native plant garden. The new Ethnobotany Garden is the result of a two-year makeover of an existing garden space. Chinese willow

have been replaced with coyote willow; Shasta daisies have been replaced with Rocky Mountain penstemon, scarlet gilia, and more; and nearly all non-native plants have been removed and replaced with native plants.

Plant stakes paid for by a generous CoNPS Mission Grant have been installed to inform visitors about the names of plants in the garden.

Interpretive signage is currently being designed and will be installed this fall. Curriculum is also being developed to educate school children and museum visitors about Ute ethnobotany. For more information and to RSVP, contact mary.t.menz@gmail.com

Southeast Chapter

Watershed Restoration in Action! Colorado Springs Saturday, August 15 8:30–10:00 AM

Join local citizen scientist and CoNPS member Gary Rapp for an engaging discussion and demonstration of how riparian forests can be restored to protect us from stormwater damage and enhance native pollinator and songbird habitat.

Please meet at the Shooks Run Agroforestry Project terrace garden at the north end of North Shooks Run Park, about a 200-yard walk north from on-street parking near 653 N. Franklin St. (just west of its intersection with N. Prospect St.). Please observe City Park and Recreation rules for COVID-19 posted at: <https://coloradosprings.gov/parks>

Cross-Pollination Events

October 1-November 3
Colorado Parks & Recreation Annual Conference
<https://www.cpra-web.org/page/SessionProposals>

October 6-8
Sustaining Colorado Watersheds Conference
Avon, CO
<https://www.watereducationcolorado.org/programs-events/conferences/>

CoNPS Webinars

CoNPS offers webinars on a variety of native plant topics. Sign up for these webinars on the CoNPS website (<https://conps.org/mfm-event-calendar/#!calendar>). New webinars are constantly being added to the calendar.

Ecosystems in Colorado's Southeast Prairie **Saturday, August 15; 9:00 AM–NOON** **Presenter: Carol English, MS**

This workshop focuses on several ecosystems within the Western Great Plains Ecoregion including the shortgrass prairie, shale barrens, sandhill shrubland, playas, and southwestern great plains canyon areas. Carol will cover the native plants and animals that are dependent on plant species in this region. Participants will also learn about the different types of rare plant communities that occur in these areas.

Carol English has been involved in the field of natural resources and education for more than 30 years. She holds a BS in earth science, teaching certificate, and MS in biology. Carol has presented natural resource classes and programs at Yosemite Institute, Outward Bound, and Jefferson County Open Space. She is a certified Native Plant Master® and taught Native Plant Master courses for nine years. In addition, Carol has worked as a botanist for the Colorado Natural Heritage Program, Yosemite National Park, and Colorado State Land Board. She has owned her natural-resource based business for seven years.

Learn How to Use iNaturalist **Tuesday, August 25, 2020 9:30–11:00 AM** **Presenter: Audrey Spencer**

Looking to contribute to citizen science? Or maybe you just want to share your observations and connect with scientists who can identify the plants, animals and other organisms you observe? Learn how to use this dynamic tool at a free webinar sponsored by the CoNPS education and outreach committee. Sign up at [CoNPS.org](https://conps.org). A link will be sent to registrants by or before Monday, August 24.

Wildscaping 101—Native Plants for Birds **Sunday, August 30; 1:00–3:30 PM** **Presenter: Kate Hogan, MS**

Are you passionate about native plants and want to learn more about the ecological connections between our natives and our Colorado bird life? Join Kate for an engaging and exciting webinar on ways to diversify the birds found in your own yard, using a variety of food groups provided by our native plants. Participants will review the newly created Native Plants for Birds handout designed in partnership with Denver Audubon, CoNPS, Audubon Rockies, and CSU Extension. This webinar is designed for all skill levels of native plant enthusiasts. Kate will review some of the science behind the essential need for native plants in our landscape, and some of the native plants that can be planted in the fall.

Kate Hogan has worked in the field of ecology for more than 20 years. She holds a BS in natural science and biology from the University of Puget Sound and an MS in nonprofit management from Regis University. For the last five years, Kate has worked at Denver Audubon as the community outreach coordinator, where she presents outreach programs throughout the Denver metro area and manages the Audubon Center at Chatfield.



◀ “Steve Olson”... continued from page 29

He grew up in the Chicago suburbs, too “clumsy and inept” for sports, by his own description. So he gravitated to nature. First the birds in his backyard. Then, while attending Southern Illinois University, the world of plants. It was a fragile and intricate world, he came to learn. There were layers. Understory and overstory. And everything was connected. For an early job out of college, Olson embarked into the cypress swamps of southern Illinois with a team. Someone noted the things that crept, crawled and buzzed. Someone noted the things that slithered and swam. Another noted the things that scampered, another the things that flew. And then there was Olson, who noted the things that grew, of which everything else was dependent upon.

He eventually had enough of the swamps and flatlands. “A change of scenery was necessary,” he says. And Colorado was that. The milkweed of the plains. The colorful cacti of the desert. Forests of ponderosa pine. Meadows of wildflowers, every color of the rainbow. Rugged cliffs and their surprising, persevering vegetation. The surprises found at every elevation range, from montane to subalpine to the extreme tundra, where flora somehow found a way.

Olson’s curiosity doesn’t require surprises. Here in an aspen grove on Pikes Peak, he observes things he’s come to expect. “All kinds of good stuff here,” he says, returning to his cross-legged position on the ground. Something grows below a rusted pipeline. “*Besseyia plantaginea*,” Olson says, referring to the flower commonly known as kittentails. Near it is one that goes by pussytoes, for its pad shape. Olson carefully peels back grass. “For people with sharper eyes,” he says, revealing a minuscule blossom that upon closer look appears to be a perfect, white diamond. “Rock jasmine.” He picks a furry-feeling strand and smells a scent that recalls some cleanse. He picks sage to smell that soothing scent, too. He moves onto a perennial, starry solomon’s seal. He admires the flower barely in bloom. “Nothing spectacular,” he says. “Just kind of nice.” 🌀

In Memorium: Stanley Smookler

January 23, 1929–March 28, 2020

Amateur Botanist, Friend, and Mentor

By Denise C. Wilson

I first met Stan in 2006, when I was looking for a local expert on the plants of Golden Gate Canyon State Park. The Chicago Botanic Garden had hired me to collect native, wild seed for the Kew Gardens Millennium Seed Bank. GGCSP had recommended I contact Stan, because he had established an herbarium starting in 1991. In it was more than 600 voucher specimens; and boy, he knew the plants of that park!

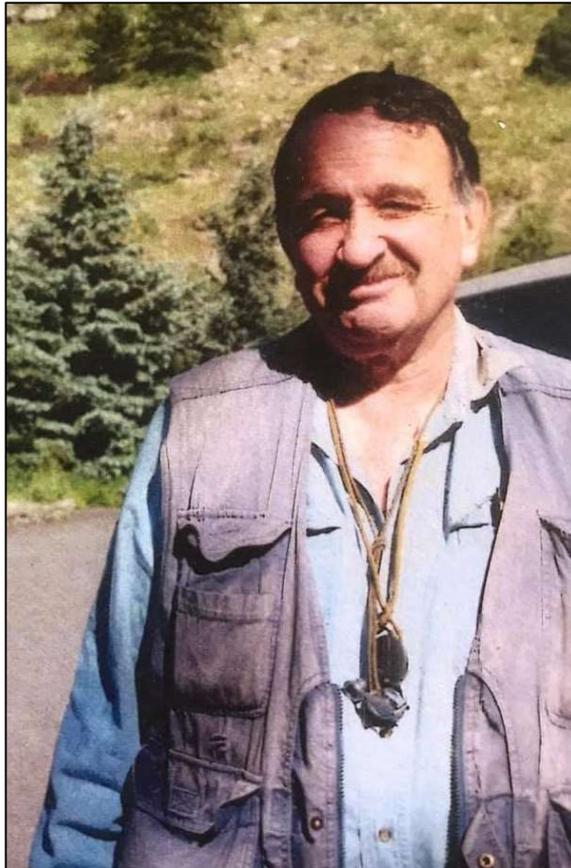
On one of our forays near a trailhead at the southern end of the park, Stan found a substantial population of early cinquefoil (*Potentilla concinna*). It's a relatively small plant, so we were on hands and knees with noses to the ground, when a hiker came up and asked, "Did you lose a contact lens?" Stan replied, "No, we're collecting seeds of *Potentilla concinna*!" The man replied, "Oh yeah, I thought that!"

Stan had been working with long-time companion Linda Senser and Steve Austin on the GGCSP identification and voucher project for some time. Still, he liked to tell me that the seed collection work was his first paid botany gig. I remember him calling out the species name as we found them. Sure enough, when we ran the plant through the key, he'd be right. When he wasn't, he would sulk like Walter Matthau in the movie "*Grumpy Old Men*." He thought he should be right all the time.

SEINet has 805 voucher collections by Stan and those do not include vouchers from the GGCSP herbarium, because it hasn't digitized them. Stan took it upon himself to make a list of local plants that the Denver Botanic Gardens Kathryn Kalmbach Herbarium did not yet have. He then worked with Linda Senser for fifteen years collecting those vouchers.

The GGCSP herbarium collection eventually topped 800 voucher specimens. Throughout the years, Stan and Linda maintained a species list with all the locations and descriptions.

Stan was a patient mentor, always sharing his knowledge. Together we eventually collected more than 100 seed accessions within GGCSP for the original seed bank, the Seeds of Success Program, and the Dixon National Tallgrass Prairie Seed Bank.



Stan Smookler at Golden Gate Canyon State Park. © Linda Senser

Panayoti Kelaidis once verified that Stan had found alpine aster (*Aster alpinus*), not in its typical location, which is the tundra, but at GGCSP! This plant is native to the mountains of Europe (including the Alps) with a subspecies in Canada and Alaska.

However, Stan's greatest find was in 1982. It was the Ute lady's tresses (*Spiranthes diluvialis*), which is now a US Fish & Wildlife threatened species. He alerted Dr. Bill Weber, who called in Charles Sheviak, a prominent native orchid specialist from New York. Charles subsequently described this species with the type locality from the area where Stan had found the plants.

Stan passed away March 28 in Boulder. He loved to encourage people to study plants and to share knowledge.

I will always be grateful and carry the memories of our fieldwork. I owe him a great debt, but Stan would never want to be paid back.

Denise is the CoNPS marketing and events coordinator, in addition to running Wilson Associates, Inc., a botanical contracting firm specializing in native seed collection for the National Park Service. She worked for Chicago Botanic Gardens for twelve years, contributing to three of their seed banks while taking seasonal positions in plant vegetation. Her botany master's degree was completed May 2009 from the University of Colorado, Denver, with a geographic information systems certificate. ☺

Member Profile: Steve Olson

Colorado's Go-to Botanist Steve Olson Sets Sights on Retirement By Seth Bolster

Editor's note: the following is reprinted with permission from the June 24, 2020, edition of The Gazette. Steve Olson is a long-time CoNPS member, chair of the field studies committee, and a member of the Aquilegia review board.



US Forest Service botanist Steve Olson looks at lichen he found on Pikes Peak on Tuesday, June 2, 2020. Olson is the lone botanist for the Pike and San Isabel national forests and Cimarron and Comanche grasslands. Photo by Christian Murdock, *The Gazette*.

Steve Olson stops along the Pikes Peak Highway and enters a spruce forest to see what he can find. "Let's see what this is," he says, crossing his long, skinny legs and folding downward to the ground. His arms are gangly, like branches, his fingers spindly, and they gently inspect this green patch. It's not an inch away from his glasses, which rest at the crook of his nose. He sits as if in communion, or like a kindergartner at story time. "A-ha," he softly remarks. It's Pikes Peak parsley. One of a kind, Olson explains, found only around this summit and the neighboring slopes of Almagre. It's something about this particular soil, Olson says, this crumbly granite. "It's been suspected in a few other places," he says, "but nothing definitive."

It would be easy to confuse. Pikes Peak parsley looks like some clump you might find in your backyard. That is if you're someone without the analytical eye of Olson. He's the US Forest Service botanist assigned to the 3 million acres defining the Pike and San Isabel national forests and Cimarron and Comanche grasslands. You can find Pikes Peak parsley—its scientific name is *Oreoxis humilis*—within a database of some 2,200 other hard-to-pronounce plant names that Olson has compiled. This has been a project of his for the nearly 20 years he's spent at his Forest Service post in Pueblo.

In the broader management of the PSICC, his duty is to protect rare life that grows from the earth. Permit renewals will reach his desk. A continued request for an overhead power line, for example. Or a new permit proposing construction, or logging, or mining, or gas and oil exploration. Olson will turn to his database to see what flora might be harmed.

Here on the side of the Pikes Peak Highway, it's easy to imagine a cement drainage — built as part of the mountain's long history of development and commercialism — consuming ground where Pikes Peak parsley might have once sprouted. True, Olson says. Pikes Peak parsley was probably impacted. But for as globally rare as it is, "within this place, it's clearly not hard to find," Olson says, "and it seems to be fairly happy." Here in early June, he's pleasantly surprised to see some yellow already bursting forth, not long after snow melted from these high elevations. "It's just getting started," he says.

Olson, however, is wrapping up. He's looking to retire in the coming months. He'll leave behind that database for his successor — assuming there will be one. It's hard to know for sure amid ongoing uncertainties with the Forest Service's budget, which has been increasingly consumed by wildfire management. For fiscal year 2021, the agency's proposed cuts were described as "an improvement over past years' recommendations" by the National Association of State Foresters, "but nearly all of those proposed cuts would be made to state and private forestry programs."

Olson has been the lone botanist assigned to the entire PSICC. He concedes those 2,200 plant entries hardly scratch the surface of the vast and varied beauty and mystery of his assigned "unit," covering the Kansas prairie, the canyonlands of southern Colorado, the famed rivers of Chaffee and Fremont counties, the foothills of America's Mountain and other 14,000-foot peaks spread across the Sangre de Cristo, Sawatch and Mosquito ranges. Olson's database "is one of those things that'll never be complete," he says. "Because there's always something new showing up."

He often roams like this, quick to curl himself down to the ground for investigation. He brings binoculars, because he's a bird aficionado. "But also it's a labor-saving device," he says. He might train the binoculars on a distant ridge and decide he need not go there.

Efficiency has been key to his job. A permit request comes, and "the ultimate goal is to look at every single site," Olson says. "But the reality is, for as big a place as the Pike and San Isabel and Cimarron and Comanche is, you have to find ways to do it more efficiently." Hence his database, which he can refer to from his desk. It was mostly built from his desk, using past research and other available online catalogs.

The project started as he had to learn about what he called "a whole new world." That was Colorado compared to the Midwest.

"Steve Olson"... continued on page 27 ►

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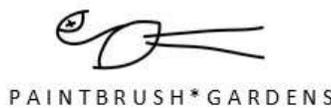
DUES include the electronic version of the *Aquilegia* newsletter, published quarterly.

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Can You ID these Flowers?



Answers: (clockwise, from upper left): golden saxifrage (*Saxifraga chrysantha*), tufted alpine saxifrage (*Saxifraga cespitosa*), spotted saxifrage (*Saxifraga bronchialis* var. *austroriparia*), splash saxifrage (*Saxifraga flagellaris* ssp. *crandallii*), alpine brook saxifrage (*Saxifraga rivularis*), nodding saxifrage (*Saxifraga cerna*); all in the Saxifragaceae family. © Anna Wilson

Colorado Native Plant Society



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**The Annual Photo Contest
and Silent Auction are online
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See page 10 for details.

44TH Annual CoNPS Conference “Peaks to Prairies



**Plants in the
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Developing Metapopulation Connectivity Criteria from Genetic and Habitat Data to Recover the Endangered Mexican Wolf

CARLOS CARROLL,* RICHARD J. FREDRICKSON,† AND ROBERT C. LACY‡

*Klamath Center for Conservation Research, P.O. Box 104, Orleans, CA 95556, U.S.A., email carlos@klamathconservation.org

†1310 Lower Lincoln Hills Drive, Missoula, MT 59812, U.S.A.

‡Chicago Zoological Society, Brookfield, IL 60513, U.S.A.

Abstract: Restoring connectivity between fragmented populations is an important tool for alleviating genetic threats to endangered species. Yet recovery plans typically lack quantitative criteria for ensuring such population connectivity. We demonstrate how models that integrate habitat, genetic, and demographic data can be used to develop connectivity criteria for the endangered Mexican wolf (*Canis lupus baileyi*), which is currently being restored to the wild from a captive population descended from 7 founders. We used population viability analysis that incorporated pedigree data to evaluate the relation between connectivity and persistence for a restored Mexican wolf metapopulation of 3 populations of equal size. Decreasing dispersal rates greatly increased extinction risk for small populations (<150–200), especially as dispersal rates dropped below 0.5 genetically effective migrants per generation. We compared observed migration rates in the Northern Rocky Mountains (NRM) wolf metapopulation to 2 habitat-based effective distance metrics, least-cost and resistance distance. We then used effective distance between potential primary core populations in a restored Mexican wolf metapopulation to evaluate potential dispersal rates. Although potential connectivity was lower in the Mexican wolf versus the NRM wolf metapopulation, a connectivity rate of >0.5 genetically effective migrants per generation may be achievable via natural dispersal under current landscape conditions. When sufficient data are available, these methods allow planners to move beyond general aspirational connectivity goals or rules of thumb to develop objective and measurable connectivity criteria that more effectively support species recovery. The shift from simple connectivity rules of thumb to species-specific analyses parallels the previous shift from general minimum-viable-population thresholds to detailed viability modeling in endangered species recovery planning.

Keywords: *Canis lupus baileyi*, circuit theory, conservation planning, Endangered Species Act, least-cost distance, metapopulations, population viability

Desarrollo de Criterios de Conectividad Metapoblacional a Partir de Datos Genéticos y de Hábitat para Recuperar al Lobo Mexicano en Peligro de Extinción

Resumen: Restaurar la conectividad entre poblaciones fragmentadas es una herramienta importante para aliviar las amenazas genéticas para las especies en peligro. A pesar de esto, los planes de recuperación típicamente carecen de criterios cuantitativos para asegurar la conectividad de dicha población. Demostramos cómo los modelos que integran los datos de hábitat, genéticos y demográficos pueden ser utilizados para desarrollar criterios de conectividad para el lobo mexicano (*Canis lupus baileyi*) que se encuentra en peligro y actualmente está siendo reintroducido a la vida silvestre a partir de poblaciones cautivas que descienden de 7 fundadores. Usamos el análisis de viabilidad poblacional, que incorporó datos del árbol genealógico, para evaluar la relación entre la conectividad y la persistencia para una metapoblación restaurada de lobo mexicano con 3 poblaciones de igual tamaño. La disminución de las tasas de dispersión aumentó el riesgo de extinción de poblaciones pequeñas (<150–200), especialmente cuando las tasas de dispersión bajaban más allá de 0.5 migrantes genéticamente efectivos por generación. Comparamos tasas de migración observadas en la metapoblación de lobos de las Montañas Rocallosas del Norte con 2 medidas efectivas de distancia

basadas en el hábitat, de menor costo y de distancia de resistencia. Después usamos la distancia efectiva entre dos poblaciones potenciales de núcleo primario en una metapoblación reintroducida de lobo mexicano para evaluar las tasas potenciales de dispersión. Aunque la conectividad potencial fue más baja en los lobos mexicanos frente a la metapoblación de lobos de las Rocallosas del Norte, una tasa de conectividad de >0.5 migrantes genéticamente efectivos por generación puede obtenerse por medio de dispersión natural bajo las actuales condiciones de paisaje. Cuando hay suficientes datos disponibles, estos métodos permiten a los planificadores moverse más allá de las metas de conectividad esperadas o de reglas generales para el desarrollo de criterios objetivos y medibles de conectividad que apoyen con mayor eficiencia la recuperación de la especie. El cambio de reglas generales de conectividad simple a análisis específicos de especies es similar al cambio previo de umbrales de mínimos generales de viabilidad de población a modelos detallados de viabilidad en la planificación de la recuperación de especies en peligro.

Palabras Clave: Acta de Especies en Peligro, *Canis lupus baileyi*, distancia de menor costo, metapoblaciones, planificación de conservación, teoría de circuitos, viabilidad poblacional

Introduction

Efforts to recover endangered species increasingly involve measures to ensure population connectivity between core habitat areas to enhance population persistence and maintain evolutionary potential (Lowe & Allendorf 2010). The U.S. Endangered Species Act (ESA) requires that recovery plans define “objective and measurable” recovery criteria that comprehensively address the threats that led to listing of the taxa as threatened or endangered (16 U.S.C. §1533 [f][1][B][ii]). However, recovery plans that mention connectivity typically include only aspirational objectives or general rules of thumb (USFWS 1987). Here, we used a case study of recovery planning for the endangered Mexican wolf (*Canis lupus baileyi*) to demonstrate why quantitative connectivity criteria can form an important element of recovery plans and how such criteria can be developed and implemented.

As descendants of the first wave of colonization of North America by the gray wolf (*Canis lupus*), Mexican wolves represent the most genetically unique New World wolf lineage and one of the most endangered mammals in North America (Vonholdt et al. 2011; Wayne & Hedrick 2011). One population of approximately 75 individuals currently exists in the wild, with approximately 300 additional individuals maintained in captivity (Siminski 2012). Genetic threats are greater for the Mexican wolf than for other wolf subspecies because 7 wild founder individuals were the source of all wolves in both the captive and reintroduced populations (Hedrick & Fredrickson 2008). Negative effects of inbreeding on litter size are evident in captive and wild populations of Mexican wolves (Fredrickson et al. 2007). In other small and isolated wolf populations in Europe and North America, inbreeding accumulation has reduced litter size and increased incidence of skeletal defects (Liberg et al. 2005; Rääkkönen et al. 2009). Dispersal of even a single migrant into such inbred populations can dramatically affect genetic structure and population performance (Vilá et al. 2003).

Wolves are among the most vagile of all terrestrial mammals and can disperse over 800 km (Forbes & Boyd 1997). Wolves were historically present throughout their

range in the contiguous 48 states as a largely continuous population with some degree of genetic isolation by distance and additional heterogeneity reflecting ecological factors (Vonholdt et al. 2011). Due to habitat loss, over-exploitation, and other factors, future wolf distribution in the United States outside of Alaska is likely to consist of many relatively disjunct subpopulations, and these subpopulations will be small relative to historic population sizes ($>300,000$; Leonard et al. 2005). However, given the species' vagility, achieving connectivity via natural dispersal may be feasible within such a metapopulation. Rigorous assessment of the influence of connectivity as well as population size on viability is thus a necessary component in wolf recovery planning (Wayne & Hedrick 2011).

We demonstrate how results from population viability analyses can be combined with habitat data to develop quantitative recovery criteria for population connectivity. We used population viability analysis (PVA) that incorporated pedigree data to address the relation between connectivity and persistence for the species. Pedigree data for the existing wild population and for new populations founded by hypothetical captive pairings designed to minimize relatedness allowed us to realistically assess the effects of genetic management on restoration success. We then used habitat-based effective-distance metrics to determine the level of natural dispersal feasible given expected management and landscape characteristics. These models also allow identification of specific linkage areas in which connectivity conservation efforts can be focused. When sufficient data are available, these methods allow planners to move beyond general aspirational connectivity goals or rules of thumb to develop objective and measurable connectivity criteria that more effectively support species recovery.

Methods

Context of Case Study

We used information from previously published studies to determine what areas within the southwestern United States and northern Mexico contained sufficient habitat

to support populations of Mexican wolves. The majority of the subspecies' historic range occurred in Mexico (Leonard et al. 2005). However, high human-associated mortality risk and low prey density within potential core areas in Mexico suggests that these areas are unlikely to support populations of over 100 individuals (Araiza et al. 2006). Therefore, we also considered potential reintroduction areas in the southwestern United States that were outside the historic range of the Mexican wolf but within the historic zone of genetic intergradation between Mexican wolves and more northerly wolf populations (Leonard et al. 2005). Projections of increasing aridity in the southwestern United States due to climate change (Notaro et al. 2012) suggest that establishment of populations at or beyond the northern extreme of the historic range may be an appropriate strategy to increase metapopulation resilience.

We used a 2-stage process to evaluate potential recovery criteria for the Mexican wolf. Stage 1 consisted of a PVA in which population performance across a range of scenarios was compared with alternative population size and connectivity criteria. In stage 2, we used effective-distance metrics derived from habitat data to evaluate what rates of dispersal could be expected between the reintroduced populations. By combining information from these 2 stages, we were able to evaluate what combination of population size and connectivity criteria allowed recovery of a metapopulation of Mexican wolves given current habitat conditions.

PVA is a structured method of integrating information on diverse threats to a population's persistence. Due to the magnitude of genetic threats to the Mexican wolf, we used an individual-based population simulation model (Vortex) (Lacy 2000; Lacy & Pollak 2012) that allows exploration of how genetic threat factors vary with population size and metapopulation structure. We combined the Vortex results with data from a previously published model (Carroll et al. 2006) that evaluated the distribution of potential wolf habitat in the southwestern United States.

Carroll et al. (2006) used a spatially explicit population model that allowed detailed treatment of spatial population dynamics and habitat configuration but lacked consideration of genetic issues. Their results suggest that the southwestern United States has 3 core areas with long-term capacity to support populations of several hundred wolves each. These 3 areas, each of which contains a core area of public lands subject to conservation mandates, are in eastern Arizona and western New Mexico (i.e., Blue Range, the location of the current wild population), northern Arizona and southern Utah (Grand Canyon), and northern New Mexico and southern Colorado (Southern Rockies) (Carroll et al. 2006). Based on the number and location of potential core areas, we structured our analysis to evaluate performance of a metapopulation of 3 populations and varied population

size and connectivity across a range of plausible recovery criteria.

Vortex Simulations of Population Viability

The Vortex model simulates the effects of both deterministic forces and demographic, environmental, and genetic stochastic events on wildlife populations (Lacy 2000; Lacy & Pollak 2012). Vortex simulates a population by stepping through a series of events that describe an annual cycle of a sexually reproducing, diploid organism. Vortex tracks the sex, age, and parentage of each individual in the population as demographic events are simulated. Vortex allows the user to specify the pedigree of the starting population and uses the genetic relationships among founders to derive inbreeding coefficients and other genetic metrics in subsequent simulated generations. Vortex allows tracking of both demographic metrics (population size, time to extinction) and genetic metrics (heterozygosity, allelic diversity, and inbreeding coefficient).

We adapted the Vortex model structure to make it appropriate for analysis of connectivity effects for a species with a complex social breeding system. We incorporated into the model the persistent monopolization of breeding opportunities by male and female alpha individuals. Once an individual achieves alpha status it will generally retain that status until death. This aspect of the wolf social system reduces genetic effective population size (N_e) and thus may enhance inbreeding effects. We also modified Vortex to track the observed number of genetically effective migrants per generation (here termed *migrant* and defined as the total number of individuals from all other populations that produces at least one offspring in the recipient population). These results were used to assess the effects of dispersal on population persistence and inform development of a recovery criterion for population connectivity. Alternative recovery criteria for population size were evaluated by creating a numeric threshold above which a percentage (10–16%) (Table 1) of any surplus individuals were removed annually. Further details, metadata, and sample input files documenting model structure are provided in Supporting Information.

We parameterized Vortex with available information from the wild Mexican wolf population (Fredrickson et al. 2007), the Northern Rocky Mountains (NRM) metapopulation (Smith et al. 2010), and other wolf populations (Supporting Information). We did not base model parameterization solely on data from the existing wild Mexican wolf population for 2 reasons. First, we analyzed potential persistence of populations reintroduced to new areas whose demographic rates may not match those of any extant population. Second, the existing wild population remains heavily manipulated via management removals and re-releases. Human-caused wolf mortalities in the existing wild population constituted 81% of the

Table 1. Results of sensitivity analysis of Vortex population model assessed using standardized coefficients from logistic regression of parameter sets against probability of extinction and quasi extinction.

Parameter	Minimum	Maximum	<i>z</i> value for probability of		
			extinction	quasi-ex.-150	quasi-ex.-250 ^a
Adult mortality ^b	18.32	27.48	167.46	162.48	111.15
Percentage of females in breeding pool	40	60	-160.67	-156.80	-104.49
Population size threshold	50	350	-158.63	-136.53	-72.03
Strength of inbreeding depression ^c	6.586	9.789	152.81	141.54	92.90
Density dependent reproduction	categorical		-92.42	-54.95	-8.35
Effective migrants per generation	0.0	2.4	-88.13	-56.17	-35.49
Average number of years between disease events	4	6	76.54	81.23	41.31
Pup mortality ^b	19.52	29.28	75.37	60.22	43.56
Variation between existing and new populations ^d	categorical		-34.12	-32.62	-24.79
Carrying capacity buffer ^e	1.07	1.60	-5.44	-51.50	-52.47
Harvest efficiency ^f	6.4	9.6	-3.86	-2.44	-12.65

^aQuasi-extinction occurs when the 8-year running mean population size falls below 150 or 250. All regressions are based on 1000 scenarios derived from randomized parameter sets, with 100 replicate runs per scenario. Standardized regression coefficients (*z* values), created by dividing a regression coefficient by its standard error, are unitless values whose magnitude indicates the relative importance of a parameter in the model.

^bFrom Smith et al. (2010) for Greater Yellowstone Area wolf population.

^cSlope parameter in equation of Fredrickson et al. (2007) relating litter size to inbreeding coefficient.

^dVariation in population performance arising from contrasts between populations in initial pedigree.

^eRatio of ecological carrying capacity to the population size threshold parameter.

^fReciprocal of proportion of the population above the population-size threshold that is removed annually.

mortalities with known causes from 1998 to 2011, primarily due to illegal shooting (43%), vehicle collisions (14%), and lethal management removals (12%) (Turnbull et al. 2013). However, since 2009, when revised management protocols restricted management removals, the wild population has shown positive demographic trends, growing from 42 to 75 individuals (USFWS 2012). Demographic rates in the wild population, particularly survival rate, thus remain highly contingent on management policy regarding removals. Our goal here was not to review the current status of the existing wild Mexican wolf population, but to assess what conditions would allow recovery of the subspecies as a whole.

Analysis of the potential effects of stochastic factors on viability requires the assumption that demographic rates alone will not cause deterministic population decline. However, demographic data collected over the last decade for the wild Mexican wolf population imply an intrinsic population growth rate of <1 (USFWS 2012). We therefore used mortality rates from the wolf population in the Greater Yellowstone Ecosystem (GYE) because mortality rates there (24.4% and 22.9% for pups and nonpups [yearlings and adults], respectively [Smith et al. 2010]) are intermediate among the 3 NRM core populations and represent a plausible goal for mortality rates after recovery actions are implemented but before delisting (Smith et al. 2010). Our baseline demographic parameter set resulted in a deterministic lambda of 1.23, which is similar to that used in previous Mexican wolf PVAs (Seal 1990; IUCN 1996). We evaluated the effect of alternate assumptions concerning mortality rates as part of the sensitivity analysis described below.

All simulated populations were started with wolves produced from the existing Mexican wolf pedigree (Siminski 2012). Founders of the existing wild (Blue Range) population were based on the known 2013 composition of the population projected forward 9 years to a starting population of 122 wolves (Supporting Information). The 2 other simulated populations were founded by assuming 2 pairs would be released each year from 2018 through 2022 into each population. We selected individuals for release from a hypothetical new generation of captive-born wolves that were minimally related and collectively represented genetic variation in the existing captive and wild populations. Released individuals produced offspring and experienced mortality after release, and surviving founders and offspring formed new pairs such that at the start of 2022 each of the 2 new populations contained 50 wolves and 10 pairs (Supporting Information).

Sensitivity Analysis

Although wolves are among the best studied of large mammals, substantial uncertainty exists on how to appropriately parameterize demographic models. We performed a global sensitivity analysis by generating 1000 sets of parameters in which values for 9 key parameters were drawn from a random uniform distribution with a range equal to $\pm 20\%$ of the mean value ("relative sensitivity analysis" [Cross & Beissinger 2001]) from their best estimates (Table 1). We also varied target population size and connectivity rates across a uniform distribution spanning a range of recovery criteria values (Table 1).

Each of the 1000 parameter sets was evaluated based on 100 replicate simulations of 100 years each.

We used a relative sensitivity analysis because several parameters were either aspects of model structure for which empirical distributions do not exist (carrying-capacity buffer [i.e., the proportion by which ecological carrying capacity exceeds the population size parameter] and harvest efficiency [i.e., proportion of the population above the population size parameter that is removed in a particular year]) or would be difficult to derive from the literature (Seal 1990; IUCN 1996) (see Supporting Information for references for demographic parameters in Table 1).

We used standardized coefficients from logistic regression of parameters against extinction and quasi-extinction outcomes to rank the effect of parameters on outcomes (Cross & Beissinger 2001). Dividing a regression coefficient by its standard error results in a standardized regression coefficient or z value, which expresses the unique contribution of that parameter scaled by the variability of the parameter (Cross & Beissinger 2001). The resulting z values (Table 1) are unitless and interpretable only in comparison with other z values in the same model. Significance tests and associated P values would be uninformative because the large number of scenarios considered (1000) arbitrarily inflates sample size.

Following the global sensitivity analysis, we generated 1000 scenarios of parameters in which population size and connectivity rates were again drawn from a random uniform distribution but other parameters were fixed at their mean values (Table 1). We used locally weighted regression (loess) (Cleveland & Devlin 1988) to evaluate in more detail the relation of extinction and quasi extinction to population size and connectivity rate.

Endangered and Threatened Status under the ESA

The ESA defines an endangered species as “at risk of extinction throughout all or a significant portion of its range” (16 U.S.C. §1532[3.6]) and a threatened species as “likely to become endangered in the foreseeable future” (16 U.S.C. §1532[20]). The statute does not provide a quantitative definition of *at risk of extinction*. Recovery plans typically include risk thresholds of 1% to 10% over periods ranging from several decades to a century. There is less agreement over interpretation of the statute’s definition of threatened status. Angliss et al. (2002) proposed that, to be consistent with the statute, criteria for threatened status should be defined by reference to the criteria for endangered status rather than directly in terms of extinction risk. This approach was subsequently incorporated into recovery plans for species such as the fin whale (*Balaenoptera physalus*), which will be removed from the list of threatened species when it “has less than a 10% probability of becoming endangered (has more than a 1% chance of extinction in 100 years) in 20 years”

(NMFS 2010). We used a time frame for the foreseeable future of 100 rather than 20 years because we analyzed genetic threats that require decades to accumulate to deleterious levels.

Incorporating Multiple Persistence Thresholds

To illustrate how tiered thresholds for endangered and threatened status might be informed by quasi-extinction metrics, we selected a population threshold (150 individuals) that corresponded to adequately low extinction risk (<10%) in exploratory analyses with baseline demographic rates. We then measured the proportion of simulations with a population size criteria of >150 in which, after the initial 30 years of population establishment, the 8-year (2 generation) running mean of population size drops below 150. As with extinction probability, the metrics report the mean quasi-extinction probability across the 3 populations.

Populations of most species continue to increase under state-level management after recovery and removal (delisting) from the federal list of threatened species. However, because wolves can negatively affect other resources (livestock, wild ungulates), state agencies may seek to manage delisted wolf populations at the lowest level consistent with maintaining recovered status. Due to genetic and other issues, long-term management of populations to a harvest-imposed ceiling may result in deterioration in vital rates (Mills 2012). Population thresholds implemented by the states after federal delisting are analogously related to threatened status in that they must ensure an adequately low probability of becoming threatened in the foreseeable future. This risk can be measured by a second quasi-extinction metric based on the probability of population size dropping below the threshold dividing endangered and threatened status (which was developed as described above). Under the ESA’s framework, the thresholds that distinguish extinct, endangered, threatened, and recovered species are thus interrelated and can be quantitatively assessed with a unified set of PVA-based metrics.

Feasibility of Alternative Connectivity Criteria

We assessed what rate of natural dispersal between potential core populations could be achieved given the distribution of habitat. We projected connectivity rates between primary core populations in the Mexican wolf metapopulation by relating observed connectivity rates in the NRM metapopulation (Vonholdt et al. 2010) to habitat-based effective distance between populations in both the NRM and the southwestern United States. Because published data on effective migration rate in the NRM are insufficient to build a predictive model, this extrapolation is necessarily qualitative, but nonetheless informative in this planning context. We also compared

habitat-based distances between the Mexican wolf and NRM metapopulation with the distances within those metapopulations to evaluate potential dispersal rates between the 2 metapopulations.

We compared results from 2 contrasting effective-distance metrics based on least-cost (shortest-path) distance and resistance (current flow) distance, respectively (Carroll et al. 2012) in order to assess the robustness of conclusions to choice of connectivity metric. Both least-cost distance and resistance distance have been correlated with gene flow in several species (McRae et al. 2008). Habitat suitability index values from a previously published study (Carroll et al. 2006) were assumed to be proportional to movement cost and conductance (see supplementary material S2 for description of habitat model). Least-cost distance, calculated using the Linkage Mapper software (McRae & Kavanagh 2011), represents cost of movement as distance, and identifies the single optimal path between two predetermined endpoints that has the shortest total distance (least total cost). In contrast to least-cost distance, resistance (current flow) distance integrates the contributions of all possible pathways across a landscape or network. We used Circuitscape software to calculate a resistance distance statistic that summarizes overall connectivity between each pair of core areas (McRae et al. 2008). Additionally, Circuitscape produced maps of current flow that can help planners direct conservation measures toward areas important for connectivity.

Results

Effects of Population Size and Connectivity on Extinction and Endangerment

Population size and dispersal rate interacted to influence probability of extinction and quasi extinction (Table 1, Figs. 1 & 2). Dispersal rate strongly affected extinction probability at population criteria below 200 but decreased in importance at larger population sizes (Fig. 1a). Dispersal rates of <0.5 migrants greatly increased extinction risk (Fig. 1b). Extinction risk continued to decrease at rates between 0.5 and 1 migrants for populations of <150, but there was less effect of increased dispersal on persistence for larger population sizes (Fig. 1b).

Dispersal rate had less effect on probability of endangerment (defined here by a quasi-extinction threshold of 150) than on probability of extinction (Table 1). Higher dispersal rates reduced the probability of endangerment in 2 ways. First, and most importantly, higher dispersal rates reduced the population size threshold corresponding to an extinction probability that was adequately low to merit downlisting (Fig. 1a). Second, higher dispersal rates reduced the probability of a downlisted population again dropping below that threshold and becoming en-

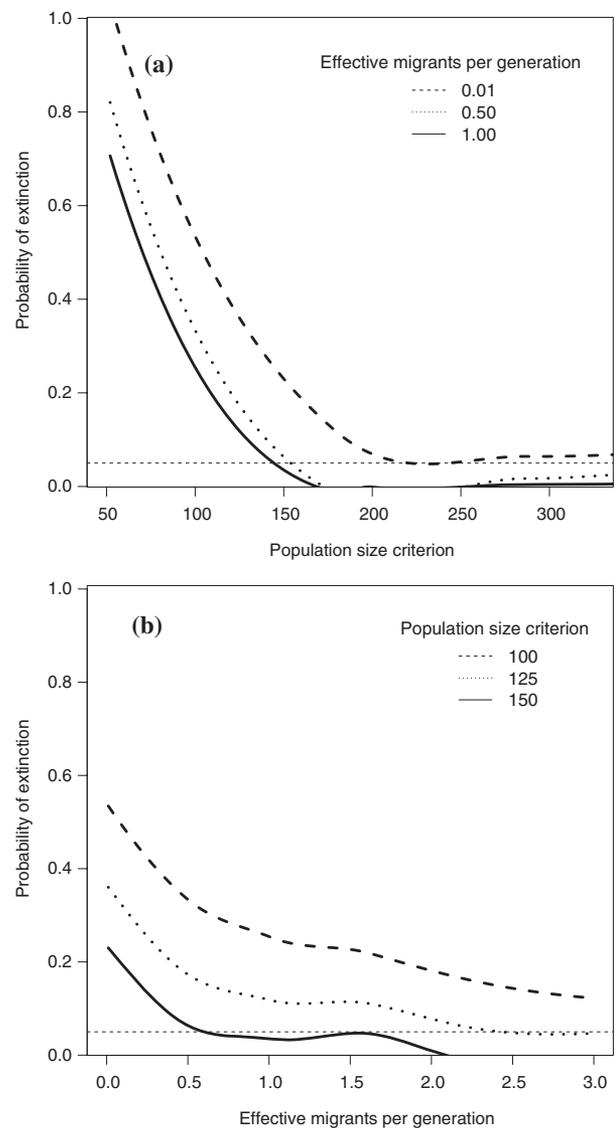


Figure 1. Relation of probability of extinction of Mexican wolf populations to (a) population size criterion and (b) dispersal rate (effective migrants per generation) on the basis of Vortex population simulations of a metapopulation of 3 subpopulations of the specified size. Sensitivity analysis is based on 1000 scenarios derived from randomized combinations of population size and dispersal rate, with 100 replicate runs per scenario. Continuous parameters are set at their mean value and results from categorical variables are averaged. Horizontal dotted line identifies a 5% population extinction-risk threshold commonly used in recovery plans.

dangered in the future (Fig. 2). Connectivity had less influence on persistence at the 250 quasi-extinction threshold (Table 1). Simulation results suggested that a buffer for each population of 50–100 individuals above the delisting threshold was needed to adequately reduce the risk that

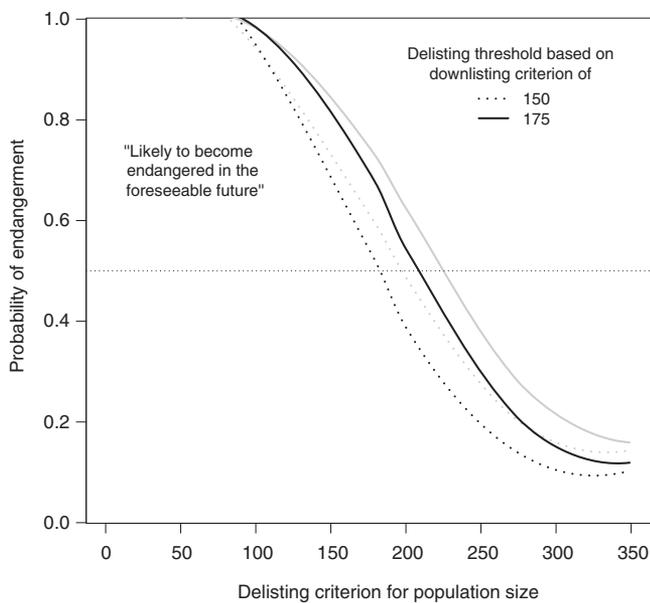


Figure 2. Relation of probability of endangerment (i.e., quasi extinction) to population-size criterion, as derived from the logistic regression of simulation results in the sensitivity analysis (black lines, results given dispersal of 1 effective migrant/generation; grey lines, dispersal of 0.5 effective migrants/ generation). Probability of endangerment is based on the proportion of simulations in which the 8-year running mean of population size drops below a threshold based on analysis of extinction risk (Fig. 1) at any time after year 30 in the simulation. A population is classified as threatened when probability of endangerment exceeds a threshold (e.g., 50%, horizontal dotted line).

delisted populations would fall below that threshold in the foreseeable future.

Effects of Demographic Parameters on Persistence and Relisting

Results of the sensitivity analysis suggested that the most important parameters (absolute value of standardized coefficient > 100) were adult mortality, proportion of females in the breeding pool, and strength of inbreeding effects (Table 1). Parameters of intermediate importance (absolute value of standardized coefficient 70–100) were density-dependent reproduction, frequency of disease outbreaks, and pup mortality. Between-population variation was of lower importance. Carrying-capacity buffer and harvest efficiency were the least important parameters. Logistic regression of randomized parameter sets on probability of quasi extinction at either the 150 or 250 population thresholds yielded similar results, except that the effect of the carrying-capacity buffer increased and that of density-dependent reproduction de-

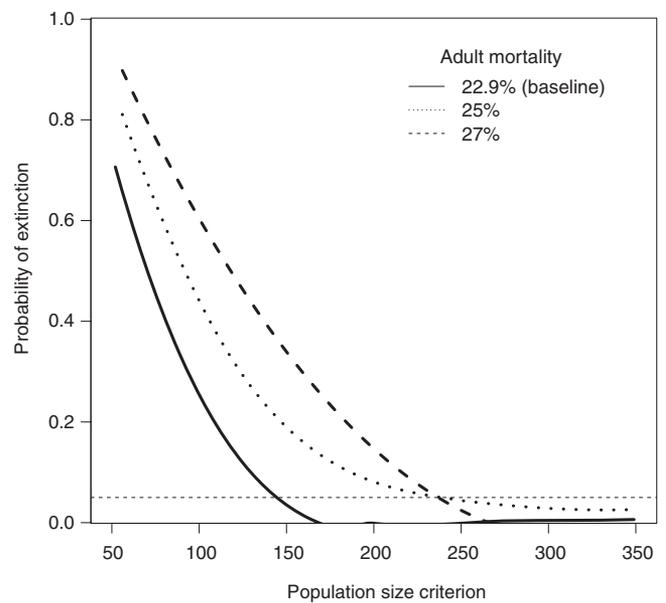


Figure 3. Relation of probability of extinction to population-size criterion under differing levels of adult mortality, as derived from the logistic regression of simulation results in the sensitivity analysis. Dispersal is assumed to be 1 effective migrant/generation. Horizontal dotted line identifies the 5% population extinction-risk threshold.

creased at these larger population thresholds (Table 1). The population-size criterion had as large an effect as the most influential demographic parameters on extinction and on quasi extinction at the 150 threshold but had lower effect at the 250 quasi-extinction threshold. The dispersal-rate criterion was of intermediate importance (Table 1). Conclusions regarding what population-size and connectivity criteria corresponded to a specific extinction risk were contingent on demographic parameters such as adult mortality, which had large z values in the sensitivity analysis (Fig. 3).

Determining Inappropriate Connectivity Criterion

Linkages between primary core populations were ranked similarly (Pearson correlation = 0.85, Spearman rank correlation = 0.72, $n = 9$) under both least-cost distance and resistance distance metrics (Supporting Information). For those linkages ranked more favorably based on resistance distance than based on least-cost distance, multiple linkages may allow more dispersal between those areas than expected based on their single shortest connection (Supporting Information). Projected connectivity between the Blue Range and both the Grand Canyon and Southern Rockies primary core populations was less than that of the 2 best NRM linkages (Supporting Information) but greater than that between the Grand Canyon and

Southern Rockies populations or between Yellowstone and northwestern Montana. When considered in the context of observed NRM migration rates (Hebblewhite et al. 2010; Vonholdt et al. 2010), this comparison suggests that it may be more difficult to achieve a connectivity criterion of 1 migrant/generation for the Mexican wolf in the southwest than for wolves in the NRM.

Because both distance metrics suggest that few direct migrants would be expected between the Grand Canyon and Southern Rockies, we structured the Vortex PVA to assume dispersal would occur along a chain of 3 populations rather than directly between all pairs of populations. This metapopulation structure provides the most dispersal to the centrally located Blue Range population, which otherwise would perform poorly relative to new populations derived from less-related individuals.

Least-cost and resistance distances between the Mexican wolf and NRM metapopulation were greater than any distances within those metapopulations. Mean intermetapopulation resistance distance was 1.23 and 1.34 that of intrametapopulation resistance distance for the NRM and Mexican wolf metapopulations, respectively. Mean intermetapopulation least-cost distance was 2.59 and 1.81 that of intrametapopulation resistance distance for the NRM and Mexican wolf metapopulations, respectively. Current maps suggest that a potential core area in northern Utah could serve as a key stepping stone to enhance connectivity between metapopulations (Fig. 4).

Discussion

Recovery plans for endangered species frequently include either aspirational objectives for maintaining connectivity or general rules of thumb rather than specific quantitative criteria (USFWS 1987). Results from our analysis demonstrate that, where sufficient data exists, quantitative connectivity criteria based on species-specific demographic and habitat data can form an objective and measurable component of recovery plans. Use of pedigree data for the existing wild population, as well as new populations founded by hypothetical captive pairings, allowed us to realistically incorporate genetic effects on restoration success. Results from recent advances in measurement of genetically effective migration rates (Vonholdt et al. 2010) were then integrated with habitat connectivity modeling to predict migration rates and target recovery actions at specific habitat linkages. The shift from simple connectivity rules of thumb to species-specific analyses parallels the previous shift from simple rules of thumb for minimum viable population size to detailed PVA modeling in endangered species recovery planning.

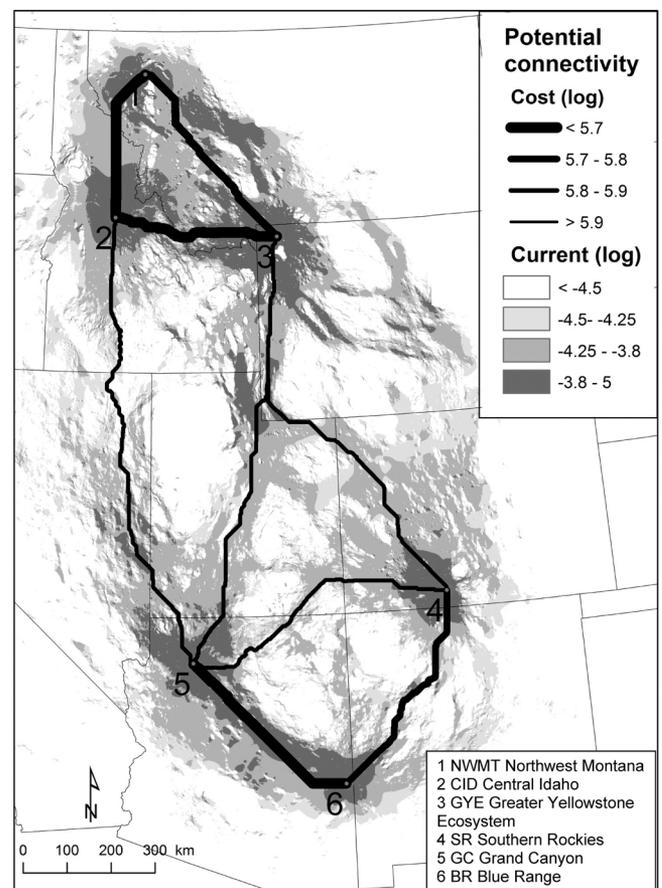


Figure 4. Potential habitat linkages between 6 existing or potential wolf-population core areas in the western United States (thickest lines, linkages with lowest least-cost distance; darkest gray shading, areas with highest importance for connectivity based on the resistance distance model; abbreviations for core areas correspond to labels in Appendix S3 in Supporting Information).

Importance of Connectivity Criteria

Population size had among the strongest influence on population persistence of any parameter evaluated in the sensitivity analysis (Table 1). Connectivity ranked among the moderately important parameters, suggesting that it also merits attention in recovery planning. The importance of connectivity suggested by our PVA results may be most relevant to other species that have been extirpated in the wild and subsequently recovered from a limited number of captive founders or to formerly widespread species that are now limited to small isolated populations. To avoid the genetic damage that may occur during demographic downturns associated with episodic events (e.g., drought, disease), a population derived from inbred and interrelated founders generally must have a larger census population size than a population derived from outbred and unrelated individuals (Allendorf et al.

2012). Similarly, a single effective migrant is more likely to increase persistence of inbred populations (Vilá et al. 2003).

Comparing general rules of thumb on adequate rates of connectivity with results from species-specific simulations can give context to PVA results. The most commonly proposed rule of thumb for connectivity states that one genetically effective migrant per generation into a population is sufficient to minimize the loss of polymorphism and heterozygosity within populations (Allendorf 1983). Our simulation results support use of this rule of thumb because population persistence declined more rapidly at rates below one migrant for smaller populations (<150) (Fig. 1b). Our results also suggest that ensuring lower but nonzero rates of connectivity (e.g., >0.5 migrants) remains important in cases where one migrant may not be achievable. The contrast between our results and previous reviews concluding that a rate of one migrant may be less than optimal for wild populations may be because in our model inbreeding affected persistence solely via effects on litter size, whereas previous reviews considered a broader suite of potential inbreeding effects (Mills & Allendorf 1996). Additionally, we did not consider what population and connectivity criteria would ensure maintenance of adaptive potential through a long-term balance between loss of alleles via genetic drift and new alleles produced by mutation (Franklin & Frankham 1998).

Although wolves are a relatively well-studied species, our simulations necessarily involved substantial uncertainty in both model parameters and structure (e.g., density dependence). Criteria such as population size and connectivity that primarily address stochastic factors remain important even when (as here) effects of deterministic factors and parameter uncertainty are large (Fig. 3). Our baseline parameters were based on the assumption that recovery actions would be effective in reducing the Blue Range population's currently high mortality rates. Alternate mortality-rate parameters would result in different population size and connectivity rates being required to achieve adequate population persistence (Fig. 3). Because metapopulations with adequate connectivity can better withstand less favorable demographic rates, inclusion of a connectivity criterion is precautionary and reduces uncertainty about the future status of a species.

In addition to evaluating extinction probability, we considered 2 quasi-extinction metrics related to probability of relisting as either endangered or threatened. The 2 metrics offered complementary insights regarding the resilience conferred by alternate recovery criteria. An exclusive focus on minimizing extinction might lead to criteria that result in a species persisting in a permanent state of endangerment, which is inconsistent with the intent of the ESA to recover self-sustaining populations (16 U.S.C. §1531[2][b], §1532 [3][3]). Use of multiple persistence metrics focuses attention on the often-ignored genetic

and other challenges inherent in managing wildlife populations to a fixed population ceiling (Mills 2012).

Mapping and Managing Population Connectivity

Previous recovery plans for wolves and other large carnivores such as grizzly bears (*Ursus arctos*) noted the importance of metapopulation connectivity but did not develop objective and measurable connectivity criteria (USFWS 1982, 1987). This may have been because at the time such plans were developed, there was less recognition of the synergistic effects of dispersal on genetic diversity and demographic performance of small populations. Due to recent advances in genetic assignment tests and other techniques that allow identification of genetically effective migrants, connectivity is increasingly measurable in wild populations (Vonholdt et al. 2010). When coupled with habitat-based connectivity models, these methods allow development of quantitative connectivity criteria and their incorporation into monitoring programs. Given evidence from other species for utility of effective-distance metrics in predicting gene flow (McRae et al. 2008), they are appropriate tools for informing wolf-recovery planning and demonstrate the utility of applying such methods to data gathered in future monitoring of reintroduced populations. Our results suggest that habitat-based metrics such as least-cost and resistance distance are useful for assessing expected migration rates, but that multiple metrics should be compared to provide a more-informative ranking of alternate linkages.

Differing levels of population connectivity imply qualitatively different genetic effects on populations. We focused primarily on recovery criteria relevant to inbreeding connectivity (Lowe & Allendorf 2010). In this context, our results suggest that viability of the existing wild population is uncertain unless additional populations can be created and linked by dispersal of >0.5 migrants/generation (Fig. 1). In contrast, adaptive connectivity (sensu Lowe & Allendorf 2010) requires only low levels of dispersal (>0.1 migrants) to spread advantageous alleles between populations. Although effective distance metrics suggest that dispersal between the NRM and Mexican wolf metapopulations may be low (<<0.5 migrants), this may be sufficient for maintenance of adaptive connectivity, with occasional dispersal maintaining a regional cline in genetic structure similar to historic conditions (Leonard et al. 2005). Recovery plans for formerly widely distributed species should consider how such broad-scale genetic structure can be restored via conservation of interregional linkages and stepping-stone habitat (Franklin & Frankham 1998).

An primary goal of the ESA in seeking to protect threatened and endangered species—as well as the ecosystems on which these species depend—is to recover these species to the point at which they are self-sustaining in

their natural habitat (U.S.C. §1531[2(b)]). Preservation of habitat connectivity and necessary levels of natural dispersal is analogous to preservation of the habitat that permits persistence of a wild population of any species. Absent a clear physical barrier to natural genetic exchange (such as a large urban area), achieving connectivity for highly vagile species such as the wolf via natural dispersal rather than artificial translocation is consistent with the intent of the ESA. Integrating PVA and connectivity models as we have done here allows planners to develop such criteria with species-specific PVA and to identify location-specific management actions necessary to meet these criteria and achieve recovery of self-sustaining populations.

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Supporting Information

A description of Vortex model structure and parameters (Appendix S1), description of habitat inputs and methods used in analysis of potential dispersal rates between populations (Appendix S2), plot of resistance distance versus least-cost distance between existing or potential wolf population core areas in the western United States (Appendix S3), and Vortex and connectivity analysis input files showing details of model structure used in simulations (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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Genetic rescue and inbreeding depression in Mexican wolves

Richard J. Fredrickson^{1,*}, Peter Siminski², Melissa Woolf³
and Philip W. Hedrick^{1,*}

¹*School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA*

²*The Living Desert, Palm Desert, CA 92260-6156, USA*

³*Turner Endangered Species Fund, Ladder Ranch, Caballo, NM 87931, USA*

Although inbreeding can reduce individual fitness and contribute to population extinction, gene flow between inbred but unrelated populations may overcome these effects. Among extant Mexican wolves (*Canis lupus baileyi*), inbreeding had reduced genetic diversity and potentially lowered fitness, and as a result, three unrelated captive wolf lineages were merged beginning in 1995. We examined the effect of inbreeding and the merging of the founding lineages on three fitness traits in the captive population and on litter size in the reintroduced population. We found little evidence of inbreeding depression among captive wolves of the founding lineages, but large fitness increases, genetic rescue, for all traits examined among F_1 offspring of the founding lineages. In addition, we observed strong inbreeding depression among wolves descended from F_1 wolves. These results suggest a high load of deleterious alleles in the McBride lineage, the largest of the founding lineages. In the wild, reintroduced population, there were large fitness differences between McBride wolves and wolves with ancestry from two or more lineages, again indicating a genetic rescue. The low litter and pack sizes observed in the wild population are consistent with this genetic load, but it appears that there is still potential to establish vigorous wild populations.

Keywords: conservation genetics; genetic rescue; inbreeding; inbreeding depression; wolves

1. INTRODUCTION

Inbreeding reduces the fitness of wild (Keller & Waller 2002), captive (Ralls *et al.* 1988) and experimental populations (Lacy *et al.* 1996), and increases the risk of population extinction (Newman & Pilson 1997; Saccheri *et al.* 1998). Inbred populations may have fitness restored by immigration of unrelated individuals (Wang *et al.* 1999; Whitlock *et al.* 2000), a phenomenon termed ‘genetic rescue’ (Tallmon *et al.* 2004). Support for genetic rescue comes from experiments in which fitness was increased following translocation of outbred individuals into small, declining wild populations with low fitness (Westemeier *et al.* 1998; Madsen *et al.* 1999, 2004; Hogg *et al.* 2006). Populations with a history of small size may have a high fixed, or nearly fixed, load of deleterious alleles, and the detrimental effect of additional inbreeding may be limited (Hedrick 1994; Hedrick & Kalinowski 2000). Small populations isolated from one another, however, are expected to become fixed for deleterious alleles at different loci. In this case, crosses between inbred populations may produce offspring with increased fitness, resulting in genetic rescue. Whereas the effects of inbreeding in small populations may be a cause for concern among conservation managers, the prospect of fitness restoration and reduced extinction risk resulting from renewed gene flow may offer new conservation opportunities.

* Authors for correspondence (richard.fredrickson@asu.edu and philip.hedrick@asu.edu).

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Mexican wolf (*Canis lupus baileyi*), an endangered subspecies of grey wolf, is the most genetically distinct subspecies in North America (Leonard *et al.* 2005). Human activities throughout its range reduced and isolated Mexican wolf populations such that by 1925 they were rare in the United States (Brown 1983), and by the 1950s their range and numbers in Mexico were greatly reduced (Leopold 1959). By 1980, fewer than 50 wild Mexican wolves were thought to remain in isolated groups spread across four Mexican states (McBride 1980). Surveys in Mexico since then have failed to detect Mexican wolves.

All Mexican wolves alive today originated from three captive lineages founded between 1961 and 1980 by a total of seven wolves (Hedrick *et al.* 1997). These lineages were managed independently until 1995 when the Aragón and Ghost Ranch lineages were merged into the McBride lineage (Hedrick *et al.* 1997). By this time, each lineage had accumulated substantial levels of inbreeding (see the electronic supplementary material, figure S1) and the heterozygosity at microsatellite markers was about one half of that observed in northern grey wolves (Wayne & Vila 2003).

Pairings between lineages began in 1995 with the first F_1 pups (those resulting from pairings between lineages) being born in 1997 (figure S1). Since then, F_1 wolves have been bred among themselves, backcrossed to McBride wolves, and bred with cross-lineage wolves (wolves with ancestry from two or more lineages other than F_1 wolves). The initial goal was for the merged population to have 10% of its ancestry from each of the Aragón and Ghost Ranch lineages. Upon review of the fitness effects of the

merger, ancestry from these lineages could be increased to a maximum of 25% each. At the end of 2005, the captive population numbered about 300 wolves, held in 48 facilities throughout the USA and Mexico. Releases of captive-bred Mexican wolves to re-establish a wild population began in Arizona in 1998 (Interagency Field Team 2005). Initial releases were from the McBride lineage and releases of cross-lineage wolves began in 2000. At the end of 2006, there were nine known packs of wild Mexican wolves in Arizona and New Mexico (Hedrick & Fredrickson *in press*).

Here, we used data collected over 44 years from the Mexican wolf captive breeding program and from the first 9 years (1998–2006) of the reintroduction program to look for evidence of inbreeding depression among Mexican wolves as well as genetic rescue from merging the three lineages. We addressed four questions: (i) did captive wolves from the McBride and Ghost Ranch founding lineages show inbreeding depression? Furthermore, did inbred descendants of crosses between lineages show inbreeding depression? (ii) Did crosses between captive wolves of different lineages produce wolves with increased fitness? (iii) Did Mexican wolves in the reintroduced population show inbreeding depression? (iv) Did cross-lineage wolves have greater fitness than McBride lineage wolves in the reintroduced population?

2. MATERIAL AND METHODS

For the captive population, we investigated the effects of demographic and inbreeding variables on three fitness traits: the probability of live birth, litter size and pup survival to 180 days. We determined values of fitness traits and demographic covariates from the Mexican wolf studbook (Siminski 2005). Demographic covariates and inbreeding models considered in the analyses of each trait are listed in the electronic supplementary material, table S1.

For the reintroduced population, ‘litter size’ was the maximum number of pups observed with a pair from April through November. For two litters, the number of pups was determined from post-mortem examination of the mother. Only pairs that were free-ranging during the breeding season, and at least the month before, were included. Three females that conceived in the wild were captured and brought into captivity shortly before giving birth, and the numbers of pups in these litters were determined while in captivity. Inbreeding coefficients (f) for captive and wild wolves were estimated from pedigree information. Parentage of wild-born wolves was determined from genetic markers by the US Fish and Wildlife Service (USFWS). All statistical analyses were calculated using SAS v. 9.1.3.

(a) Estimating the effects of inbreeding

We used generalized estimating equations (GEE, Hardin & Hilbe 2003) to estimate the effects of inbreeding on the probability of live birth and litter size in the captive population, and on the litter size of wild pairs. GEE is an extension of generalized linear models for use when data are longitudinal or clustered (Hardin & Hilbe 2003). Logistic and identity link functions were used to model the probability of producing live pups and litter sizes, respectively. We used PROC GENMOD to calculate GEE regressions.

To estimate the effects of inbreeding on survival of captive wolves to 180 days, we used Cox’s proportional hazards models

with standard errors adjusted for non-independent failure times within litters using the method of Lee *et al.* (1992). We implemented Cox regressions using SAS PROC PHREG. For additional information on estimation, see the electronic supplementary material. To model the effects of inbreeding on fitness, we used a multi-model approach in an information-theoretic context (Burnham & Anderson 2002), in a two-step process (see the electronic supplementary material). We assessed the weight of evidence in support of the selected best model being the actual best model within the set using Akaike weights (w , Burnham & Anderson 2002), which range from 0 to 1, with greater values indicating greater support.

(b) Identifying fitness differences among Mexican wolves

We looked for evidence of genetic rescue by comparing values of fitness traits between F_1 wolves (all resulting from crosses between McBride and Ghost Ranch or McBride and Aragón) and wolves from the McBride and Ghost Ranch lineages. Genetic rescue occurred if the F_1 fitness was greater than that of inbred wolves from the founding lineages. For these comparisons, we used two groups of McBride pairings. ‘Contemporary McBride’ pairings occurred from 1999 to 2003, had the greatest inbreeding levels, and coincided with the cross-lineage pairings in this study (1999–2005). ‘Early McBride’ pairings included those from 1981 to 1993 or a minimally inbred subset. Similarly, Ghost Ranch litters were divided into three groups: litters born to the founding female; litters with intermediate levels of inbreeding; and maximally inbred litters. Similar information was not available for analysis of Aragón wolves.

3. RESULTS

(a) Inbreeding effects on the captive founding lineages

Inbreeding appeared to have weak or no fitness effects on captive wolves from the founding lineages. For McBride wolves, there was some evidence that inbreeding in the sire and dam had small effects on the probability of producing live pups ($N=180$ pairings, 89 litters). The best model included only two dichotomous demographic variables, but support for this model was weak ($w=0.22$). The second and third best models included the level of inbreeding in the sire ($w=0.19$, electronic supplementary material, figure S2a) and the mean of inbreeding levels in the dam and sire ($w=0.17$), respectively, in addition to the two demographic variables from the best model. The odds ratios for the two models indicated that the odds of failing to produce live pups increased by factors of 1.76 and 2.11, respectively, for an increase in f by 0.1.

To investigate effects of inbreeding on the probability of live birth within Ghost Ranch and Aragón lineage wolves, we used 51 pairings between McBride and Ghost Ranch or McBride and Aragón wolves (MB \times GR, MB \times AR) and 52 contemporary pairings among McBride lineage wolves (31 total litters produced). The results provided some evidence that inbreeding in sires reduced the probability of live birth, but little evidence of inbreeding effects in the dams. The best model included inbreeding in the sire, but support was weak ($w=0.39$). This model suggested that the odds of failing to produce live pups were 1.51 and 5.68 times greater among Aragón and Ghost Ranch sires,

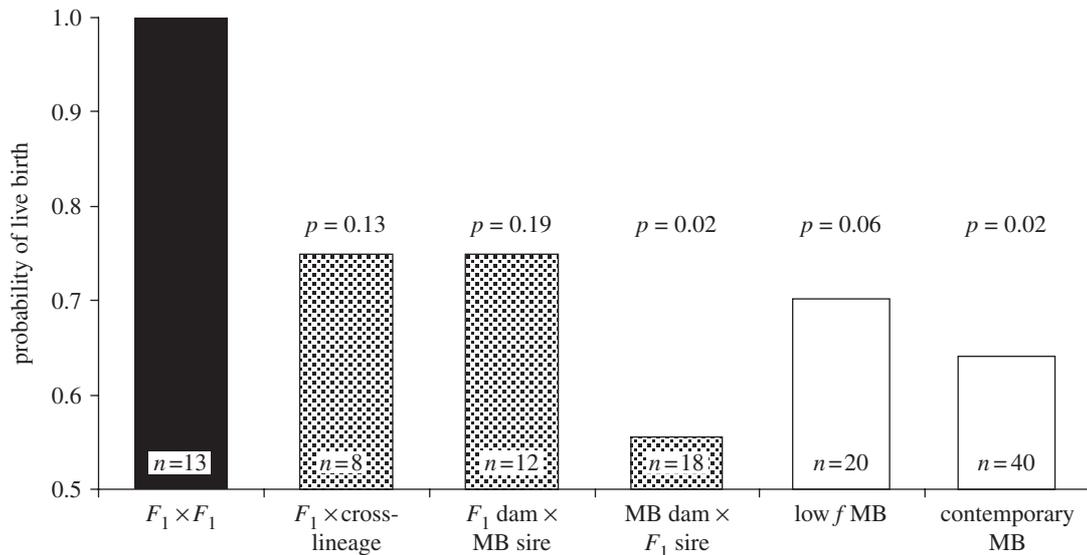


Figure 1. Probability of live birth among $F_1 \times F_1$ pairings (black bar) relative to other pairings by F_1 wolves (stippled bars) and McBride (MB) lineage pairings (open bars). The p -values are for pairwise comparisons with $F_1 \times F_1$ pairings.

respectively, than among McBride sires (electronic supplementary material, figure S2b).

Analyses of litter size within Ghost Ranch and McBride lineage wolves provided evidence for a small effect of inbreeding on Ghost Ranch litters, but no effect on McBride litters. For Ghost Ranch litters ($N=39$), the best model ($w=0.80$) and second best model ($w=0.19$) included inbreeding effects in the dam. The best model suggested litter size declined by 0.32 pups with an increase of 0.1 in the dam f . For McBride litters, the best model included only demographic variables and had strong support ($w=0.91$). Finally, there was no evidence of inbreeding effects on survival of Ghost Ranch pups and only slight evidence for inbreeding effects on McBride lineage pups (results not shown).

(b) Fitness effects of outbreeding among captive wolves

Although inbreeding appeared to have little or no effects on fitness in the founding lineages, F_1 wolves showed large fitness increases. The proportion of live births for pairings between F_1 wolves was 89% and 33% higher than that observed among contemporary McBride pairings in zoos and prerelease facilities, respectively (figure 1). Litters from $F_1 \times F_1$ pairings averaged more than twice the size of those from contemporary McBride wolves (7.5 versus 3.6, $p=0.0001$; figure 2b) and maximally inbred Ghost Ranch litters (7.5 versus 3.5, $p=0.002$; figure 2c). In addition, pups born to cross-lineage dams had 18% and 21% higher survival rates to 180 days than contemporary McBride lineage pups in zoos and prerelease facilities, respectively ($p=0.004$, figure 3).

Fitness among F_1 wolves was also higher than wolves early in the McBride lineage with low levels of inbreeding. Pairings between F_1 wolves were more likely to produce live pups than pairings among McBride wolves with little inbreeding (1.0 versus 0.7, $p=0.06$; figure 1). The $F_1 \times F_1$ and McBride pairings in this comparison were closely matched in inbreeding levels (mean $f_{\text{midparents}}=0.0$ and 0.016, respectively). Litters from pairings between F_1 wolves (mean $f=0.0$ dams, 0.057 pups), were also larger than those from early McBride lineage dams (7.5 versus

4.7, $p=0.003$; figure 2b) producing litters with low inbreeding (mean $f=0.074$ dams, 0.156 pups), and pups born to cross-lineage dams had greater survival than pups early in the McBride lineage (figure 3).

Overall, however, F_1 and cross-lineage wolves showed a range of fitness levels for the three traits examined (figures 1–3). Pairings between McBride lineage dams and F_1 sires were least likely to produce pups and produced nearly the smallest litters, and cross-lineage pups born to McBride dams had the lowest survival among cross-lineage wolves. These differences were significant when compared with $F_1 \times F_1$ pairs for birth probability (1.0 versus 0.56, one-tailed $p=0.02$) and litter size (7.4 versus 4.2 pups, one-tailed $p=0.02$). Cross-lineage pups born to McBride dams also had lower survival than pups born to cross-lineage dams (pups born in zoos 0.85 versus 0.78, one-tailed $p=0.04$). In fact, the performance of McBride dams in cross-lineage pairs showed no (birth probability) or only small improvement (litter size and pup survival) over contemporary McBride pairings (figures 1–3).

Surprisingly, pairings between F_1 and cross-lineage wolves ($F_1 \times$ cross) produced the smallest litters (4.1 pups) among cross-lineage wolves (figure 2a), even though they had a relatively high probability of producing live pups (0.75, figure 1). By contrast, pairs with F_1 dams and McBride sires averaged 5.7 pups per litter. This difference in litter size may have resulted from higher dam inbreeding in the former relative to the latter (mean $f_{\text{dam}}=0.059$ versus 0.0); litter inbreeding was similar between the two pairing types (mean $f_{\text{pup}}=0.149$ versus 0.142).

(c) Inbreeding effects among captive cross-lineage wolves

In contrast to wolves from the founding lineages, inbreeding had strong effects on the fitness of cross-lineage wolves. For the probability of producing live pups ($N=54$ pairings, 39 litters), the best two models (summed $w=0.82$) both indicated that inbreeding in the sire and dam reduced mating success. For parents with no inbreeding, the best model estimated the probability of live birth as 0.96, but for parents with mean inbreeding of 0.1 and 0.2, the probability of live birth dropped to 0.68

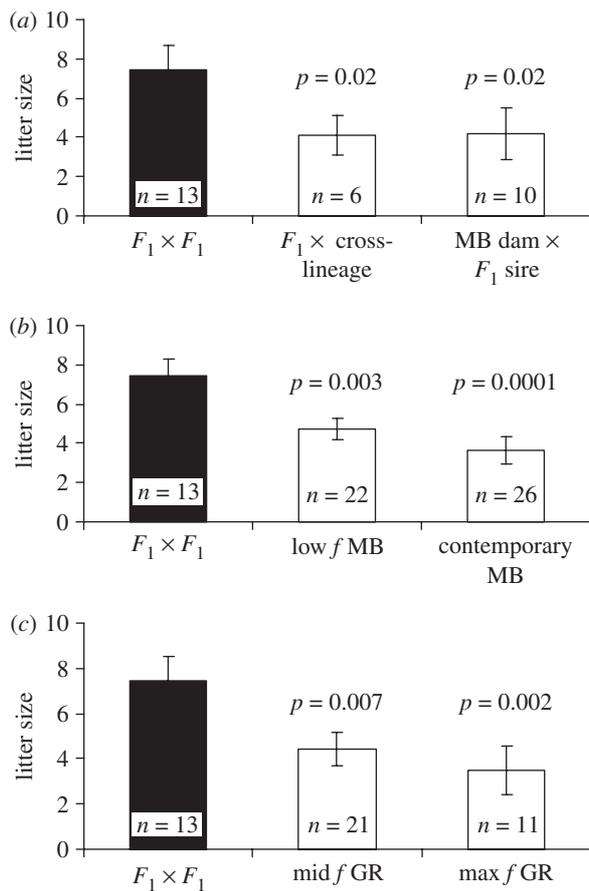


Figure 2. Litter sizes of $F_1 \times F_1$ pairings (black bars) contrasted with (a) pairings between F_1 and cross-lineage wolves (fixed effects, $p=0.016$), (b) early McBride (MB) lineage pairings with low inbreeding and contemporary McBride pairings (fixed effects, $p=0.0002$), and (c) Ghost Ranch (GR) pairings with intermediate and maximal inbreeding levels (fixed effects $p=0.003$). The p -values refer to pairwise contrasts with $F_1 \times F_1$ pairings. Error bars show standard errors.

and 0.18, respectively (figure S2c). Odds ratios from this model indicated that the odds of failing to produce live pups increased 9.9 times with $f=0.1$ in the parents and 98.5 times with $f=0.2$.

There was also evidence of strong inbreeding depression in litter size among pairs including cross-lineage wolves ($N=39$ litters). The best model ($w=0.99$) indicated that inbreeding in the dam and pups affected litter size, and that litter size declined by 2.8 pups with an increase of 0.1 in f of the dam and pups.

For pups born to cross-lineage pairs, there was some evidence that inbreeding in the dam increased mortality, but there was no evidence that inbreeding in the pups affected their survival. For dams with $f=0.1$, the best model suggested that pup survival declined 12.6% and 3.8% for pups in zoos and prerelease facilities, respectively, relative to dams with no inbreeding, but this model had weak support ($w=0.20$).

(d) Inbreeding and genetic rescue among wild wolves

Inbreeding also had strong effects on observed litter sizes in the reintroduced population. The best model describing litter sizes ($N=39$ litters) among wild pairs included only inbreeding in the pups (figure 4). This

model had strong support ($w>0.99$) and the regression coefficient ($f_{\text{pup}}\beta = -8.23$, 95% confidence interval (CI): $-12.20, -4.26$) was highly significant ($p<0.0001$). Two variables related to wolf monitoring efforts failed to substantially improve model fit relative to the constant only model (electronic supplementary material, table S1).

4. DISCUSSION

In captive wolves of the McBride and Ghost Ranch lineages, inbreeding appeared to have weak or no effects on fitness. However, crosses between lineages produced wolves with greatly increased fitness, indicating genetic rescue. Subsequent inbreeding in cross-lineage wolves resulted in reduced fitness, revealing a high genetic load, the accumulation of deleterious or lethal alleles, not apparent in the analyses of the founding lineages. In the wild population, 52% more pups were observed among packs producing cross-lineage pups than those producing pure McBride lineage pups, underscoring the low fitness of wolves with only McBride ancestry and the restored fitness among cross-lineage wolves. Our study appears to be the first to explore the fitness effects of crosses between inbred but unrelated lineages in a wild vertebrate.

The range of inbreeding effects we observed was wide, affecting the fitness of sires, dams and pups. The apparently low mating success of inbred males probably resulted in part from reduced fertility. Semen samples from Mexican and generic grey wolves suggested that inbred Mexican wolves had reduced sperm quality (morphology and motility), and that some males may have been functionally infertile (Asa *et al.* in press). This is consistent with other studies that have found inbreeding or heterozygosity effects on ejaculate quality (Roldan *et al.* 1998; Gage *et al.* 2006). Our finding that inbreeding levels in the dam and pups affected litter size is consistent with observations from mice (Lacy *et al.* 1996) and foxes (Nordrum 1994) where maternal inbreeding was found to increase loss of ova or embryos before implantation, and pup inbreeding increased post-implantation mortality and mortality in the first 3 days after birth. In contrast to the strong negative effects of pup inbreeding on prenatal survival among cross-lineage wolves, there was no evidence of an effect of litter inbreeding on pup survival, perhaps due to the relatively benign conditions in captivity (Jimenez *et al.* 1994).

(a) Lethal equivalents

Among captive cross-lineage wolves, McBride ancestry accounted for all inbreeding in dams and sires and 94.4% of the total inbreeding in pups. McBride ancestry also accounted for all inbreeding in wild litters. Consequently, we calculated lethal equivalents (Morton *et al.* 1956) and litter-reducing equivalents (Liberg *et al.* 2005) using GEE for McBride ancestry only. For the probability of live birth, we estimated 5.64 (95% CI: 0.83–10.43) and 3.65 (95% CI: 0.19–7.12) lethal equivalents for captive dams and sires, respectively, in the McBride lineage, based on 54 pairings. In this context, a lethal equivalent is the cumulative effects of deleterious alleles sufficient to prevent a dam from producing live pups or a sire from successfully mating.

To estimate the genetic load associated with litter size among captive McBride lineage wolves, we used 23 litters

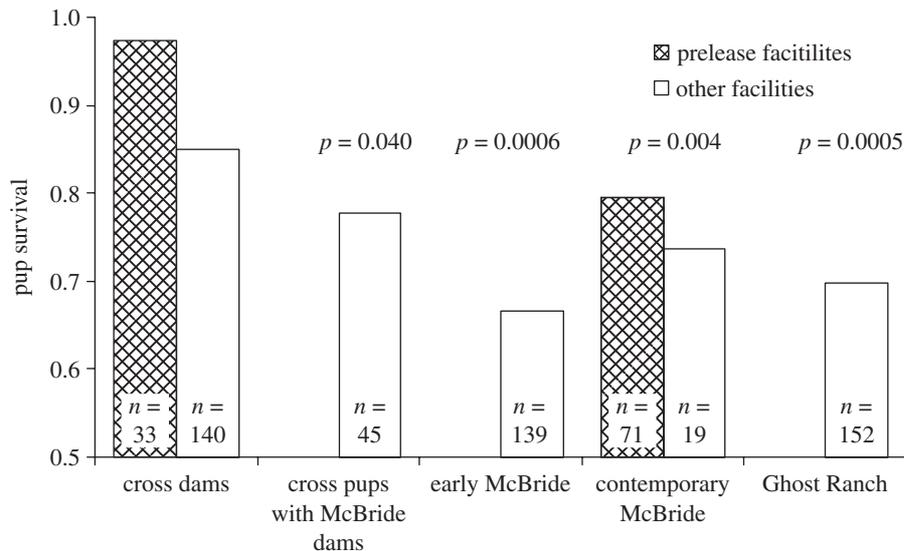


Figure 3. Survival of pups born to cross-lineage dams relative to cross-lineage pups born to McBride lineage dams, McBride lineage pairs and Ghost Ranch lineage pairs in prerelease facilities (hatched bars) and other facilities (open bars). The p -values are for comparisons with cross-lineage pups born to cross-lineage dams.

produced from dams with $f=0$ and 10 litters produced by McBride dams paired with F_1 sires ($N=33$ litters). The number of litter-reducing equivalents estimated from cross-lineage pups (6.89, 95% CI: 3.64–10.15) was nearly twice that estimated from McBride dams (3.52, 95% CI: 0.54–6.51). An analysis using only the 23 litters produced by F_1 dams provided a similar estimate for pups (7.16, 95% CI: 4.37–9.94). This suggests that about two-thirds of the litter-reducing equivalents in the McBride lineage acted to reduce prenatal pup survival, and the remainder acted to reduce fertility in dams.

For the wild population, we estimated 5.19 (95% CI: 1.95–8.44) litter-reducing equivalents among pups. Because most breeding wolves and the litters produced had only McBride ancestry, estimates of litter-reducing equivalents may increase in the future as additional cross-lineage litters with inbreeding from their McBride ancestry are born into the wild population.

The numbers of lethal equivalents estimated for captive Mexican wolf pairs (dams+sires) and litter-reducing equivalents estimated for captive litters (dams+pups) were greater than the numbers of lethal equivalents estimated by Ralls *et al.* (1988) for juvenile survival in 35 out of 40 captive mammal populations. Litter-reducing equivalents among captive and wild pups only, were greater than that of 33 and 30, respectively, out of 40 populations examined, estimated by Ralls *et al.* (1988), but similar to those estimated for wild Scandinavian wolf pups (6.04, Liberg *et al.* 2005). Liberg *et al.* (2005) also noted that inbreeding in the dam and pups reduced winter litter sizes among Scandinavian wolves.

(b) Causes of inbreeding depression and heterosis in Mexican wolves

The Mexican wolf lineages may have been primed for strong heterosis by the combination of small effective sizes (Fredrickson 2007), isolation and rapid inbreeding in captivity resulting in divergence between lineages and the fixation of large numbers of moderately deleterious alleles within lineages (Wang *et al.* 1999; Whitlock *et al.* 2000). Before being merged, the Aragón and Ghost Ranch

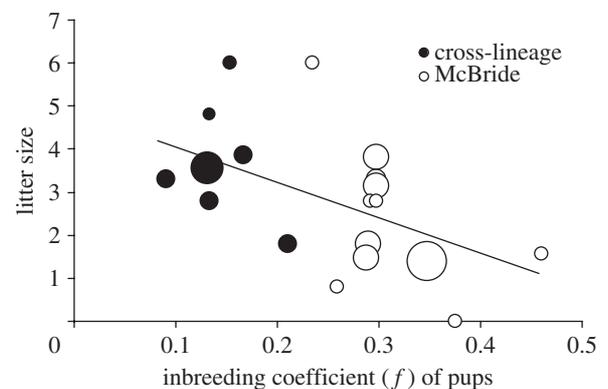


Figure 4. Numbers of pups observed with wild wolf pairs and estimated regression function. For pairs with more than one litter, circles show mean litter sizes. Circle size reflects sample size (1–5), with a total sample size of 39 litters.

lineages were fixed at 45% of microsatellite loci surveyed, each lineage had substantial numbers of unique alleles, and levels of genetic differentiation between the three lineages were comparable to that seen between different populations of northern grey wolves (Hedrick *et al.* 1997). The presence of large heterotic effects among F_1 wolves suggested that many loci in the founding lineages were differentially fixed or nearly fixed for deleterious alleles (Whitlock *et al.* 2000), and that the increased fitness among F_1 offspring relative to contemporary inbred individuals resulted from the masking of deleterious alleles in heterozygotes (Wang *et al.* 1999). The weak inbreeding depression observed in the McBride and Ghost Ranch lineages coupled with the strong inbreeding depression within cross-lineage wolves is also consistent with a high fixed load within each of the founding lineages. Theory further predicts that the numbers of lethal equivalents will be elevated in the F_1 offspring, providing the potential for strong inbreeding depression with renewed inbreeding (Wang *et al.* 1999).

In addition to the fitness increases among F_1 wolves relative to inbred wolves from the McBride and Ghost Ranch lineages, F_1 wolves showed greater fitness than

earlier outbred Mexican wolves. Litter sizes among $F_1 \times F_1$ pairings (mean = 7.45 pups) exceeded those from the McBride and Ghost Ranch founding females (mean = 3.40 and 6.71, respectively), pairings between outbred offspring of the McBride founding female (5.12 pups), and a sample of unborn wild litters from Mexico in the 1970s (mean = 6.75 pups, electronic supplementary material, table S2). In addition, survival of pups born to cross-lineage dams greatly exceeded survival of pups born early in the McBride and Ghost Ranch lineages (figure 3), but the greater survival of contemporary McBride versus early McBride pups suggested that some of the observed survival increases may have resulted from improvements in animal husbandry over time. Although we have focused on genetic rescue in Mexican wolves resulting from the masking of deleterious alleles, it is hoped that the merging of the three founding lineages also restored neutral genetic variation and retained adaptive variation, thereby allowing selection to act on the full range of genetic variation in future generations, a phenomenon termed 'genetic restoration' (Hedrick 2005).

(c) Reintroduction prospects for Mexican wolves

Thus far, the wild population has produced smaller pack and litter sizes than other grey wolf populations in North America, despite abundant prey in the reintroduction area (Interagency Field Team 2005). Our results suggest that this may result largely from the high fixed genetic load in McBride lineage wolves. By the end of 2006, relatively few cross-lineage wolves had been introduced, and one half of the alpha wolves had only McBride ancestry. The heterotic effects we observed suggested that cross-lineage wolves have the potential to increase the population growth rate and initiate a high effective migration rate of Ghost Ranch and Aragón ancestry into the wild population (Ingvarsson & Whitlock 2000; Saccheri & Brakefield 2002; Vila *et al.* 2003). This, however, has not occurred largely due to high rates of human-caused mortality and removals for management reasons (USFWS 2005). Therefore, it currently appears that there is the biological potential in Mexican wolves to establish vigorous wild populations if conflicts with humans can be resolved.

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Letter to the editor

Genetic rescue, not genetic swamping, is important for Mexican wolves

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The recent article by [Odell, Heffelfinger et al. \(2018\)](#) (hereafter OH) is another effort to limit and stymie Mexican wolf recovery by state game and fish ungulate biologists and their allies. OH advocate relying substantially on Mexico for recovery, a possibility that is very uncertain due to a largely unquantified but limited natural prey base and widespread killing of predators in the Mexican areas suggested for recovery. On the other hand, the reintroduced Mexican wolf population in Arizona and New Mexico, outside what OH state is historical range, now numbers over 100, and preys primarily on elk despite the availability of white-tailed deer. Further, it is unlikely that there will be effective natural interchange between Mexican and USA populations because of the proposed construction of a border wall and inhospitable habitat between the two countries.

A new aspect of the anti-wolf arguments in OH is that if Mexican wolves are successful in expanding their range northward they would be in danger from “genetic swamping” by northern gray wolves. However, such contact would actually reinstate the historical situation where wolves once occupied the geographic range in the western United States from Mexico to Canada and formed a continuous population with clinal variation in phenotypic traits ([Wayne and Shaffer, 2016](#)) and gene flow between wolf subspecies ([Leonard et al., 2005](#)).

Wolves in the reintroduced Mexican wolf population, descended quite unequally from three lineages (0.78 McBride, 0.07 Aragon, 0.15 Ghost Ranch) with seven total founders, have an average pedigree inbreeding coefficient of 0.20 and the estimated number of remaining founder genome equivalents is only 2.0 ([Siminski and Spevak, 2017](#)). [Fredrickson et al. \(2007\)](#) found that there was inbreeding depression for litter size in both captive and wild Mexican wolves and also found that crosses between the three lineages showed an increased fitness, resulting in temporary genetic rescue.

Since 2009, there has been artificial supplemental feeding of wild denning Mexican wolves that has greatly increased the survival of pups. It is probable that this supplemental feeding masks some of the detrimental effects of inbreeding, an impact that would be evident if feeding is discontinued. The present-day descendants, three generations later than examined by [Fredrickson et al. \(2007\)](#), are in need of genetic rescue again and crosses with northern gray wolves would provide an appropriate cross to increase fitness.

OH suggested that the somewhat smaller body size and smaller pack size in Mexican wolves than in other wolves would make Mexican

wolves at a disadvantage when interacting with northern gray wolves. However, the smaller body size and smaller pack size might actually be adaptive characteristics that allowed Mexican wolves to survive where there was more limited and smaller prey, and where larger body size might be disadvantageous. Because of their predatory flexibility, Mexican wolves can use larger prey, such as elk, where the current wild population exists, and potentially other areas.

Eight Texas cougar females were translocated to Florida to breed with Florida panthers because Florida panther numbers were very low and they showed several traits indicating inbreeding depression. There was concern that adaptive traits that allowed Florida panthers to successfully survive in the Florida environment would be eliminated by this translocation. As a result, the number of animals translocated were at a level such that expectations were that detrimental traits accumulated by inbreeding would be eliminated but traits adaptive to Florida would be retained ([Hedrick, 1995](#)), a prediction that has generally been proven correct ([Johnson et al., 2010](#)). As precedent, descendants of Texas cougars and Florida panthers are considered Florida panthers and are therefore protected as endangered species.

Using genomic analysis, Mexican wolves have the lowest genetic variation of any wolves ([vonHoldt et al., 2011](#)), indicating that there is limited standing variation for future adaptation to environmental challenges, such as new diseases and climate change. Two other sources of adaptive variation are from mutation and from crosses with related subspecies or species, called adaptive introgression. Generating adaptive variation from mutation generally takes very many generations and often has negative pleiotropic effects. On the other hand, variants present in other related subspecies or species should be adaptive in those species and are more likely to be adaptive when introgressed.

There are a number of examples, including humans, in which genetic variation that has been naturally introduced from other animal subspecies or species has been adaptive ([Hedrick, 2013](#)). For a genetically depauperate subspecies such as Mexican wolves, crosses with another subspecies, such as northern gray wolves, might restore variation and provide a source of genetic variants that would allow future adaptation.

Overall, given the discussion of the relevant evolutionary biology principles here, genetic rescue from crossing with northern gray wolves would likely facilitate Mexican wolf recovery. Unfortunately, the goal of OH appears to be to put as many roadblocks in the way of Mexican wolf recovery as possible, including now the specter of genetic

swamping, because of their short-sighted view that fewer wolves will provide more ungulates for hunters.

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Philip Hedrick^{a,*}, Robert Wayne^b, Richard Fredrickson^c

^a School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

^b Department of Ecology and Evolutionary Biology, 610 Charles E. Young Dr. South, University of California, Los Angeles, CA 90095, USA

^c Missoula, MT, USA

E-mail address: philip.hedrick@asu.edu

* Corresponding author.

Genetic Aspects of Mexican Wolf Expansion into Colorado **by Phil Hedrick (February 18, 2023)**

Although Mexican wolves are recognized as a subspecies, the present population is descended from a very small number of individuals and as a result has low genetic variation. Because of this restricted ancestry and subsequent mating between relatives, Mexican wolves also have significant inbreeding depression. In addition, Mexican wolves presumably have adaptive variation that increases their viability in parts of Mexico and the United States. Therefore, for successful long-term reintroduction of Mexican wolves and expansion northward, genetic goals should include increasing genetic variation, reducing inbreeding depression, and retaining adaptive variation. (As a result, retaining all the genetic integrity of Mexican wolves is not appropriate because some of the genetic variants in Mexican wolves are detrimental.)

Low Genetic Variation

Mexican wolves have a very low base level of genetic diversity in the captive population compared to other wolf populations because of a low number of founders as documented by both genomic data and pedigree analysis and their subsequent small population numbers. The amount of genetic variation from genomic analysis for Mexican wolves is lower than that for other wolves and the observed genomic heterozygosity in Mexican wolves is only 0.12, only about 55% that of northern gray wolves which has a genomic heterozygosity of 0.22. This is about the amount of genetic variation expected if a pair of wolves were taken at random from northern gray wolves, this pair reproduced and had two progeny, and those two progeny had offspring to produce a population. In other words, the level of genetic variation in Mexican wolves already is that expected from an extreme two-generation bottleneck of two individuals each generation from northern gray wolves. In other words, the very low level of genetic variation in Mexican wolves is a fundamental problem for their recovery.

Significant Inbreeding Depression

As the result of past small population size and mating between relatives, there is inbreeding depression in Mexican wolves, that is, low fitness because of an increase in homozygosity. It has been documented that there was inbreeding depression for litter size in both captive and wild Mexican wolves and crosses between the Mexican wolf lineages showed an increased fitness, a further confirmation of the documentation of significant inbreeding depression. The present-day members of the captive and wild Mexican wolf populations, which are at least three generations advanced from those previously examined, would be expected to exhibit as much or more inbreeding depression as found earlier. Other factors that influence fitness, such as viability, mating success, and probability of reproduction, might also be impacted by inbreeding. In other words, it is highly likely in a population with only two founder genome equivalents remaining that there would be inbreeding depression and ignoring inbreeding depression might further imperil the population.

Since 2009, there has been extensive artificial supplemental feeding of a high percentage of wild denning Mexican wolves, feeding that has greatly increased the survival of pups. This

supplemental feeding very likely masks the detrimental effects of inbreeding in the wild population, an impact that would be evident if feeding was discontinued.

Adaptive Variation

Genetic analysis has shown that Mexican wolves are differentiated from other wolves and should be considered as a valid subspecies. In addition, their historical geographic range, as well as their habitat and prey base, were different from other wolves. Because of these factors, it is assumed that Mexican wolves have adaptive variation that makes them successful in the areas and habitats where they were historically found. Although the adaptive variants that are important in making Mexican wolves an important subspecies to protect have not been specifically identified, any such genetic variants should have a selective advantage over genetic variants from other wolf populations in ancestral Mexican wolf areas or areas that share similar environmental factors.

Genetic Goals for Mexican Wolves

As a result of these considerations, the genetic goals for wild populations of Mexican wolves, including those in Colorado, should include increasing genetic variation, reducing inbreeding depression, and retaining adaptive variation. Breeding in the captive population is focused on maintaining genetic variation and minimizing inbreeding as much as possible but as discussed above genetic variation has been lost and inbreeding depression has developed in the captive population.

A way to reach these goals is to have introgression from other wolves, even those from another subspecies. As an example, Texas cougars were moved to Florida to mate with the endangered Florida panthers (Texas cougars and Florida panthers are considered different subspecies). (Mating between coyotes (*Canis latrans*) and red wolves (*Canis rufus*) is quite different because they are different species and should not be allowed to interbreed.)

In the Florida panther example, all of the progeny between Texas cougars and Florida panthers were considered Florida panthers and were protected. It was concluded that with limited introgression of Texas cougar ancestry that genetic variation in Florida panthers would be increased, detrimental Florida genetic variants contributing to inbreeding depression would be reduced, and adaptive Florida genes would be retained. Similarly for Mexican wolves, introgression of genetic variation from northern gray wolves would increase genetic variation, reduce inbreeding depression, and retain adaptive variation. That is, gene flow from northern gray wolves would be good genetically for the Mexican wolf population and the adaptive variation present in Mexican wolves that makes them unique could be retained.

As further support of this approach, genetic data and analysis have supported that the range of Mexican wolf genetic ancestry was widespread. Before extirpation of wolves from the western United States, wolves formed clines of genetic ancestry and morphology south to north. In other words, restoration of this pattern should be the goal of long-term recovery, not the isolation of Mexican wolves in the southwest and northern gray wolves in the northern Rockies. This range expansion would also facilitate a connection to northern gray wolves, which would provide both a way to increase genetic variation and reduce inbreeding depression. In addition, if this expansion was managed then adaptive variation present in Mexican wolves would be retained as it was for Florida panther adaptive variation when there was an introduction of Texas

cougars into Florida. The location of the cline in genetic variation between the subspecies would be determined by the success of the genetic variants from the two subspecies in different areas.

It is very unlikely that there would be any problem with matings between wolves from the north and Mexican wolves. Before wolves were extirpated in the 20th century, they often moved substantial distances and mated with other wolves. Having these type of matings would be restoring what was the natural scheme before extirpation.

Genetic rescue guidelines with examples from Mexican wolves and Florida panthers

Philip W. Hedrick · Richard Fredrickson

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Abstract In populations or species with low fitness (high genetic load), a new management strategy called genetic rescue has been advocated to help avoid extinction. In this strategy, unrelated individuals from another population are introduced into the population with low fitness in an effort to reduce genetic load. Here we present ten guidelines that can be used to evaluate when genetic rescue is a good management option, the appropriate procedures for genetic rescue planning and management, and the potential negative genetic consequences of genetic rescue. These guidelines are then used to evaluate the genetic rescue aspects of the recovery programs for the Mexican wolf and the Florida panther.

Keywords Florida panthers · Gene flow · Genetic load · Genetic restoration · Inbreeding depression · Mexican wolves

Introduction

Although positive ecological factors are essential for the persistence of endangered species, genetic factors may also be significant in a number of situations. In general, genetic factors important in conservation can be divided into those with immediate effects, such as inbreeding depression, and

those with longer term effects, such as genetic load and loss of genetic variation for future adaptation. Perhaps the most significant early contribution of genetics to conservation was the recognition of the importance of inbreeding depression (for reviews, see Hedrick and Kalinowski 2000; Keller and Waller 2002). Inbreeding depression is an effect on fitness thought to be due to an increase in homozygosity of detrimental alleles from inbreeding (Charlesworth and Charlesworth 1999).

Several management approaches are generally accepted and commonly used in conservation genetics to manage these genetic impacts. First, in captive or controlled populations, inbreeding can be avoided as much as possible in order to minimize inbreeding depression (Hedrick and Kalinowski 2000). In captive pedigreed populations, avoiding inbreeding generally is managed by minimizing mean kinship between parents (Ballou and Lacy 1995). Second, in both captive and wild populations that can be managed, the effective population can be maximized to retain genetic variation (this strategy also minimizes fixation of detrimental variation). For example, the supplementation program in the endangered winter run Chinook salmon uses a breeding protocol that attempts to maximize the effective population size of released smolts (Hedrick et al. 1995, 2000). Third, the population can be divided into groups with limited gene flow between them; this both provides genetic connections among the subpopulations and can result in greater overall retention of variation than in an undivided population of the same total size (Fernández et al. 2008). Other management approaches that have been recommended in conservation genetics are more controversial, such as purging inbreeding depression (Boakes et al. 2007) or supplementation of natural populations with captive-raised individuals (Ryman and Laikre 1991). Here we will discuss genetic rescue, a relatively

P. W. Hedrick (✉)
School of Life Sciences, Arizona State University, Tempe,
AZ 85287-4501, USA
e-mail: philip.hedrick@asu.edu

R. Fredrickson
College of Forestry and Conservation, University of Montana,
Missoula, MT 59812-0596, USA

new management option that seeks to increase fitness in a population by introducing unrelated individuals.

Inbreeding depression, genetic load, and genetic rescue

Let us begin this discussion by differentiating between inbreeding depression and genetic load. Inbreeding depression can be defined as the reduction in fitness (or fitness-related traits) for progeny from matings between relatives, as compared to those from matings between unrelated individuals. This is in contrast to the lowered mean population fitness, called genetic load, that can occur over time when detrimental mutations with a small selective disadvantage in a small population become fixed or of high frequency by genetic drift much as if they were neutral (Wang et al. 1999; Kirkpatrick and Jarne 2000). More specifically, genetic load can be defined as the reduction in mean fitness in a population due to high frequencies or fixation of detrimental variants, as compared to a population without lowered fitness from detrimental variation.

In a large population at equilibrium, substantial standing detrimental genetic variation is expected and, consequently, a large reduction of fitness is expected if inbreeding occurs. However, there may be little genetic load, because, due to the efficacy of selection in large populations and the fact that most detrimental variants are recessive, detrimental variants are in low frequency. If the population declines in number, purging of detrimental variation should take place, especially for alleles of large detrimental effect, thereby reducing inbreeding depression. However, some detrimental variants might become fixed, particularly those of smaller effect, causing an increase in genetic load (Hedrick 1994; Wang et al. 1999). If the population remains small for an extended period, more detrimental variation could be purged, further reducing inbreeding depression, but more detrimental variants could be fixed, causing higher genetic load. Such a population might show no lowered fitness upon further inbreeding, that is, no inbreeding depression, but owing to fixation of detrimental variation, all individuals in the population might have a low fitness and the population might have a high genetic load.

Several caveats should be mentioned. First, if the population declines in numbers, some populations (or even species) might become extinct and the ones going extinct could be the ones with higher genetic load or lower population fitness. As a result, the remaining populations might not have as high a genetic load as would be expected from the initial amount of standing detrimental genetic variation before the population decline. Second, genetic load might be documented as a low estimate of fitness compared with

other populations, or by crossing with individuals from another population and observing an increase in the fitness of their progeny compared with progeny of within-population crosses. However, making such crosses experimentally might not be possible, or the groups might differ in other characteristics as well (Wang 2000).

In the fruit fly, *Drosophila melanogaster*, approximately half the effect of inbreeding depression is thought to be from nearly recessive lethals and half from detrimental variants of small effect but with higher dominance (Wang et al. 1999; Lynch et al. 1999). However, *D. melanogaster* generally has a very large effective population size ($>10^6$) and the genetic architecture of the detrimental genetic variation in this species probably reflects that of a large population near equilibrium. Alternatively, for many endangered species, genetic drift has been important, either because of a current small population size or a history of bottlenecks. As a result, endangered species might have a very different genetic architecture with fewer segregating variants of large detrimental effect (Hedrick 2002), lower inbreeding depression, and perhaps higher genetic load, than do species with histories of larger population size. With advances in identifying genes negatively influencing fitness and measuring their effect in other organisms, the genetic architecture of detrimental variation in endangered species should become known in the future (Charlesworth and Willis 2009).

In some populations or species with low fitness (high genetic load), as indicated by low numbers caused by low reproduction and/or high mortality, a new management strategy called genetic rescue has been advocated recently to help avoid extinction. In this strategy, unrelated individuals from another population are introduced into the population with low fitness in an effort to reduce genetic load caused by high frequency detrimental variants that have accumulated in the population. Note that genetic rescue is intended to reduce the amount of genetic load, not the amount of inbreeding depression. Two useful general reviews of the introduction of genetic variation to promote genetic rescue are by Tallmon et al. (2004) and Edmands (2007). More specifically, genetic rescue has been examined theoretically (Wang et al. 1999; Whitlock et al. 2000) and experimentally (Newman and Tallmon 2001; Saccheri and Brakefield 2002; Swindell and Bouzat 2005, 2006), has occurred naturally in a few situations (Vilà et al. 2003), and has been the result of management actions in several others (Madsen et al. 1999, 2004; Westemeier et al. 1998; Bouzat et al. 2009; Land and Lacy 2000). In addition, genetic rescue has seen widespread application in plant species, see Tallmon et al. (2004), Bossuyt (2007), and Willi et al. (2007).

Here we will focus on planning and carrying out genetic rescue as a management action and provide an ideal set of ten guidelines for implementation of genetic rescue. Then

we will examine these guidelines in two case studies, Mexican wolves and Florida panthers, in which management for genetic rescue (or genetic restoration, see below) has been carried out.

Guidelines for genetic rescue

(a) When genetic rescue may be a good management option?

Before genetic rescue as a management strategy is begun, the overall situation and other management, including doing nothing, should be carefully evaluated. In other words, the potential benefits of genetic rescue to help avoid extinction should be documented to be greater than the risks from translocation of individuals into the endangered population. In reality, some aspects of these suggested guidelines may not be possible to carry out before the action is begun but they should be part of the context for examining genetic rescue management. The following guidelines present three conditions under which genetic rescue may be a recommended option.

(1) There should be *evidence of low fitness* in the endangered population (or high inbreeding from either pedigree or molecular data) so that making an introduction is likely to increase fitness, that is, reduce genetic load in the endangered population. It may be possible to compare fitness parameters to either historical data from the population or species or to compare fitness measures to populations of a closely related organism. Low numbers in the population for a long time may potentially be of ecological causation but if the possibility of extinction appears to be high, then experimental examination of fitness in crosses may be informative as to whether the low fitness has a genetic basis.

(2) For successful genetic rescue, a *closely related donor population* should be available so that likelihood of outbreeding depression (low fitness in offspring, or future generations, from crosses) is low (Tallmon et al. 2004; Edmands 2007). In general, the donor population should be from the same species as the endangered population and either from a nearby area and/or from a similar habitat.

(3) Before the actual genetic rescue is attempted in the wild, endangered population, there should be *experimental data* from a captive population to support validity of genetic rescue. In other words, there should be experimental demonstration of successful mating, good reproduction, and good survival in crossed progeny and no evidence of outbreeding depression between these populations. This is particularly important if molecular data suggest the donor and endangered populations are not closely related. However, in species with a longer generation time or ones that are difficult to breed in captivity, it

may not be possible to carry out a complete experimental evaluation in captivity before initiation of genetic rescue management. Given evidence of low fitness and the presence of a closely related donor population, managers could then consider the tradeoff between the time, money, and effort needed for this evaluation and the risk of extinction and argue for immediate genetic rescue management.

(b) Genetic rescue planning and management

Once a decision is made to carry out genetic rescue, then the basic protocol for introduction and procedures in the subsequent generations should be established. These procedures should be as well thought out as possible but they also need to have some flexibility if there are unpredicted outcomes. The following represent three recommended conditions for genetic rescue and management.

(4) There should be an established and cautionary *translocation protocol* so that the introduction of outside individuals makes negative non-genetic effects unlikely. For example, the risk of the introduction of disease from the donor population or detrimental changes in the behavior of the endangered population should be minimal. Further, detrimental behavioral effects might be minimized by only introducing females, introducing groups of animals, or only introducing animals to unoccupied territories or locations.

(5) There should be a detailed *monitoring plan* for the introduction and its consequences in succeeding generations. This would entail identification of individuals by some established means and monitoring of their movement, mating behavior, survival, and reproduction as thoroughly as possible. In addition, the relationships between individuals, such as maternity, paternity, and other close genetic relationships, should be determined and verified by genetic means in a timely manner so that an accurate and complete pedigree of the rescued population can be constructed and potentially used in management.

For some populations or types of organisms, however, construction of complete pedigrees may not be possible. In these cases, the change in genetic composition of the population over time could be estimated based on classes of admixed (e.g., F₁ and backcross individuals) and non-admixed individuals (pure individuals from the donor and endangered populations). Ideally measures of individual fitness indexing demographic rates having the greatest effects on population growth would be quantified for classes of admixed and non-admixed individuals. However, if this is not possible, estimates of the population growth rate over time combined with information on the change in proportion of admixed and non-admixed classes could be used to gauge success of management efforts.

(6) There should be the potential for *management continuation* over time, even several generations, and the

possibility to modify management when necessary. For example, if the initial success is less than expected, then further management, including further introductions in future generations should be possible. Or if the initial amount of ancestry from the donor population is more than expected or wanted, then further introductions in future generations should be restricted. In other words, there should be flexibility if there are unpredicted outcomes but changes should be implemented only when they are based on scientifically credible information.

(c) Potential negative genetic consequences

Although genetic rescue should have positive genetic effects by increasing the fitness and population persistence of the rescued endangered population, it may have genetic effects that could be detrimental. Some of these potentially negative effects may be difficult to document initially and may be of less significance than the positive impact of genetic rescue. The following are four negative genetic consequences that may be potentially associated with genetic rescue.

(7) If the introduction from the donor population results in a very high donor population ancestry, then there may be *swamping of local genetic variation* and traits (some of which may be adaptive). To counteract this effect, the level of introduction should be kept at a level low enough so that the genetic rescue effect will be successful (elimination of detrimental variation at high frequency) but not too much to completely change allele frequencies and eliminate locally adaptive variation (Hedrick 1995). Further, if the level of the introduction is too large or the introduced animals are very reproductively successful, then the ancestry of the endangered population may actually be replaced by ancestry from the donor population. Obviously, this would result in the genetic replacement of the population (maybe an appropriate outcome in some cases), not in genetic rescue, and all local variation may be eliminated (e.g., Hogg et al. 2006).

(8) If a high proportion of ancestry after genetic rescue is from a few individuals from the donor population (and/or from a few individuals from the endangered population), then in the next few generations there could again be a *small effective population size* (N_e). This effect could be minimized if more migrants from the donor population are included and they contribute fairly equally to the genetically rescued population. Also inclusion of as much of the endangered population as possible in the initial crosses from the donor population should reduce this effect. If the initial F_1 and backcross individuals have a high fitness, it may be difficult to keep the effective population size high because a large part of the population may be descended from these few individuals.

(9) Bringing in individuals from the donor population may result in a relatively *short-lived fitness increase*. For example, the first generation may have a higher fitness because recessive, detrimental variants will be covered up. However, in second generation and succeeding generation crosses, there may be segregation that again results in recessive homozygotes and lowered fitness (e.g., Liberg et al. 2005). In fact, inbreeding depression may be increased in the rescued population (Wang et al. 1999) relative to that of the endangered population before rescue. If the endangered population is considered unique (e.g., the last remaining population of a subspecies), following initial genetic rescue there may be a desire to increase the ancestry of the endangered subspecies in the rescued population by creating backcrosses with individuals that have ancestry only from endangered population. This however runs the risk of reconstituting the genetic load that contributed to the endangerment of the subspecies and may lead to failure of the rescue program.

(10) The possibility of genetic rescue in a given endangered population from a given donor population may be a one-time option and/or have the *most effect in the first attempt*. If genetic rescue is attempted more than once using the same pair of endangered and donor populations, then the impact could be substantially less in successive attempts because detrimental variants at particular loci that caused the genetic load would have been reduced in frequency in the endangered population by the introduction of non-detrimental variants from the donor population. For the detrimental variants remaining in the endangered population, there may not be non-detrimental variants in the donor population or the donor population itself may have some detrimental variants that could reduce the fitness of the endangered population.

Genetic rescue in Mexican wolves and Florida panthers

Mexican wolves

The Mexican wolf (*Canis lupus baileyi*), an endangered subspecies of the gray wolf, is the most genetically distinct wolf subspecies in North America (Leonard et al. 2005). Human activities and killing throughout its range reduced and isolated Mexican wolf populations so that by 1925 they were rare in the United States (and probably extinct by the 1970s) and by the 1950s, their range and numbers in Mexico were greatly reduced. As a result, the Mexican wolf subspecies was listed as endangered in 1976. Only a few Mexican wolves remained in isolated groups in Mexico by 1980 and surveys since then have not detected any wild Mexican wolves in Mexico.

All Mexican wolves alive today descend from three captive lineages founded between 1960 and 1980 from a total of seven wolves (Hedrick et al. 1997). In 1995, after genetic evaluation of the existing captive Mexican wolves, the Aragón and Ghost Ranch lineages were merged into the larger captive McBride lineage to increase founder number, heterozygosity, and fitness, and to reduce inbreeding. (The McBride lineage was originally named the certified lineage but was renamed after Roy McBride, who caught the founders of this lineage and whose work on Florida panthers is discussed below.) The McBride, Ghost Ranch, and Aragón lineages were founded by 3, 2, and 2 different, unrelated individuals, respectively. The estimated average inbreeding coefficients within the McBride, Ghost Ranch, and Aragón lineages were 0.18, 0.61, and 0.26, respectively, before they were merged. The initial goal, based on a number of factors, was for the merged population to have 10% of its ancestry from each of Aragón and Ghost Ranch lineages and 80% from the McBride lineage, and upon review of the fitness effects of the merger, ancestry from the Ghost Ranch and Aragón lineages could be increased to a maximum of 25% each with McBride ancestry reduced to 50% (Hedrick et al. 1997).

The captive population has been examined for the effects of inbreeding level and crosses between lineages for several fitness-related traits (Fredrickson et al. 2007). For example, Fig. 1 gives the mean number of pups for wolves in the McBride lineage with low and high levels of inbreeding and cross-lineage wolves. There is a decline in the number of pups in the McBride lineage between those with low and high inbreeding levels but this effect on inbreeding depression was not statistically significant. However, the cross-lineage wolves had a significantly higher number of pups than the contemporaneous high inbreeding group (similar results were found for survival). This recovery in fitness in crosses between lineages indicates genetic rescue of the captive Mexican wolf population (for a similar finding for sperm quality, see Asa et al. 2007).

In 1998, a population of Mexican wolves was reintroduced into eastern Arizona and western New Mexico. Initially these wolves had only ancestry from the McBride lineage, but starting in 2000 wolves with ancestry from more than one lineage have been released (Hedrick and Fredrickson 2008). As of January, 2009, there were 52 wolves in this reintroduced population, a number that had not changed significantly for several years. It is more difficult to obtain data on reproduction in wild wolves than in captive wolves because the first measurement of reproduction is usually some time after the pups emerge from the den at around 10 weeks of age. However, Fig. 2 gives the data for reproduction in the reintroduced population as a function of the inbreeding coefficient of the progeny. The

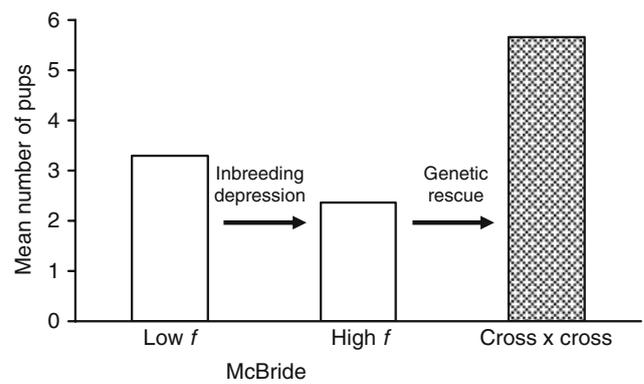


Fig. 1 The mean number of Mexican wolf pups in litters from the McBride lineage with either a low or high inbreeding coefficient and in litters from cross-lineage by cross-lineage wolves (Hedrick and Fredrickson 2008)

cross-lineage wolves (indicated by closed circles) have a lower inbreeding coefficient and higher number of pups than the highly inbred McBride wolves. In other words, as in the captive population, there appears to be genetic rescue for reproduction in the reintroduced population.

Florida panthers

Pumas (*Puma concolor*) were aggressively hunted by Europeans in North America and bounties were offered for hides. In the eastern United States by the late 1920s, pumas were present only in central and south Florida and possibly along some river drainages in Louisiana (Young and Goldman 1946). The numbers in Florida continued to decline because of continued persecution (Tinsley 1970) and in 1967, the puma subspecies called the Florida panther (*P. concolor coryi*) was federally listed as endangered.

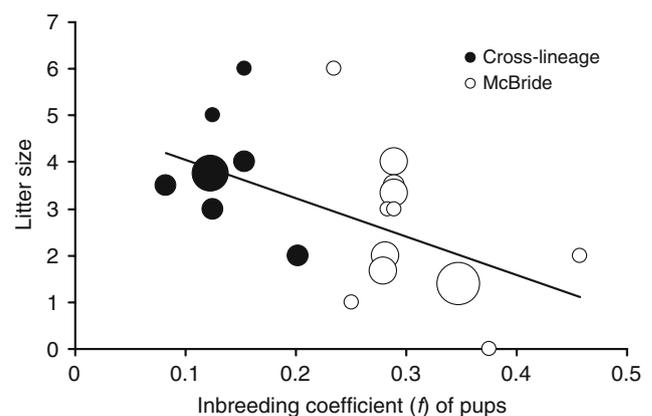


Fig. 2 The maximum number of progeny observed in litters in the reintroduced Mexican wolf population as a function of the inbreeding coefficient of the progeny. Open and closed circles indicate McBride and cross-lineage wolves, circle size indicates the number of litters with a given number of progeny, and the line indicates the linear regression between these points (Fredrickson et al. 2007)

In the early 1970s, the Florida panther was believed extinct and no breeding population was known (Nowak 1993). However, several animals were treed by dogs in 1973 and 1974 in southern Florida (Nowak 1993). The numbers found over the next few years were small but for the 1980s and early 1990s, the general claim was that the census population number was between 30 and 50 (Seal 1994; Maehr et al. 2002) although detailed monitoring suggests that the numbers were lower (McBride et al. 2008). In addition to this low population size, a suite of previously rare and potentially deleterious traits were observed in the population that suggested that genetic drift had nearly fixed detrimental variants. These traits, which are found in high frequency only in the Florida panther and are unusual in other puma subspecies, include high frequencies of cryptorchidism (unilateral or bilateral undescended testicles), kinked tail for the last five vertebra, cowlick on the back (this is not assumed to be deleterious), atrial septal defects, and the poorest semen quality recorded in any felid (Roelke et al. 1993; Table 1). In addition, a large survey of microsatellite loci has shown that Florida panthers have much lower molecular variation than other North American populations of pumas (Culver et al. 2000; see also Driscoll et al. 2002).

A program to release females from the closest natural population from Texas was initiated in 1995 to genetically restore fitness in the Florida panther with an initial goal of 20% Texas ancestry (Seal 1994; Hedrick 1995). Five of the eight introduced Texas females produced offspring with resident Florida panther males and subsequently F_2 and backcross offspring were produced (Land and Lacy 2000). Although little has been published about Florida panther genetics in recent years, even a decade ago Land and Lacy (2000) estimated that approximately 20% of the overall ancestry was from the introduced Texas cougars.

One of the initial findings was that the frequency of the rare and potentially deleterious traits dramatically declined in animals with Texas ancestry (Mansfield and Land 2002; Land et al. 2002; Table 1). For example, of the animals with Texas ancestry, only 7% had a kinked tail (compared to 77% without Texas ancestry) and the animals with

kinked tail were all progeny from backcrosses to Florida cats. Similarly, but not as dramatic, only 24% of the animals with Texas ancestry had a cowlick (compared to 80% without Texas ancestry). Only seven males with Texas ancestry had been evaluated for cryptorchidism and all had two descended testicles, in other words, a reduction from 49 to 0% cryptorchidism because of Texas ancestry. In other words, the introduction of Texas cougars initially resulted in a substantial reduction of the frequency of the rare and/or detrimental traits that had accumulated in the Florida panther but a current analysis remains to be published.

Recently, McBride et al. (2008) published the number of Florida panthers documented by physical evidence from 1985 to 2007 (Fig. 3; 2008 number, R. McBride, pers. comm.). Notice that the numbers from 1985 to 1995 fluctuated between 19 and 30 animals before mortalities (15–27 after mortalities were removed) and did not increase. However, after the introduction of the Texas pumas in 1995, the numbers grew very quickly to 104 in 2008 (84 after mortalities were removed). Using a simple logistic model, it is estimated that the population grew at a rate of approximately 13% per year over this period (12% per year using the after mortality data). In other words, it is compelling that the substantial increase in numbers after the introduction of the Texas pumas demonstrates genetic rescue. In addition, McBride et al. (2008) documented an increase in the number of dispersing individuals and road kills over this period, both indirect indicators of an increase in population density. Pimm et al. (2006) also observed an increase in survival after the introduction of the Texas animals although a study of survival based on genetically determined ancestry remains to be published.

Using the guidelines to evaluate genetic rescue in Mexican wolves and Florida panthers

Now let us evaluate both the Mexican wolf and Florida panther genetic rescue actions in light of the guidelines given above (Table 2 gives a summary). Although both

Table 1 The frequencies of three traits found in high frequency in Florida panthers with no Texas puma ancestry and data from F_1 , F_2 , and backcrosses to Texas and Florida with Texas ancestry (Mansfield and Land 2002; Land et al. 2002; sample size in parentheses)

	No Texas ancestry	Texas ancestry				Mean
		F_1	F_2	BC-TX	BC-FL	
Cryptorchidism	0.49 (49)	0.00 (2)	0.00 (2)	–	0.00 (3)	0.00 (7)
Kinked tail	0.77 (176)	0.00 (17)	0.00 (7)	0.00 (3)	0.20 (15)	0.07 (42)
Cowlick	0.80 (115)	0.20 (10)	0.00 (5)	0.00 (1)	0.60 (5)	0.24 (21)

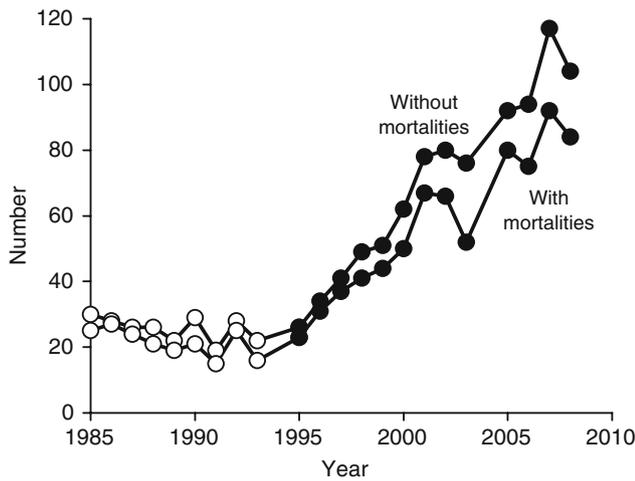


Fig. 3 The number of Florida panthers documented by physical evidence annually from 1985 to 2008, both without including mortalities and after including all known mortalities for the year (McBride et al. 2008; R. McBride pers. comm.; D. Land pers. comm.). Data for the years before the translocation are indicated by open circles and data for those after the translocation by closed circles

actions were started only 15 years ago, or around 4–5 generations in both species, there is enough information to give general evaluations.

Mexican wolves

(a) *Was it a good option to plan genetic rescue for the Mexican wolf?* First, in the mid-1990s, the inbreeding coefficient of the McBride captive lineage was around 0.18

Table 2 An evaluation of the genetic rescue programs for the Mexican wolf and Florida panther and whether they met the guidelines discussed

	Mexican wolf	Florida panther
(a) Good option?		
(1) Low fitness	Yes	Yes
(2) Donor population	Yes	Yes
(3) Experimental data	Yes	No
(b) Management		
(4) Introduction protocol	Yes	Yes
(5) Monitoring plan	Yes (genetic?)	Yes (genetic?)
(6) Continued management	No	No
(c) Negative genetic effect		
(7) Swamping local	No	No
(8) Small N_e	Yes	Maybe
(9) Short-lived fitness increase	Yes?	Not known
(10) One time or most effect in first attempt	Yes?	No

Evaluations in *boldface* did not meet the guidelines or are questionable

and was predicted to increase quickly, even though there was good captive management to keep the inbreeding level as low as possible. Although the data were published after the crossing of the lines were initiated, there did not appear to be inbreeding depression for viability or fecundity (Kalinowski et al. 1999) but there did appear to be inbreeding depression for body size (Fredrickson and Hedrick 2002). In particular, the body size of captive animals appeared to be smaller than that of wild animals caught before the captive program was initiated, suggesting genetic load.

Second, there were only two other known captive lineages, Ghost Ranch and Aragón, of putative Mexican wolves (no wild animals were known), making these the only donor populations of the same subspecies. These two lineages were evaluated using genetic markers, specifically mtDNA and microsatellite loci (García-Moreno et al. 1996; Hedrick et al. 1997). Both lineages appeared to be of Mexican wolf ancestry (and without dog or coyote ancestry) and to have been founded from animals unrelated to the founders of the McBride lineage. Finally, crosses between the McBride and Ghost Ranch lineages, and McBride and Aragón lineages were made in captivity to evaluate their potential. Both types of crosses were successful and the progeny survived, grew, and reproduced well.

(b) *Was the management adequate and appropriate?* First, because the initial crosses were made in captivity, progeny with ancestry from more than one lineage were available to release into the reintroduced population. In other words, a separate protocol for introduction was not necessary beyond the protocol already in place for releasing animals from captivity. Second, there was already a program in place to physically monitor animals released into the reintroduced population and this program was used to monitor reproduction and survival of animals of cross-lineage ancestry as well as pure McBride ancestry. In addition, the program includes annual counts that can be used as an index of population size. Although genetic analysis of paternity and relatedness has been implemented for monitoring in the reintroduced Mexican wolf program, genetic analysis has not been done in a timely manner by the National Forensics Laboratory. Finally, management to continue the genetic rescue program after the initial releases was in place and the ancestry from the Ghost Ranch lineage averaged around 13% in alpha wolves from 2006 to early 2009 but the ancestry from the Aragon lineage averaged only 6% over the same period—below the suggested minimal level of 10%. However, mainly because of non-scientific considerations, further releases were greatly reduced in the 4-year period 2005–2008 to only a total of five wolves. At this point, both the reintroduced population and the program of genetic rescue are presently

at great risk because of the low growth rate of the wild population (see “Discussion”).

(c) *Have there been negative genetic effects of the management?* First, since the level of ancestry of Ghost Ranch and Aragón lineages are still very low, any particular alleles in the McBride lineage could not have been greatly influenced, and certainly not swamped. Further, from both genetic and morphological evaluation, there do not appear to be characteristics only found in the McBride lineage. Second, because of the continued low numbers of packs and breeding individuals in recent years, it appears that there is a low effective population size and a very high ancestry of the inbred McBride lineage. Although there has been no formal estimation of the effective size of the introduced population, it appears to be at most 10 but may be less because the average number of pairs with at least one pup surviving to 8 months of age for the period 2006–2008 was only 5.3 and over the last two breeding seasons at least half of the packs had an alpha wolf descended from a single highly successful pack. Already in backcrosses the inbreeding coefficient is around 0.13 (see Fig. 2) and the average expected inbreeding coefficient of progeny from known pairs is 0.2. Nearly all of this inbreeding is because of identity by descent from the McBride lineage but also somewhat from the Ghost Ranch or Aragón lineages when, for example, both parents shared ancestry from either of these lineages.

Finally, there may not be a second chance for genetic rescue here because none of lineages are still maintained separately in captivity. Although semen was collected from one Aragón and six Ghost Ranch wolves from 1996 to 2000, the efficacy of artificial insemination using frozen semen from strongly inbred wolves is uncertain. It was assumed that the increase in fitness from lineage crossing would be used expeditiously to enhance the numbers of wild wolves and that a second round of crosses would not be necessary. However, mainly because of non-scientific reasons, the cross-lineage wolves were not incorporated into the reintroduced population in a timely manner and this opportunity may have been lost. If the reintroduced population does not increase soon, it may be necessary to consider extraordinary measures, such as introducing northern gray wolves, a closely related subspecies (Leonard et al. 2005), into the reintroduced Mexican wolf population.

Florida panther

(a) *Was it a good option to plan genetic rescue for the Florida panther?* First, as we discussed above there was a suite of traits, including several that appear to have strong negative effects on fitness, that suggested that Florida panthers had experienced genetic drift and had been

isolated from other puma populations. In addition, the very low genetic variation, compared to other puma populations, suggested that the Florida panther population had been through an extreme bottleneck (Culver et al. 2008).

Second, although the nearest donor population in the United States was relatively far away in west Texas, as recently as the late nineteenth century there probably was genetic exchange between these populations through intermediate populations (Seal 1994). In addition, Culver et al. (2000) found that although the Florida panther (*P. concolor coryi*) and the Texas puma (*P. concolor stanleyana*) are classified as different subspecies, it appears that the genetic variation found in the Florida panther is a subset of that found in other North American pumas (they also suggested that all North American pumas be subsumed into one subspecies). Although the arid, west Texas desert habitat is quite different from the humid, tropical habitat of south Florida, there is no United States puma population presently living in a tropical habitat. Finally, it was recommended that experimental crosses between Texas cougars and Florida panthers be carried out in captivity before the translocation of Texas animals into the wild population (Seal 1994) to check for the potential success of genetic rescue and the lack of outbreeding depression. However, for non-scientific reasons, this was never done.

(b) *Was the management adequate and appropriate?* First, the protocol for translocation to prevent introduction of disease was thorough and effective and no known diseases were introduced into Florida. Further, all eight animals (all females) were successfully transferred although three died before they could reproduce. Second, the monitoring of the introduced animals was carried out as for the existing wild Florida panther population (McBride et al. 2008). Samples for genetic analysis have been taken for all the animals that have been captured and sent to Steve O’Brien and his colleagues at the National Cancer Institute. Although it appears that these samples have been analyzed, and paternity and relatedness will be assessed (D. Onorato, pers. comm.), these data have not been published and are not available.

Finally, the recommendation by Seal (1994) was to introduce eight animals in the first generation so that gene flow would be about 20%, and then introduce a single new animal each generation thereafter. The first generation release was generally successful (however, only 5 of the 8 animals produced two or more offspring, rather than the 8 recommended by Seal 1994) but there have been no further introductions and there are no plans that have been made public for further introductions.

(c) *Have there been negative genetic effects of the management?* First, were some adaptive traits lost by swamping the ancestry (for this concern, see Maehr and Caddick 1995)? No such adaptive genetic traits have ever

been identified, either before or after the translocation of Texas pumas. Furthermore, as discussed above the overall fitness of the Florida panther population has greatly improved because of the introduction of the Texas animals.

Second, two of the five Texas females that had offspring contributed approximately 40 and 30% of the Texas ancestry in the present population. As a result, Land and Lacy (2000) suggested that the Texas ancestry was represented in an amount reflecting only three effective founders. In other words, although there was a genetic rescue effect, the limited contributions of the different female Texas pumas may result in a “bottleneck” for this part of the Florida panther population. However, the potentially low effective population size from the Texas pumas (and the Florida panthers to which they mated) has not yet been evaluated because genetic analysis is still in preparation.

Third, it is not yet clear how long the increase in fitness from genetic rescue will endure. Although there have been several generations since the introduction of Texas pumas, genetic pedigree analysis and Texas ancestry in different individuals is still in preparation. Combining this genetic information with survival and reproduction data would provide an evaluation of the extent of the genetic rescue effect.

Finally, there are many more unrelated animals from Texas (or other areas) that could be introduced if necessary. In other words, it is possible if the fitness of the Florida panther does begin to decline because of small population size that more unrelated individuals could be introduced for an additional program of genetic rescue. Although it appears that the Florida panther population may be reaching carrying capacity in its present environment (McBride et al. 2008), a genetically healthy population with a higher intrinsic rate of increase would better be able to rebound in response to a future catastrophe.

General discussion

Although genetic rescue has become an important management option in some cases, and probably will become more important as endangered populations become smaller and more isolated, it is essential to realize that it is not the ultimate solution for the recovery of endangered species. What genetic rescue may do is temporarily increase the population size, which may in turn temporarily reduce the probability of extinction and increase the probability of establishment of an introduced or endangered population. This may give more time to fix environmental and other problems that have caused endangerment. For the long term genetic health of endangered species, recovery of populations to an effective population size resembling that before endangerment is necessary, a goal that is dependent

upon eliminating, or greatly reducing, the factors that caused endangerment.

As discussed specifically above, the implementation of genetic rescue management should be undertaken only when it is clear that the benefits outweigh the risks. For example, when there is a demonstrated low population fitness making the risk of population extinction high in the near future, and risks from disease and outbreeding depression appear small, then genetic rescue management is a reasonable option. In choosing management options, Edmands (2007) stated that, “managers should strive to do no harm.” However, doing no harm should not be used as a reason to do nothing, often the option chosen by managers that are seeking to avoid decisions that could result in criticism, lawsuits, or other negative reactions.

The 10 guidelines given above provide a framework for consideration of genetic rescue management in other situations besides that of the Mexican wolf and Florida panther. Species such as fish, amphibians, and plants may differ enough in various life history attributes, such as number of offspring and/or dispersal, or the ability to monitor nearly every individual, that the guidelines may have to be significantly modified. However, the evaluation of these two programs shows that many of the factors contained in these guidelines were utilized in the planning and implementation of genetic rescue. Unfortunately, this evaluation also suggests that some aspects of genetic rescue should have been carried out in a more timely manner and that for Mexican wolves, the recovery of the reintroduced population is now in jeopardy, partly because of poor implementation of genetic rescue management.

A similar but more comprehensive approach to genetic rescue has been called genetic restoration. This approach seeks to restore neutral genetic variation and maintain adaptive genetic variants, as well as eliminate the effects of detrimental variants (Seal 1994; Hedrick 2005; Bouzat et al. 2009). The more comprehensive view of genetic restoration, which includes maintaining and/or recovering both adaptive and neutral variation, may result in a longer term improvement in population persistence and the potential to adapt to future environmental changes than genetic rescue.

The potential positive and negative genetic effects of introducing individuals from genetically diverse but geographically isolated populations into apparently inbred population was theoretically evaluated before the introduction of Texas pumas into Florida (Hedrick 1995) and this analysis, or an expansion of it, could be used to examine other potential genetic rescue (or restoration) situations. Hedrick (1995) found that 20% gene flow from outside in the first generation and 2.5% every generation thereafter, as recommended in Seal (1994), resulted in quickly improved fitness or elimination of genetic load.

This was part of the basis for the recommendation that 20% ancestry level (10% each from the Ghost Ranch and Aragon lineages) was an appropriate initial goal for genetic rescue in Mexican wolves. As we mentioned above, one concern about introducing outside animals was that any locally adapted alleles would be swamped by outside gene flow. However, as evaluated theoretically by Hedrick (1995), an initial level of 20% gene flow only resulted in a slightly reduced in fitness by lowering the frequency of locally advantageous alleles and restored the variation for neutral variants.

Both the Mexican wolf and Florida panther nearly became extinct because of killing by humans. Both have been protected species for many years and efforts have been made to increase their population numbers. However, human-caused mortality is still a major factor limiting the recovery of these two species. Since the release of Mexican wolves in 1998, 31 have been illegally shot and 12 have been killed by vehicles (Table 3a). In addition, 144 Mexican wolves have been removed from the population because of rules that require removal for depredation of livestock and straying beyond designated boundaries. On the other hand, only two Florida panthers have been shot since the translocation of Texas pumas but 105 have been killed by vehicles (Table 3b). In other words, both species continue to have high human-caused mortality and Mexican wolves have the high added cost to the population of management removals which have removed many wolves and packs of high genetic value. The high cost of human-caused failures (mortality and removals) raises serious doubts about the success of recovery and population persistence, as well as the ultimate success of genetic rescue in both species.

For two other wolf populations, genetic rescue has or could have a substantial impact. First, the contemporary Scandinavian wolf population in Sweden and Norway appears to have been established by a pair of migrants from the large Finland–Russia population in the early 1980s. The number remained at less than ten individuals in a single pack during the 1980s. A third founder naturally migrated from the Finland–Russia population around 1990, resulted in genetic (or behavioral) rescue, and the population increased to around 100 individuals (Vilà et al. 2003). Subsequently, the amount of inbreeding greatly increased, as determined by a near-complete pedigree, and there was a significant decline in the number of surviving pups per litter (Liberg et al. 2005). As a result of this subsequent decline in fitness and other factors (for example, the Norwegian government has killed a number of wolves in the part of the population residing nearby in Norway), the annual population increase has declined and inbreeding has continued to rise. Several other migrants from Finland have reached northern Sweden (Seddon et al. 2006) but they

Table 3 Cause of mortality and management removals by year in the (a) reintroduced Mexican wolf population (USFWS at <http://www.fws.gov/southwest/es/mexicanwolf/>) and cause of mortality in the (b) Florida panther population (Florida Fish and Wildlife Conservation Commission, unpubl. data, pers. comm. E. D. Land 2009)

Year	Shooting	Vehicle	Natural	Unknown	Total	Removals
(a) Mexican wolf						
1998	4	–	–	–	5	6
1999	–	1	2	–	3	12
2000	1	2	1	–	4	23
2001	4	1	2	1	9	10
2002	3	–	–	–	3	7
2003	7	4	–	1	12	15
2004	1	1	1	–	3	7
2005	3	–	–	1	4	21
2006	1	1	1	2	6	18
2007	1	–	1	2	4	23
2008	5	2	2	2	13	2
2009	1	–	–	–	1	–
Total	31	12	10	9	67	144
(b) Florida panther						
1995	–	2	–	1	3	
1996	–	2	–	1	3	
1997	–	1	2	1	4	
1998	1	4	3	1	9	
1999	–	3	4	–	7	
2000	–	7	1	5	13	
2001	–	7	2	2	11	
2002	–	6	7	1	14	
2003	–	10	8	6	24	
2004	–	11	5	4	20	
2005	–	9	2	1	12	
2006	–	11	3	5	19	
2007	–	15	7	3	25	
2008	–	10	6	6	22	
2009	1	7	2	–	10	
Total	2	105	52	37	196	

have either been killed or disappeared before they became part of the breeding population except for one recent migrant (O. Liberg, pers. comm.) To an outside observer, this situation suggests that some artificial gene flow of several animals for several generations would increase the founder number, result in a second episode of genetic rescue (or even genetic restoration), and potentially increase the number of wolves in this population to a self-sustaining number.

Second, the population of wolves on Isle Royale, a United States national park in Lake Superior, has long been offered as an example of a small population that has existed

for many decades without detrimental genetic effects. However, a recent study by Rääkkönen et al. (2009) has shown that 58% of the Isle Royale wolves exhibited some kind of congenital abnormality in the vertebral column compared to around 1% in outbred populations. Although the clinical significance of these defects is not known in wolves, similar abnormalities in dogs are associated with debilitating effects (Rääkkönen et al. 2009). The Isle Royale population is thought to have been founded by one female and one or a few males in the late 1940s and its longterm effective population size has been estimated as around 3.8 (Peterson et al. 1998). Because of global climate change, the likelihood of new natural founders coming across winter ice to the island is very unlikely. Again, this situation suggests that artificial gene flow from only a few successful animals could result in genetic rescue or restoration. However, before the extreme deterioration was documented by Rääkkönen et al. (2009), it had been suggested that it would be inappropriate to introduce unrelated wolves for esthetic, ecological, and political reasons (Peterson 1995; Rääkkönen et al. 2009), an opinion that deserves reconsideration at this point.

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Letter To The Editor

Defense of an Expanded Historical Range for the Mexican Wolf: A Comment on Heffelfinger et al.

SARAH A. HENDRICKS , Department of Biological Sciences and Institute of Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID, USA

STEPHAN KOBLMÜLLER, Institute of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria

RYAN J. HARRIGAN, Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, 300 LaKretz Hall, Los Angeles, CA 90095, USA

JENNIFER A. LEONARD, Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana (EBD-CSIC), 41092 Sevilla, Spain

RENA M. SCHWEIZER, Division of Biological Sciences, University of Montana, 32 Campus Dr., Missoula, MT 59812, USA

BRIDGETT M. VONHOLDT, Department of Ecology and Evolutionary Biology, Princeton University, M151 Guyot Hall, Princeton, NJ 08544, USA

ROLAND KAYS, North Carolina Museum of Natural Sciences, Biodiversity Lab, Raleigh, NC 27601, USA; and Department of Forestry & Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA

ROBERT K. WAYNE,¹ Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 610 Charles E. Young Drive East, Los Angeles, CA 90095, USA

In a recent review, Heffelfinger et al. (2017) question the utility of currently available genetic data and habitat suitability evidence as being sufficient justification for a proposed historical range expansion of the Mexican wolf (*Canis lupus baileyi*). According to these authors, the opinions of experts who observed wolves in the wild prior to extirpation and subsequent morphologic analysis of historical specimens should have more weight than genomic data in designation of a historical range. We assert that reintroductions and wildlife management plans should develop definitive expectations based on evolutionary hypotheses, and use genetic data to test them. The field of evolutionary genetics has experienced a revolution, given genome-wide typing and sequencing approaches, and we hope that Heffelfinger et al. might value this dramatic shift when considering conservation recommendations and future research possibilities. We have 4 main concerns with the work of Heffelfinger et al., which could be detrimental to the successful conservation and management of the subspecies.

MORPHOLOGY AND EVOLUTIONARY INFERENCE

Heffelfinger et al. (2017: 770) conclude that “differences in skull morphometrics are valuable indices to the geographic barriers to gene flow.” However, skull size, which is the principle phenotypic character used to deduce the original

historical distribution of Mexican wolves (Bogan and Melhop 1983, Nowak 1995), is not a reliable indicator of evolutionary distinction at the subspecies level because it can be plastically altered by habitat or prey size and abundance, or simply vary through time (Gortázar et al. 2000, Huston and Wolverton 2011, McNutt and Gusset 2012, Meachen and Samuels 2012). Taxonomy is best served by a focus on characters that have near perfect heritability, such as DNA sequence changes, which define evolutionary groups in a web-of-life framework (Arnold 2016, vonHoldt et al. 2017). The use of size as a diagnostic indicator of ancestry for Mexican wolves presumes that it maps to phylogeny. However, this is often not the case in wolves (Fan et al. 2016). In fact, none of the 5 currently supposed North American gray wolf subspecies based on morphologic characters are well matched with partitions based on genome-wide nuclear genetic markers (vonHoldt et al. 2011, Schweizer et al. 2016). A single genetic unit may contain wolves of varying size; hence, slightly larger wolves outside the original Mexican wolf range may share a common ancestry with them, which is consistent with the genetic findings of Leonard et al. (2005) and Hendricks et al. (2016).

THE OPINION OF EXPERTS AND TYPOLOGICAL THINKING

Heffelfinger et al. (2017) suggest that field observations made during the period of extirpation and subsequent cranial morphometric studies should dominate definitions of geographic range. However, early historical observations are weak data for range inference and opinions of experts (as defined by Heffelfinger et al. [2017]) were developed under a typological framework in large part prior to acceptance of the

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¹E-mail: rwayne@eeb.ucla.edu

modern evolutionary synthesis and did not incorporate evolutionary thinking. Even minor variations, often observed in just a few specimens, were used to define subspecies and races, and this Victorian legacy has carried over into modern times. For example, in the southern United States, historically black wolves were morphologically defined as a distinct species and then subspecies (*Canis rufus niger*). However, genetic analysis of gray wolves and closely related canids now suggest that the black coat coloration came from past hybridization with dogs, and gray and black wolves are the same species (Leonard et al. 2002, Anderson et al. 2009). The modern systematic framework for taxonomy uses shared-derived traits to define clades, such as those based on mtDNA or nuclear sequence data. These clades are then the basis for evolutionary taxonomic units (Moritz 1994). As such, size is a dubious character for evolutionary inference at best and average size may differ between populations depending on how they are defined and sample size. The classic text by (Young and Goldman 1944) cited by Heffelfinger et al. (2017) advocated 24 subspecies based on the sort of traits Heffelfinger et al. (2017) suggest can be used to define the Mexican wolf. This number was reduced to only 5 in recent analyses using a subset of skull measurements (Bogan and Melhop 1983, Nowak 1995). Some populations, such as the Mexican wolf, may be so narrowly defined that size does not overlap with other populations despite sharing a close evolutionary history. Under a modern view of admixture in current wolves, larger wolves observed by past naturalists may have been admixed or, despite size differences, are genetically and evolutionarily Mexican wolves. Large intergradation zones likely existed between Mexican wolves and other adjoining populations as suggested by the historical genetic data (Leonard et al. 2005, Hailer and Leonard 2008). Hence, a simple typological model as advocated by Heffelfinger et al. (2017) is not appropriate for informing either conservation or reintroduction decisions.

LIMITATIONS OF GENETIC DATA

Heffelfinger et al. (2017) indicate that the sample sizes included in Leonard et al. (2005) and Hendricks et al. (2016) are inadequate for genetic data to delineate historical range. Specifically, the authors suggest that it is impossible to rule out the process of genetic drift and incomplete lineage sorting to explain the distinct mtDNA clade for Mexican wolves. We maintain the Mexican wolf is one of the best defined groupings below the species level of any large North American vertebrate, which is supported by mtDNA sequence, microsatellite loci, genome-wide single nucleotide polymorphisms, and complete genome sequence data (Hedrick et al. 1997, Leonard et al. 2005, vonHoldt et al. 2011, 2016, Fan et al. 2016). The clade has a geographic coherence not expected for incomplete lineage sorting and recent analyses place the mtDNA clade in a worldwide context showing that it is highly distinct from other modern North American wolves (Koblmüller et al. 2016). The lack of geographic sampling is in part compensated by the large number of polymorphisms used to reconstruct evolutionary

history (Morin et al. 2004, Landguth et al. 2011, vonHoldt et al. 2011, 2016). In fact, a single genome can reveal much of the history of an entire species (Gronau et al. 2011, Li and Durbin 2011), and whole genome data support the distinctiveness of the Mexican wolf (Freedman et al. 2014, Fan et al. 2016, vonHoldt et al. 2016). Further, Hendricks et al. (2016) uses a combined approach of molecular, morphological (skull), and habitat suitability modeling data to incorporate evolutionary and ecological evidence to compensate for the paucity of available historical samples. Using multiple lines of scientific evidence, rather than single traits or *ad hoc* descriptions of one or few specimens, has similarly been used to characterize the taxonomic standings of the tiger (*Panthera tigris*), a system plagued with controversy that has hindered management efforts (Wilting et al. 2015).

HISTORICAL RANGE DEFINITION

In the absence of more extensive genetic data and given the questionable inferences from body size and historical observations, habitat suitability estimates in Hendricks et al. (2016) provide an alternative hypothesis for geographic range. Several conclusions by Heffelfinger et al. (2017) seem to misrepresent habitat suitability models. For instance, these models cannot provide evidence regarding the historical demography of populations. The fact that suitable habitat for Mexican wolves are predicted east and west of the Nevada and Arizona deserts implies only that suitable habitats exist for this species in these regions. Whether populations share a common history, or as Heffelfinger et al. (2017: 772) state “parallel changes driven by common ecological forces,” is not a question that these models, nor the conclusions of Hendricks et al. (2016), attempt to answer. These models do, however, identify habitat, outside the traditionally defined historical range of the Mexican wolf, that are currently suitable for this species. Heffelfinger et al. (2017) also argue against the ecological modeling results in Hendricks et al. (2016) because we predict suitable habitat exists as far north as southern Oregon. That areas far from the historical geographic range have suitable habitat, does not contradict its use for a guide to historical range, rather, criteria such as continuity or possible dispersal must be used in addition to defend an expanded historical geographic range. Much of the additional range we find in the Grand Canyon ecoregion and Southern Rockies, is confirmed by other landscape-scale analysis (Sneed 2001) and niche-based studies (Carroll et al. 2014) and with observations of long-range dispersal and admixture zones in modern wolves. Further, a web-of-life framework explains the evidence for a historical hybridization of *C. l. baileyi* and *C. l. mogollonensis*, which extends the historical range northwards, and allows for future admixture in this region (vonHoldt et al. 2017). Natural admixture zones should be part of reintroduction plans and admixed individuals providing ecosystem functionality should receive protection (Arnold 2016, Wayne and Shaffer 2016, vonHoldt et al. 2017).

Given the difficulty of establishing Mexican wolves in the United States and Mexico, which contrasts with the

considerable success of Yellowstone-Idaho reintroduction (Wayne and Hedrick 2011), expanded historical range and suitable habitat is needed, and as discussed above, is supported by ecological and genetic evidence. Further, climate change is likely to increase the proportion of suitable range northwards. Contemporary species conservation needs to move beyond strict adherence to maintaining or restoring populations within their putative historical ranges. Emphasis on the realized contemporary and likely future range may enhance the long-term viability of the highly endangered Mexican wolf.

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Mexican Gray Wolves and the Ecology of Fear: A Comparative Assessment of Community Assemblages in Arizona

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MEXICAN GRAY WOLVES AND THE ECOLOGY OF FEAR: A COMPARATIVE
ASSESSMENT OF COMMUNITY ASSEMBLAGES IN ARIZONA

by

JOSHUA SCOTT HOSKINSON

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SIGNED: *Joshua Scott Hoskinson*

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:



Melanie Culver

Dr. Melanie Culver
Assistant Professor, Wildlife Conservation and Management Program
Assistant Leader, Arizona Cooperative Fish and Wildlife Research Unit, USGS
School of Natural Resources and the Environment

April 10, 2018

Date



ARIZONA

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DEDICATION

This master's thesis is dedicated to two species in particular – the Mexican gray wolf (*Canis lupus baileyi*), and the coyote (*Canis latrans*). These two species have been subjugated to the intense pressure of environmental anthropocentrism, in which the wishes of humans were violently placed onto them and other predators. This anthropocentrism continues, although not as violently, and thus hopefully one day wolves, coyotes, humans, and other predators will be able to live in ecological balance.

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ABSTRACT

The dramatic ecological influence of northwestern gray wolves (*Canis lupus occidentalis*) on the landscape of Yellowstone National Park, termed a trophic cascade, has been well-documented and a marvel of reintroduction efforts of apex predators in the United States. These wolves have initiated both a traditional trophic cascade, which spans across trophic levels (predator-prey-vegetation), and a carnivore cascade, which spans across a predator guild (wolf-coyote-fox). The present study asks whether or not the Mexican gray wolf (*Canis lupus baileyi*) can influence the distribution of coyotes, gray foxes, elk, and mule deer in the Blue Range Wolf Recovery Area (BRWRA). This was completed through means of a camera trap survey, with two experimental plots within the core home range of Mexican gray wolves in the BRWRA (i.e. the “north” and “south” plots) and one plot west of the BRWRA (i.e. the “west” plot) without sustained Mexican gray wolf activity. After the camera survey, detection rates for the coyotes, gray foxes, elk, and mule deer were calculated. Through both a generalized linear mixed modelling (GLMM) approach, and a Kruskal-Wallis analysis, detection rates were compared across the north, south, and west plots. No statistically significant differences were found in detection rates between the plots, which means there is no indication that the Mexican wolves are influencing the distribution of coyotes, gray foxes, elk, or mule deer in the region. However, since the Mexican wolf population in Arizona is heavily managed, as well as issues with missing data in the camera survey itself, it would be unwise to make broad claims about the ecological implications of Mexican gray wolf reintroduction from this study.

INTRODUCTION

Apex Predators, Trophic Cascades, and Non-Trophic Cascades

Apex predators, or those species that occupy the highest trophic level in a given environment, have been repeatedly shown to have strong effects upon their respective communities and ecosystems (Ripple et al 2014b). Attributes that are frequently ascribed to these apex predators are: body size of larger than 18-34 kg, K-selected reproductive strategy, cooperative social behaviors, “family planning” (e.g. female reproductive suppression and infanticide), and territoriality; these characteristics facilitate apex predators to intrinsically regulate their own population sizes (Wallach et al 2015). Whilst having this ability, apex predators also hold a keystone role in communities and ecosystems by extrinsically regulating the population sizes and densities of prey species as well as other species within the predator guild; this property can influence the occurrence of an ecological phenomenon known as an ecological cascade.

Ecological cascades are defined as reciprocal effects that alter the abundance, biomass, or productivity of a population, community, or trophic level across more than one link in a food web (Pace et al 1999). Two broad categories of ecological cascades occur – trophic cascades and non-trophic cascades. Trophic cascades are those ecological cascades that occur across trophic interactions, or those interactions that involve the consumption of one species by another (i.e. predator-prey, plant-herbivore, etc), whereas non-trophic cascades would be defined as ecological cascades that occur across other types of species interactions, such as mutualism and competition (O’Connor et al 2012, Sanders et al 2013). For this discussion, it is important to note the different types of ecological cascades, as apex predators have the propensity to initiate ecological cascades across trophic levels (e.g. predator-herbivore-vegetation) or across non-trophic levels, such as within a predator guild (e.g. wolf-coyote-fox).

Ecological cascades have two mechanisms of operation, both integral to the overall cascade effect: N-driven cascades and μ -driven cascades. In N-driven ecological cascades the apex predator can directly influence the census population size of organisms. In μ -driven ecological cascades the apex predators can influence the foraging behavior of either prey species or other carnivores within their guild and thus more

indirectly influence the population density and distribution across a landscape (Brown, Laundre, and Gurung 1999). It is namely this last mechanism that can be more important in terms of optimal foraging strategy of either other carnivores or prey species, and this instills a “landscape of fear” for these species in which these species incorporate predation risk into their optimal foraging strategies (Brown, Laundre and Gurung 1999; Ripple and Beschta 2004a). Both of these mechanisms have been demonstrated to be equally important, with the μ -driven mechanism perhaps being stronger due to predation risk factor being incorporated into the optimal foraging strategy with the presence of an apex predator (Lima and Dill 1990; Schmitz, Beckerman, and O’Brien 1997).

When these apex predators are missing from their communities or ecosystems, either through natural extinction or anthropogenic local extirpation, irruptions of herbivores likely result, which would increase browsing rates and cause trophic downgrading of local environments (Estes et al 2011). This would also cause the release of mesopredators to potentially assume an “apex predator-like” role in the environment, although in some cases the mesopredator cannot attain the apex predator ecological niche (Ritchie et. al 2009; Ripple et. al 2013; Wallach et al 2015; Jones et. al 2016). Thus, the importance of maintaining the presence of apex predators within communities and ecosystems is to keep the ecosystems stable and prevent trophic downgrading.

Case Study: Gray Wolves (*Canis lupus occidentalis*) and Yellowstone National Park

Perhaps one of the most well-known apex predators in the United States is the gray wolf (*Canis lupus sbspp.*). The gray wolf was persecuted during the early 1900s due to ubiquitous misunderstanding and fear of the wolf, as well as the perceived threat of livestock depredation. Following this fear, misunderstanding, and anthropocentrism, the United States Congress established the United States Bureau of Biological Survey with the Division of Predator and Rodent Control (PARC) in 1915 which had expressed the purpose of eliminating the wolves and other large predators from the United States (Brown 1983; Mech and Boitani 2003).

As a result, not only was trophic downgrading caused due to increased browsing rate by elk (*Cervus canadensis*) and deer (*Odocoileus virginianus* and *Odocoileus hemionus*), but the tide was turning as American culture gained a more favorable opinion of wolves (Mech and Boitani 2003). As such, wolf conservation programs were initiated and wolves (*Canis lupus occidentalis*) were captured in Canada (Chambers et al 2012) and released into Yellowstone National Park in 1995 and 1996 (Merkle et al 2009). Research following the reintroduction of gray wolves into Yellowstone National Park studied how the wolves affected lower trophic levels and the abiotic environment (i.e. trophic cascade), and also included carnivore cascades (i.e. non-trophic cascade).

Researchers attempted to find evidence for occurrence of a trophic cascade in Yellowstone National Park resulting from wolf reintroduction. Ripple and Larsen(2000) documented a historic trend of aspen (*Populus tremuloides*) recruitment in Yellowstone National Park and found that aspen recruitment was diminished during the years of wolf extirpation from Yellowstone National Park, and the age class that would have been recruited during this time was missing (Larsen and Ripple 2003). Following the wolf reintroduction, studies found that reintroduction of the gray wolf contributed to increased recruitment of aspen (Ripple et al 2001), cottonwoods (*Populus spp.*) (Beschta 2003), willow (*Salix spp.*) (Ripple and Beschta 2004b), and alders (*Alnus incana tenuifoli*) (Ripple, Beschta, and Painter 2015). This trend of increased recruitment is still maintained 15 years after the initial wolf reintroduction into Yellowstone National Park (Ripple and Beschta 2012). In addition, studies have found that due to the increase tree recruitment the morphology of Yellowstone rivers have changed (Beschta and Ripple 2006; Beschta and Ripple 2012), leading to the inference that wolves had an effect on the abiotic environment of Yellowstone National Park. Thus, in terms of trophic cascades, the northwestern gray wolf effect on the Yellowstone National Park ecosystem has been quite remarkable.

This trophic cascade in Yellowstone National Park resulting from wolf reintroduction was primarily a μ -driven cascade; that is to say, it was primarily behavior-driven. Through reintroducing the wolf back into the environment, the elk (*Cervus canadensis*) changed its optimal foraging strategy by

incorporating the risk of predation and thus leaving “plant refugia” in locales that have high predation risk (Ripple and Beschta 2004a). It is these “plant refugia” locales where the greatest amount of tree recruitment (aspen, cottonwood, willow, and alder) occurred within Yellowstone National Park. Therefore, the major mechanism of trophic cascade occurrence was not through population reduction, but rather through behavior modifications that redistributed the density of elk post-reintroduction (Ripple and Beschta 2004a).

Carnivore Cascades

In addition to the remarkable trophic cascades, carnivore cascades occur within the carnivore guild that lives in sympatry with the apex predator (e.g. wolf-coyote-fox). Studies have shown that the mechanisms are the same except behavior modification is achieved by competitors of the same guild rather than at different trophic levels. For example, in Yellowstone National Park as a result of the wolf reintroduction, interference competition between coyotes (*Canis latrans*) and the northwestern gray wolves (*Canis lupus occidentalis*) causes a change, not necessarily in the population sizes of the coyotes, but in the population density and abundance of coyotes in a particular locale (Berger and Gese 2007; Berger, Gese, and Berger 2008; Merkel et al 2009).

These systems influence the distribution and abundance of coyotes, but the wolves can also extend their influence to other systems. For instance, by influencing the distribution and abundance of coyotes, wolves can affect pronghorn distributions (Berger, Gese, and Berger 2008), allow for irruptions in small mammal populations such as rodents (Miller et al 2012), and influence the distribution of foxes (*Vulpes spp.* and *Urocyon cinereoargenteus*) at the local scale (Levi and Wilmers 2012) and the continental scale (Newsome and Ripple 2014). Wolves can also allow recovery of grizzly bear (*Ursus arctos*) populations by limiting browsing of berry plants by elk (Ripple et al 2014a). Therefore, wolf reintroduction could assist in the recovery and conservation of other predator species by proxy. It is important to note that wolves involved in the carnivore cascades discussed here were *Canis lupus occidentalis*, one of the larger wolf subspecies.

Mexican Gray Wolves in Arizona and New Mexico

Mexican gray wolves (*Canis lupus baileyi*), the smallest subspecies of gray wolf, was historically distributed in the American Southwest and Mexico (Brown 1983; Beschta and Ripple 2010; Hendricks et al 2016). Beschta and Ripple (2010) was the only study to examine whether or not Mexican gray wolves initiated a trophic cascade after their reintroduction to the Apache-Sitgreaves National Forest in 1998. They analyzed aspen recruitment in the BRWRA in the Apache National Forest near Alpine, Arizona in three locales: a refugium site inaccessible to both elk and cattle, an old growth site accessible to elk, and a thinned site accessible to elk. They found aspen in the refugium site was consistent with climactic variation, and no significant difference from what aspen recruitment was expected from climactic variation post-reintroduction of the Mexican gray wolf. As this was contradictory to what Ripple and Beschta observed in previous studies with wolves in Yellowstone National Park, they concluded there was no trophic cascade occurring in the BRWRA of 2010.

Curiously, there have been no studies of whether or not N-driven or μ -driven cascades have occurred with regard to the Mexican gray wolf. In other words, there have been no studies on whether Mexican gray wolves have influenced either the population size or population density of other sympatric carnivores such as coyote or fox. In addition, there have been no studies on the interactive behavior between Mexican gray wolf and these sympatric canids. If the Mexican gray wolf can influence the population size and/or distribution of coyote and fox, it would be important to estimate whether or not Mexican gray wolves have established an ecologically effective density to initiate a carnivore cascade (Soulé et al 1999). In addition, data used in the Beschta and Ripple (2010) study is approaching 10 years old, and updated information to reassess the question of Mexican gray wolf involvement in any ecological cascades would be useful.

The Present Study

For the present study, the primary objective is to estimate whether or not the Mexican gray wolf is influencing the distribution of prey species, such as Rocky Mountain Elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), as well as competitor species, specifically coyote (*Canis latrans*) and gray fox (*Urocyon cinereoargenteus*) in the BRWRA. In concordance with previous studies, the hypothesis of this study is that Mexican wolves have influenced the distribution of both prey and competitor species, and therefore we predict that areas without Mexican gray wolves would have a higher abundance of prey and competitor species than areas with Mexican gray wolves.

METHODS

Study Site Description

The present study was conducted within the confines of the BRWRA and adjacent areas, which are all within the larger Mexican Wolf Experimental Population Area (MWEPA). The MWEPA is defined by the geographical area that encompasses the states of Arizona and New Mexico, bounded by Interstate 40 (I-40) to the north and the international United States-Mexico border to the south (US Fish and Wildlife Service, 2017). The MWEPA itself contains a variety of biotic communities as defined by Brown and Lowe (1994), ranging from Subalpine Grassland in the White Mountains of Arizona, to the Sonoran Desertscrub that is characteristic of Southern Arizona.

This study primarily dealt with the Rocky Mountain (Petran) Montane Conifer Forest and all cameras are at elevations between 1900m-2900m. This biotic community is semi-ubiquitous across the Mogollon Rim of Arizona – this is a major biotic community that is found within the BRWRA, and as such a majority (sixteen) of camera sites are within this biotic community, specifically located in pure stands of Ponderosa Pine (*Pinus ponderosa*). Other adjacent biotic communities present in the BRWRA are Rocky Mountain (Petran) Subalpine Conifer Forest, a mixed conifer forest including aspen (*Populus tremuloides*), and Subalpine Grassland, and two cameras are placed within these biotic communities (Figure 1). It is useful to mention that not only wolves, but Rocky Mountain Elk (*Cervus canadensis*), mule deer (*Odocoileus hemonius*), coyotes (*Canis latrans*), and gray foxes (*Urocyon cinereoargenteus*) are ubiquitous throughout this area (Anderson and Wallmo 1984, Bekoff 1977, Fritzell and Haroldson 1982, Nowak 1999).

Wildlife Camera Experimental Design

The present study consisted of three plots (two experimental plots with high wolf activity and one control plot with no wolf activity), with six (6) cameras per plot for a total of eighteen (18) camera sites. The experimental plots, hereafter referred to as “north” and “south” plots and within the boundaries of the BRWRA, were in areas of sustained Mexican wolf activity across multiple years. The “north” experimental

plot consisted of: Burro Mountain, Mamie Creek, Roger's Marsh, Rudd Creek, Sherlock Draw, and Sizer Knoll camera sites. The "south" experimental plot consisted of: Crow Poison, Double Cienega, Foote Creek, Highline Trail, Reservation Creek, and Tenney Mountain camera sites. The control plot, hereafter referred to as the "west" plot, was located west of the experimental plots in an area of no Mexican wolf activity. The "west" control plot consisted of: Canyon Creek, Hess Draw, Hog Wash, Potato Field Draw, Upper Cherry Creek, and Willow Creek camera sites. All three plots were within the Apache Sitgreaves National Forest in Arizona, and in the same habitat type (Figure 1).

Remote digital wildlife trail cameras (Covert Deuce) were placed within experimental plots based on Mexican wolf pack home range data for 2014, 2015, and 2016 provided by wolf biologists with the Mexican Wolf Interagency Field Team (IFT). The home ranges included 90% kernels, which represented areas Mexican wolves occupied 90% of the time, and 50% kernels, which represented areas Mexican wolves occupied 50% of the time (50% kernels represent the "core" home range of each pack and were always within the boundaries of the 90% kernels for a given wolf pack). Home range maps across years were compared, and locations where the 50% kernel for a given pack overlapped across multiple years were candidates for camera placements for the experimental plots. The same home range maps were referred to when deciding the camera locations for the control plot. Areas that were excluded from any 90% kernel of any given Mexican wolf pack over multiple years were candidates for the control plot. Once candidate locations were identified, and since the wolf biologists of the Mexican Wolf IFT knew each of the locations with certainty, the wolf biologists decided on exact locations for wildlife cameras in all three plots and provided GPS coordinates for each camera.

Camera settings were: 1 photo per event, 5MP photo size, 1 minute interval between photos, normal PIR sensitivity, xenon flash for night photos, and normal range for flash unless a particular camera site required adjustment in PIR sensitivity or flash range.

Each of these cameras were baited by placing scent lure (Mark June Cherry Red commercial bait) approximately 5m from the camera at ground height each time the camera was checked. This was done to maximize the number of carnivores that walked in front of the camera. Each camera was checked by the wolf biologists of the Mexican wolf IFT approximately every 2-2.5 months, for a total of one year, starting approximately December 1, 2016, and ending by November 30, 2017. In this way, data was collected for each off our seasons and for one full year.

Data Analyses

Analyses of this data was performed in two parts. First, the photos downloaded from each camera were sorted using a hierarchical method as described in Sanderson and Harris (2013). This hierarchical method involved sorting photos by plot (i.e. north, south, or west), and within each of these plots into location folders. There was a location folder for each camera, and within each location folder was a folder for each species photographed. Following photo sorting, the total number of photographs captured at each location, trap-nights (i.e. camera trap effort) for each camera location, number of independent photos of each species captured, and detection rates of each species captured were calculated with an algorithm in the program (Sanderson and Harris 2013). Photographs were considered independent if 60 minutes passed without the camera capturing another photograph of the same species – if there was another photo of the same species within that 60 minute interval, it was discounted. For further analyses, photographs that contained no species (i.e. “ghost” photographs), or photographs that contained domestic species (e.g. domestic dog *Canis lupus familiaris*, domestic cow *Bos taurus*, or humans *Homo sapiens*), were discounted. In this way, the number of independent photographs of wildlife was calculated. The independent photographs of all wildlife species, which included the independent photographs of the species of interest (i.e. coyotes, gray foxes, elk, and mule deer), were used for further statistical analyses, which consisted of analyzing differences in detection rates for species of interest between plots.

Statistical analyses were performed in two ways to provide multiple measures to determine significance for detection rates between plots. First, generalized linear mixed modelling analyses were completed using a GLMM package in the statistical program R (Knudson 2017). Secondary analyses were the Shapiro-Wilk normality test, the Kruskal-Wallis test, and Conover's test of multiple comparisons with a Holm-Bonferroni correction using the PMCMR package in the statistical program R (Thompson et al 2010; Bates et al 2011; Pohlert 2014; Li et al 2014; Gustavo Pinoargote, personal communications).

RESULTS

Results of Camera Trap Analysis

In the north plot, there were a total of 10,034 photographs captured, 313 of which were independent photographs of wildlife species, across a total of 1,679 trap nights. In the south plot, there were a total of 7,342 photographs captured, 295 of which were independent photographs of wildlife species, across a total of 1056 trap nights. Lastly, in the west (control) plot, there were a total of 21,444 photographs captured, 616 of which were independent photographs of wildlife species, across a total of 1,782 trap nights. This results in a total of 38,820 total photographs captured across all 18 camera locations, 1,224 of which were independent photographs of wildlife species, across a total of 4,517 trap nights (Table 1, Table 2, Table 3).

Further analyses were completed by the software program in Sanderson and Harris (2013). These analyses calculated detection rates of each species (i.e. coyotes, gray fox, elk, and mule deer) by dividing species abundance (i.e. the number of photos of that species) by camera trap effort (i.e. trap-nights). In this way, we are able to analyze the abundance of each species per unit camera effort. In the north plot, the average detection rate was 1.44 coyotes/unit effort for coyotes, 0.05 gray foxes/unit effort, 12.52 elk/unit effort, and 3.16 mule deer/unit effort. In the south plot, the average detection rate was 0.788 coyotes/unit effort, 0.16 gray foxes/unit effort, 24.03 elk/unit effort, and 5.05 mule deer/unit effort. Lastly, for the west (control) plot, the average detection rate was 5.38 coyotes/unit effort, 0.97 gray foxes/unit effort, 14.01 elk/unit effort, and 8.95 mule deer/unit effort (Table 4). These detection rates were then statistically analyzed.

Results of Statistical Analysis

Statistical analyses consisted of first using a generalized linear mixed modelling (GLMM) approach to test for significant differences in the detection rates amongst the north, south, and west plots. The GLMM analyses for coyotes ($p = 0.604$), gray foxes ($p = 0.259$), elk ($p = 0.480$), and mule deer ($p = 0.270$) all resulted in non-significant differences between the three plots for any species of interest.

Supplementary statistical analyses were performed to provide additional measures for significant differences between the three plots. A Shapiro-Wilk normality test was performed to see if the distribution of detection rates was normally or non-normally distributed, and the distributions of detection rates for coyotes ($p = 1.402e-07$), gray foxes ($p = 2.33e-07$), elk ($p = 0.0002399$), and mule deer ($p = 0.002226$) were all determined to be non-normal due to all p-values being below the threshold of 0.05. Next, a Kruskal-Wallis test was performed for each species, and the differences between plots for coyote ($p = 0.2686$), gray fox ($p = 0.2668$), elk ($p = 0.8948$), and mule deer ($p = 0.1923$) were all determined to be non-significant. Lastly, a Conover's test for multiple comparisons, with Holm-Bonferroni corrections, was performed between each plot for each species of interest to provide a post-hoc analysis of each pairwise comparison. For coyote, the north-west ($p = 0.64$), south-west ($p = 0.37$), and north-south ($p = 0.64$) pairwise comparisons all resulted in non-significant differences between plots. For gray fox, the north-west ($p = 0.36$), south-west ($p = 0.65$), and north-south ($p = 0.65$) pairwise comparisons all resulted in non-significant differences between plots. For elk, the north-west ($p = 1.00$), south-west ($p = 1.00$), and north-south ($p = 1.00$) pairwise comparisons all resulted in non-significant differences between plots. Lastly, for mule deer, the north-west ($p = 0.24$), south-west ($p = 0.50$), and north-south ($p = 0.51$) pairwise comparisons all resulted in non-significant differences between plots.

DISCUSSION

According to these analyses, it is a reasonable suggestion that Mexican gray wolves have yet to be successful in achieving their ecological role as top predator in the BRWRA. This conclusion is reached by the lack of significant difference of detection rates between plots with Mexican gray wolves (as measured by overlap of 50% kernels, which represent the sustained presence of a core home range for one or multiple packs of Mexican gray wolves) compared to plots without Mexican gray wolves. These non-significant differences were also true for coyotes, gray foxes, elk, and mule deer. From statistical analyses, another reasonable suggestion is that Mexican gray wolves do not affect the distribution of coyotes, elk, or mule deer. Given that the Mexican gray wolves have been consistently been in the area for 20 years, there is some question of why they have not yet achieved their ecological role as top predator. This result is contradictory to the patterns observed in Yellowstone National Park and other areas of wolf reintroduction, yet the Mexican wolves not having an effect on ecological cascades was observed with prior data collected for trophic cascade (Beschta and Ripple 2010). Though, this relationship between the north, south, and west plots only implies a correlation, not a causation, between wolf presence/absence and detection rates of elk, mule deer, coyote, and gray fox. From this study, we can say that there is no relationship between wolf presence/absence and the detection rates of the species of interest, but we cannot say that the lack of significant difference in detection rates between the plots was because of wolf presence/absence. This lack of significant difference between the plots may be due to a variety of factors.

One plausible explanation for the lack of significance may be due to the Mexican gray wolf population size and range. In 2016, the Mexican gray wolf population was estimated to be around 113 wolves occupying 41,735 km², primarily in New Mexico (US Fish and Wildlife Service, 2016) whereas the wolf population of approximately the same size in Yellowstone National Park occupied 8989 km² (Smith et al 2017). For a landscape of fear to be instituted in coyotes, gray foxes, elk, and mule deer, these species would have to encounter the Mexican wolves, in either a competitive or preyed upon manner, often enough for the Mexican wolves to be incorporated into each species' optimal foraging strategy (Lima and Dill 1990,

Brown, Laundré, and Gurung 1999) as happened in the Yellowstone National Park system (Ripple and Beschta 2004a). If these species were to encounter the Mexican gray wolf on a consistent basis, it would be reasonable to assume a modified distribution of these species, as measured by detection rate, such that a lower detection rate is observed for coyotes, elk, and mule deer, but a higher detection rate for gray foxes, in areas consistently occupied by Mexican wolves. If, however, a Mexican gray wolf is encountered only occasionally, not consistently, then the risk of predation/competition by the Mexican gray wolf would not be incorporated into the species' optimal foraging strategy. Therefore, in this case, we should not see differences in distribution of these species as measured by detection rate. According to our analyses there is no statistically significant difference in detection rate between plots with Mexican wolves and plots without, thus we suggest that a landscape of fear has not been instituted in the Blue Range Wolf Recovery Area.

Another reasonable explanation for the lack of significance is the reintroduced habitat itself. Brown (1983) states that the Mexican gray wolves historically have been associated with montane forests and woodlands within vegetation such as oak woodlands, pinyon-juniper forests, and adjacent grasslands while avoiding the Mohave, Sonoran, and Chihuahuan deserts and adjacent semi-desert grasslands. These habitats would be consistent with the habitats of Madrean Evergreen Woodland, Interior Chaparral and Montane Grasslands of Brown and Lowe (1994), which are found in the Sky Islands of Southern Arizona and New Mexico and extends well into the Sierra Madre Occidental and Sierra Madre Oriental mountain ranges of Mexico. Historically, there was perhaps another subspecies of wolf (*Canis lupus youngi* or *Canis lupus mogollonensis*) that occupied Petran Montane Conifer Forests of the Blue Range Wolf Recovery Area (Brown 1983). Both Brown (1983) and Hendricks (2016) support the notion that current territory occupied by the reintroduced Mexican gray wolves would have been the extreme farthest north of the historical distribution. In addition, according to Brown (1983) the presumed main prey item of the Mexican wolf would have been Coues' White-tailed deer (*Odocoileus virginianus couesi*) and possibly the mule deer (*Odocoileus hemionus*), not elk (*Cervus canadensis*). Thus, the lack of significance could be partially due

to the reintroduction effort being in non-historical habitat, which may affect hunting behavior, as well as presumably a non-historical prey base.

It is also worth mentioning that as part of the management plan of the Mexican wolf, supplementary food caches (i.e. road-killed native prey carcasses provided to wolf packs to assist in feeding young) and diversionary food caches (i.e. road-killed native prey carcasses provided to wolf packs to reduce potential conflicts with livestock) are provided to aid the Mexican wolves (US Fish and Wildlife Service, 2016). This provision of food caches may affect the hunting behavior of the wolves, and thus may have some effect on the ecological implications of the reintroduction effort. Though, this has not been directly studied, and so an ecological study needs to be done to assess the ecological implications of these food caches.

In any case, the Mexican gray wolf could be argued to have no significant effect on coyotes, gray foxes, elk, or mule deer for any of these reasons, or for reasons that we have not considered here. Though, due to these concerns, one should be cautious about making such claims, as this study only indicates that Mexican gray wolves have yet to achieve their top predator status. The present study is not complete in its assessment of the ecological implications of the reintroduction efforts of the Mexican wolves and further examination of a variety of questions need to be addressed before making any kind of claim as to the ecological implications of the Mexican wolf.

These further examinations of effects could take a variety of forms. First, there could be further analyses on the effects across the prey guild, which would include the prey base of not only the Mexican wolves (e.g. ungulates), but also the prey base of competitors (e.g. coyotes, pumas), as well as a vegetation assessment to analyze bottom-up trophic effects. Second, there should be a more complete analysis on other potential prey items of Mexican wolves, rather than solely focusing on elk and mule deer. One notable instance that would be necessary to look at would be the effects that Mexican gray wolves have on pronghorn (*Antilocapra americana*), as they were detected at three cameras: Hess Draw (1.32 pronghorn/unit effort), Potato Field Draw (3.21 pronghorn/unit effort), and Willow Creek (0.36 pronghorn/unit effort), which results in an average detection rate of 0.79 pronghorn/unit effort. Pronghorn

were only found in the west plot, and thus were not able to be analyzed in the present study. Though, pronghorn are known to be ubiquitous both historically and currently (Hall and Kelson 1959; Nowak 1999), and so it would be interesting to look at potential reasons as to why no pronghorn were detected at all within the 50% kernels of the Mexican gray wolf home range.

In addition to these assessments, it would be ideal to compare the results of this study to that of historical records and other Mexican gray wolf reintroduction efforts. For the historical assessment, it would be crucial to know how the Mexican wolf has historically affected the distribution of coyotes, gray foxes, mule deer, and white-tailed deer (*Odocoileus virginianus*) through trapping records (Brown 1983) in the American Southwest and Mexico. In this way, we would develop a metric by which to compare current distributions of these species of interest to their historical distributions. As for other Mexican gray wolf reintroduction sites, through a binational effort there were Mexican wolves reintroduced in the northern Sierra Madre Occidental of Chihuahua and Sonora in Mexico, and 41 wolves have been reintroduced in this area. As of 2017, these wolves are still in the establishment phase and approximately 31 wolves inhabit the northern Sierra Madre Occidental (US Fish and Wildlife, 2017). With this particular reintroduction site being in historical Mexican wolf territory (Brown 1983, Hendricks et al 2016), this would be perhaps a more suitable location for studies into the ecological effects of Mexican wolves. After all, this is the similar habitat that Roy T. McBride trapped the seven Mexican wolves in 1980 which founded the captive breeding program the ancestors to all modern Mexican wolves (Brown 1983).

In any case, whether it's continuing this study in the northern Sierra Madre Occidental in Mexico, to analyzing historical records in both Mexico and the American Southwest, to broadening the scope and looking at other competitors, prey bases, and vegetation assessments, there are far more questions to pursue before a proper conclusion on the ecological impact of the Mexican wolf reintroduction can be obtained.

FIGURE

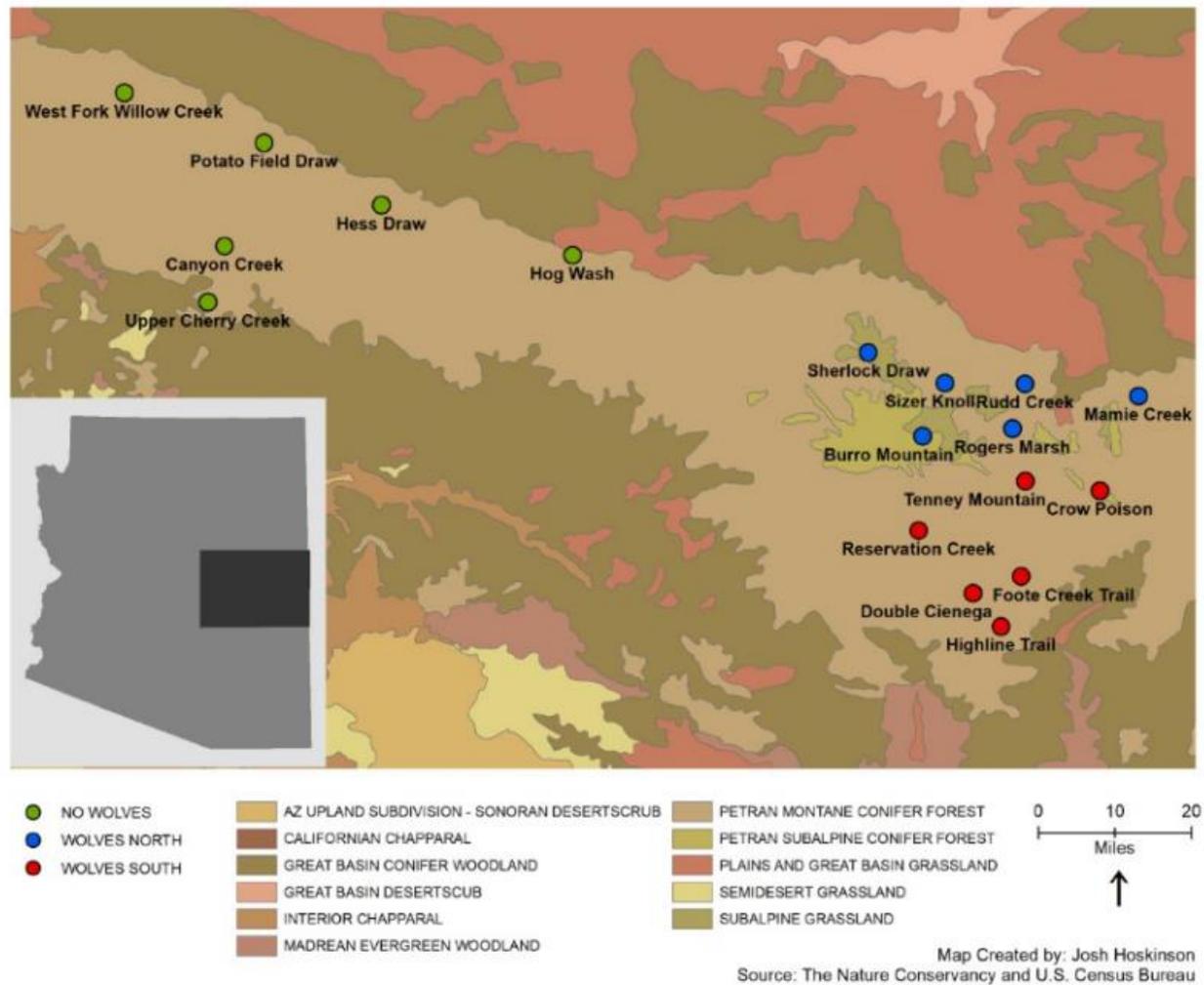


Figure 1. Map of camera locations overlaid onto the biotic communities map from Brown and Lowe (1994). The study area in relation to the entire state of Arizona is depicted in the insert map in the bottom left hand corner. Legend includes the biotic communities, camera locations for the no wolves plot (i.e. west plot) labeled in green, the north plot labeled in blue, and the south plot labeled in red.

TABLES

Table 1. North study plot wildlife pictures. Description of the start/end dates, number of trap-nights, the total number of pictures, and the total number of independent pictures of wildlife for the north plot. Four of the six cameras were down for a period of time, and so each start/end date for each period that the camera was actively capturing photographs is described. Trap-nights, or camera effort, are the number of 24 hour periods that the camera was actively capturing photographs. Total number of pictures includes all of the photographs obtained by that particular camera. The total number of independent pictures of wildlife excludes multiple photographs of the same species taken within a 60-minute period, photographs with no species (i.e. “ghost” photos), and photos of domesticated species (e.g. domestic dogs, domestic cows, and humans). This column includes, but isn’t limited to, the species of interest.

Location	Start Date	End Date	Trap Nights	Total Number of Pictures	Total Number of Independent Pictures of Wildlife
Burro Mountain	1. 11/6/2016 2. 7/5/2017	1. 4/19/2017 2. 8/10/2017	268	908	22
Mamie Creek	1. 11/15/2016 2. 6/8/2017	1. 5/15/2017 2. 10/12/2017	332	3155	43
Roger’s Marsh	1. 11/5/2016 2. 7/3/2017	1. 3/28/2017 2. 10/26/2017	349	2539	49
Rudd Creek	11/25/2016	6/24/2017	97	113	46
Sherlock Draw	11/6/2016	10/16/2016	344	1688	91
Sizer Knoll	1. 11/6/2016 2. 8/1/2017	1. 4/15/2017 2. 8/18/2017	289	1634	62
Total	-	-	1679	10034	313

Table 2. South study plot wildlife pictures. Description of the start/end dates, number of trap-nights, the total number of pictures, and the total number of independent pictures of wildlife for the south plot. Two of the six cameras were down for a period of time, and so each start/end date for each period that the camera was actively capturing photographs is described. Trap-nights, or camera effort, are the number of 24 hour periods that the camera was actively capturing photographs. Total number of pictures includes all of the photographs obtained by that particular camera. The total number of independent pictures of wildlife excludes multiple photographs of the same species taken within a 60-minute period, photographs with no species (i.e. “ghost” photos), and photos of domesticated species (e.g. domestic dogs, domestic cows, and humans). This column includes all wildlife photos, not just the species of interest.

Location	Start Date	End Date	Trap Nights	Total Number of Pictures	Total Number of Independent Pictures of Wildlife
Crow Poison	1. 1/13/2017 2. 7/26/2017	1. 3/1/2017 2. 8/27/2017	178	215	11
Double Cienega	11/5/2016	7/26/2017	257	527	167
Foot Creek	11/16/2016	5/19/2017	179	3774	73
Highline Trail	11/22/2016	6/23/2017	157	2260	14
Reservation Creek	1. 11/30/2016 2. 7/26/2017	1. 3/24/2017 2. 9/9/2017	282	514	28
Tenney Mountain	11/30/2016	1/13/2017	3 [†]	52	2
Total	-	-	1056	7384	295

† There was a malfunction in the camera at Tenney Mountain, South Plot. Thus, even though it was out longer than three nights, it only had pictures for three nights.

Table 3. West study plot wildlife pictures. Description of the start/end dates, number of trap-nights, the total number of pictures, and the total number of independent pictures of wildlife for the west plot. Three of the six cameras were down for a period of time, and so each start/end date for each period that the camera was actively capturing photographs is described. Trap-nights, or camera effort, are the number of 24 hour periods that the camera was actively capturing photographs. Total number of pictures includes all of the photographs obtained by that particular camera. The total number of independent pictures of wildlife excludes multiple photographs of the same species taken within a 60-minute period, photographs with no species (i.e. “ghost” photos), and photos of domesticated species (e.g. domestic dogs, domestic cows, and humans). This column includes all wildlife photos, not just the species of interest.

Location	Start Date	End Date	Trap Nights	Total Number of Pictures	Total Number of Independent Pictures of Wildlife
Canyon Creek	1. 2/16/2017 2. 6/9/2017	1. 5/23/2017 2. 10/5/2017	232	6065	193
Hess Draw	1. 11/27/16 2. 6/8/2017	1. 5/18/2017 2. 10/13/2017	303	2149	102
Hog Wash	1. 11/30/2016 2. 6/8/2017	1. 5/15/2017 2. 11/2/2017	332	3159	101
Potato Field Draw	11/27/2016	11/9/2017	312	767	80
Upper Cherry Creek	11/26/2016	11/10/2017	302	2849	45
Willow Creek	11/26/2016	9/23/2017	301	6455	95
Total			1782	21444	616

Table 4. Description of the camera effort and the detection rates for coyotes, gray foxes, elk, and mule deer for each of the locations at each of the study plots. Camera effort, or trap-nights, is the number of 24 hour periods that the camera was actively capturing photographs. Detection rate is the number of photographs of that species, at that location, per unit camera effort.

Plot	Location	Camera Effort	Coyote Detection Rate	Gray Fox Detection Rate	Elk Detection Rate	Mule Deer Detection Rate
North	Burro Mountain	268	0.00	0.00	7.46	0.00
	Mamie Creek	332	0.90	0.00	10.84	0.00
	Roger's Marsh	349	0.29	0.29	12.61	0.29
	Rudd Creek	97	4.12	0.00	17.53	13.40
	Sherlock Draw	344	2.62	0.00	10.76	1.45
	Sizer Knoll	289	0.69	0.00	15.92	3.81
South	Crow Poison	178	1.69	0.00	3.93	0.00
	Double Cienega	257	2.33	0.39	52.53	2.33
	Foot Creek	179	0.00	0.56	15.08	7.26
	Highline Trail	157	0.00	0.00	0.64	0.00
	Reservation Creek	282	0.71	0.00	5.32	0.00
	Tenney Mountain	3	0.00	0.00	66.67	0.00
West	Canyon Creek	232	25.43	0.86	23.28	18.1
	Hess Draw	303	1.32	0.00	23.1	6.93
	Hog Wash	332	1.51	0.00	7.23	20.48
	Potato Field Draw	312	1.28	0.00	18.27	0.32
	Upper Cherry Creek	302	1.32	0.66	6.62	4.3
	Willow Creek	301	1.44	4.31	5.57	3.59

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NEWS AND COMMENTARY

Genetics of wolf conservation

Genetics and wolf conservation in the American West: lessons and challenges

R Wayne and P Hedrick

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Top predators are endangered throughout the world because of human persecution and habitat destruction. Plans to conserve and restore predator populations are often contentious, but few species are as problematic as the gray wolf (*Canis lupus*). In the United States, wolf conservation policy and management has often been schizophrenic, ranging from predator control and open hunting to reintroduction and absolute protection. Likewise, public and scientific opinion is equally divided between those who maintain that wolves are an essential part of ecosystems and provide top-down effects contrasting with those who believe wolves cannot readily coexist with humans, especially in landscapes dominated by ranching or farming. In the American West, there have been large-scale reintroductions of the gray wolf (*C. lupus nubilus*) in the Northern Rocky Mountains (NRMs) and the Mexican wolf (*C. lupus baileyi*) in the southwest, which provide important lessons for reintroduction efforts elsewhere. In this study, we specifically discuss wolf conservation in the American West in relation to critical genetic factors that affect restoration, recovery and conservation. We also discuss the natural colonization of wolves in Sweden and Norway, and discuss a synthesis of problems and solutions in the large-scale recovery of wolves.

Wolves in the Northern Rocky Mountains

The gray wolf is an enduring symbol of the wild and once ranged throughout the Rocky Mountains and coastal ranges of Western North America. Genetic analysis suggests several hundred thousand wolves existed in these habitats and wolves now living in Canada and Alaska represent just a subset of the historical variation that once existed in the American West (Leonard *et al.*, 2005). Wolves were essentially

exterminated from the NRMs by the mid 20th century and reintroduction to Yellowstone National Park and central Idaho (see Figure 1) was initiated in 1995 with wolves from the Canadian Provinces of British Columbia and Alberta. The fact that wolves from these areas represent a subset of genetic variation found in the American West means that the reintroduced wolves are not alien species as some have asserted, rather these wolves re-established a historical legacy that derives from the Old World migration of the gray wolf to North America several hundred thousand years ago.

The NRM reintroductions used wild-caught individuals and translocated them in large numbers: 31 individuals to Yellowstone and 35 to central Idaho in 1995 and 1996. These two areas, along with a Montana population that was naturally re-established starting in 1979, define the three areas in the recovery plan for the Western wolf under the US Endangered Species Act. Recovery was to be considered successful if simultaneously each area had at least 100 individuals in 10 packs for a period of 3 years, and these populations were connected by genetically effective migration. There are now about 1600 wolves in the three areas, and they have been delisted (removed from federal protection under the Endangered Species Act) and are under state management in two of the three recovery areas. Recent research has suggested that wolves have initiated a trophic cascade in Yellowstone National Park, allowing for the restoration of forest and other native vegetation in overgrazed grassland (Ripple *et al.*, 2001; Fortin *et al.*, 2005). Nonetheless, scientific, political and moral issues continue to hamper wolf recovery for what is, otherwise, the most successful restoration of a large carnivore to its native habitat.

Several basic scientific problems need to be addressed before recovery is considered successful. First, is the total of 300 individuals in 30 packs enough

for long-term sustainability? Stochastic population models of large carnivores show that isolated populations of less than 100 individuals often have a high chance of extinction. Further, genetic loss could be consequential because wolf packs generally have only a single breeding (alpha) pair. The effective population size (N_e) determines how rapidly genetic variation is lost and is closely related to the number of breeders, and how equitably they breed. Consequently, the N_e may be only 20 or less for the 10 packs in each population. If isolated, these populations would lose $1/(2N_e)$ of genetic heterozygosity, or about 2.5%, per generation. Fortunately, the number of wolves has expanded well beyond these minimum numbers, and the loss of genetic variability has been negligible (vonHoldt *et al.*, 2008, 2010). The question is then, what will happen if western states allow the population to be hunted to the federal minimum requirement for recovery (the enacted State plans actually required a higher figure of 15 packs or 150 individuals)? Such small populations would also be more vulnerable to random demographical and genetic effects and could sink far below the minimum numbers. Unfortunately, the 10 by 10 designation for each of the three recovery areas was not based on quantitative and model-based science, but instead reflected primarily a survey of 'expert' opinion.

Second, for long-term persistence, these three sub-populations need to be connected by genetically effective migration. In a recent detailed genetic study, vonHoldt *et al.* (2010) estimated that the genetically effective migration rate between the Idaho and Montana sub populations was adequate at >3 migrants per generation. However, the Yellowstone National Park population seemed to be isolated and received no natural migrants that reproduced over a 10-year period. This may reflect the difficulty that migrant wolves have in reproducing in a saturated wolf system such as Yellowstone. This study found that migrants are successful outside the Park in the Greater Yellowstone Ecosystem where there is more turnover, but these migrants and their ancestry may not help augment the genetic diversity in the Park. Further, hunting is now allowed under State management plans to the border of the Park, and recently two radiocollared wolves were killed, one of which was a breeder. The Park population has further been reduced by 40% from

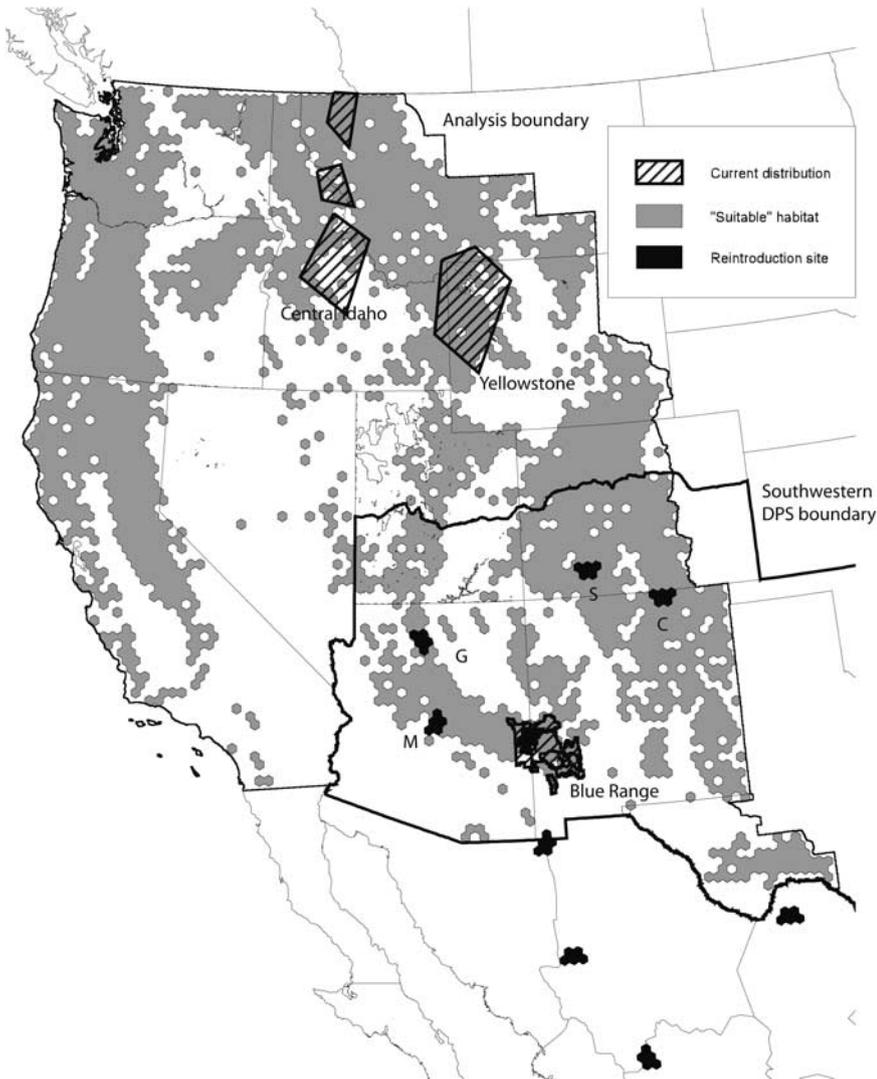


Figure 1 Areas currently occupied by Northern Rocky Mountain wolves (courtesy of C. Carroll). The three recovery areas are Northern Montana (the northmost hatched area), Central Idaho and the Greater Yellowstone Area. The reintroduction site of the Mexican wolf in the Blue Range Recovery area and the locations of four additional recovery areas on the north rim of the Grand Canyon (G), central Arizona (M) and Northern New Mexico-Southern Colorado (S and C) (Carroll *et al.*, 2006).

disease and most recently, the Druid Pack, the most notable and reproductively successful pack in the history of the re-introduction has disbanded. Whether population declines because of hunting and predator management as well as natural factors such as disease and changes in prey base, may actually improve opportunities for genetically effective migration into the Park or accelerate the loss of genetic variation and heighten the probability of local extinction is not certain. However, such concerns highlight the continued need for population management and monitoring.

Third, the three-recovery area plan needs a realistic future. Given that several hundred wolves are orders of magnitude fewer than the West sup-

ported in the past, or could support now even given the loss of habitat, what plans should be made for the long term? In Yellowstone, wolves have substantially reduced coyote numbers in many areas and may also have had top-down positive effects on the abundance of certain prey, such as pronghorn antelope (Berger *et al.*, 2008). Wolves in the West are expanding their range and have been documented in Colorado, Oregon, Utah and Washington State. A management plan is possible that transcends state boundaries and aims to re-establish genetically interconnected wolf populations that can persist into the future and focuses on areas where they provide ecosystem, societal and economic benefit with minimum human conflict.

The Mexican wolf

The history of the Mexican wolf, the smallest and most highly endangered of the North American wolves, is surrounded by controversy and mystery. The Mexican wolf, an endangered subspecies of the gray wolf, is the most genetically distinct wolf subspecies in North America (Leonard *et al.*, 2005). Landscape changes and government and private bounty hunting throughout its range reduced and isolated Mexican wolf populations so that by 1925 they were rare in the United States and extinct by the 1970s. As a result, the Mexican wolf subspecies was listed as endangered in 1976. Only a few Mexican wolves remained in isolated groups in Mexico by 1980 and surveys since have not detected any wild Mexican wolves there or elsewhere.

All Mexican wolves alive today descend from three captive lineages founded between 1960 and 1980 from a total of seven wolves (Hedrick *et al.*, 1997). In 1998, a population of 13 Mexican wolves was introduced to Eastern Arizona and Western New Mexico (known as the Blue Range population or BRP) and 65 wolves in total were introduced from 1998 to 2001 (Figure 1). Initially these wolves had only ancestry from one of the lineages with only three founders, but starting in 2000 wolves with ancestry from more than one lineage have been released (Hedrick and Fredrickson, 2008). As of January, 2010, there were only 42 wolves that could be detected in this reintroduced population (a very small number compared with the two introduced NRM populations, which had minimum numbers of 739 and 390 for the Yellowstone and Idaho populations at an equivalent time after translocation), a number that has declined from 59 in 2006. Further, there were only two breeding pairs in the population at the end of 2009 (defined as a pair with at least two young-of-year pups present at the end of the calendar year).

The initial reintroduction in 1998 was followed by further releases in subsequent years, but no documented successful reproduction and recruitment in the wild until 2003. Part of the initially slow success seems to have been because the reintroduced individuals were drawn from a naïve captive population, but after reproduction started it seemed that the population would continue to grow and become self sustaining. However, from 2005 to 2007, 62 animals were removed, many from the most successful packs, primarily because of depredation claims. These large-scale removals and the near cessation of

reintroduction actions fundamentally altered the trajectory of the population. In addition, Fredrickson *et al.* (2007) showed that part of the slow increase in population size was attributable to the low fitness in the population. Evaluation of litter size and other fitness components showed that crosses between the lineages had increased fitness both in captivity and in the reintroduced population (Fredrickson *et al.*, 2007), but management actions did not capitalize on this benefit from genetic rescue (Hedrick and Fredrickson, 2010). Further, since 1998, at least 32 animals have been illegally killed and in only two cases has the killer been identified and successfully prosecuted (four more alpha males have been killed or have gone 'missing' in the late spring-early summer of 2010). Overall, human-caused mortality from illegal killing and road kills, and removals mainly due to human conflict, have severely impacted the ability of this population to increase.

Although genetic considerations are important in the recovery of the reintroduced Mexican wolf, some management policies and actions have had quite detrimental effects on the reintroduced population. First, the reintroduced population is limited in range and individuals that leave the recovery area are generally caught or killed. Second, initial releases of captive wolves with no previous wild experience is limited to a small area in Arizona and not permitted in New Mexico, which contains some of the best wolf habitat. The cumulative effects of wolf removals primarily because of boundary issues and livestock depredations, caused the overall removal/mortality rate (64%) to exceed that predicted (47%) for the reintroduced population in the first 5 years. For the BRP to compensate for the high mortality, the recruitment rate needs to be quite high. Because there have been few new introductions recently, in combination with the low fitness of some of the alpha wolves from a single lineage, persistence of the reintroduced population is in question.

Although the problems faced with the single reintroduced population are great, for long-term recovery, the success of this population is only a start. In our opinion, the recovery objectives of the NRM wolves can only be used as a starting point for recovery of Mexican wolves. There is no recent recovery plan for Mexican wolves and two more recovery team efforts in the mid 1990s and 2000s to write recovery plans were aborted. For example, having three populations connected by significant gene flow seems to be a reasonable recovery objective for Mexican wolves.

However, Mexican wolves are quite different from NRM wolves because they all descend from captive animals, have initially a much higher level of inbreeding, suffer a higher rate of human-caused mortality, and from the recent experience with the Blue Range population, have a much more precarious probability of persistence. Given expected rates of wolf removal and killing, we suggest that for recovery of Mexican wolves three populations, each simultaneously having 250 animals for 8 years (approximately two generations) is the minimum necessity. These recovery goals need to be supported with rigorous demographical models and investigation. Because new wolves come from captivity and there is a small founder number from three different lineages, extensive management is necessary for successful recovery. The negative impacts on the population from the moratorium that was placed on reintroductions and the large-scale removals during the period from 2005 to 2009 shows a critical need for scientifically based management.

Using a sophisticated landscape analysis, potential sites for the additional populations have been identified (Carroll *et al.*, 2006) (Figure 1). In particular, the north rim of the Grand Canyon (indicated as G) and Northern New Mexico Southern Colorado sites (S and C) seem most appropriate for these two additional populations. The experience of introducing wolves to the Blue Range identifies the need to control human-caused mortality and initially intensive management in these populations. In addition, if natural gene flow does not occur between these populations, then artificial movement between the populations may be necessary.

The Scandinavian recolonization

Perhaps, the gray wolf situation with the most parallels to the NRM and Mexican wolf cases is the contemporary Scandinavian wolf population in Sweden and Norway that seems to have been established by a pair of animals that naturally immigrated in the early 1980s. The number remained at less than ten individuals in a single pack during the 1980s. A third male founder naturally migrated from the Finland–Russia population around 1990, resulted in genetic (or behavioral) rescue, and the population increased to around 100 individuals (Vilá *et al.*, 2003). Subsequently, the amount of inbreeding greatly increased and there was a significant decline in the number of

surviving pups per litter (Liberg *et al.*, 2005). Two additional male migrants started breeding in 2008 and have produced three litters apiece with a total of about 40 pups (Olof Liberg, personal communication). The estimated total number in early 2010 is 200–240 with about 26–32 in Norway. The Norwegian government has killed a number of wolves in the small part of the population residing in Norway in 2001 (9 out of 28 killed) and 2005 (5 out of 18–21). The Swedish government has now authorized hunting to maintain a limit of 210 wolves in Sweden. The Swedish population has been closely monitored at substantial expense, and remains the clearest example of the importance of genetically successful migration for recovery. However, management actions act to counteract such benefits. The contrast with the American West where genetically effective migration is a requirement for delisting, argues for the importance of strong scientifically based legislation for endangered species recovery elsewhere.

Lessons and challenges

The reintroduction of gray wolves to the NRM and Mexican wolves to the BRP provide extreme outcomes for wolf reintroduction. The NRM wolves, which derive from wild populations, grew rapidly and have generally met the recovery goals in little more than a decade. In contrast, the lone Mexican wolf population, originally derived from a single inbred population, is not increasing and its long-term survival is in question. In both cases, scientific priorities have at times been ignored given political considerations. From the NRM reintroduction, it is clear that wolves are resilient and have the potential for population growth, dispersal and adaptability. The challenge will be to harness these characteristics in a scientifically justified plan that we have the will and political acumen to implement. Genetic rescue is a reality in large carnivores and genetically effective migration is a critical variable in population management, given that large carnivores demand so much pristine habitat that is rapidly becoming subdivided by roads and human development.

Conflict of interest

The authors declare no conflict of interest. Correspondence: Professor R Wayne is at the Department of Ecology and Evolutionary Biology, University of California-Los Angeles, Los Angeles, CA, USA and Professor PW Hedrick is at the School of Life Sciences, Arizona State University, Tempe, AZ, USA.

e-mail: rwayne@ucla.edu and philip.hedrick@asu.edu

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MEXICAN WOLF RECOVERY: THREE-YEAR PROGRAM REVIEW AND ASSESSMENT¹

*Prepared by the Conservation Breeding Specialist Group (CBSG), Apple Valley, Minnesota;
for the United States Fish and Wildlife Service, Albuquerque, New Mexico:*

PAUL C. PAQUET
UNIVERSITY OF CALGARY &
CONSERVATION SCIENCE INC.

JOHN A. VUCETICH
MICHIGAN TECHNOLOGICAL UNIVERSITY

MICHAEL K. PHILLIPS
TURNER ENDANGERED SPECIES FUND

LEAH M. VUCETICH
MICHIGAN TECHNOLOGICAL UNIVERSITY

Conservation Breeding Specialist Group
12101 Johnny Cake Ridge Road
Apple Valley, MN 55124-8151
tel: 1-952-997-9800
e-mail: office@cbsg.org
June 2001

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1. INTRODUCTION

Herein we assess the progress of efforts to reestablish Mexican wolves (*Canis lupus baileyi*) in the Blue Range Wolf Recovery Area (BRWRA). This review is a direct result of an Environmental Impact Statement (EIS) concluded by the U.S. Fish and Wildlife Service (USFWS) in 1996 (U.S. Fish and Wildlife Service 1996). The EIS and associated final rule (Parsons 1998) call for the USFWS to reestablish Mexican wolves to the BRWRA. The recovery area encompasses 17,752 km² (6,854 mi²) of the Apache National Forest in southeastern Arizona and the Gila National Forest in southwestern New Mexico.

Specifically, the U.S. Department of Interior has authorized the USFWS to reintroduce about 15 wolves every year for 3 to 5 years in the BRWRA primary recovery zone. The primary recovery zone comprises about 2,664 km² (1,029 mi²) of the Apache National Forest (Groebner *et al.* 1995). The remainder of the Apache National Forest and all the Gila National Forest make up the secondary recovery zone. The USFWS may conduct re-releases in the secondary recovery zone and wolves that move from the primary recovery zone can inhabit the secondary zone.

The USFWS began reintroductions with the release of 11 wolves in March 1998. From then until March 2001 the USFWS released another 45 individuals on 61 occasions. An Interagency Field Team comprising employees from the USFWS, Wildlife Services (U.S. Department of Agriculture), Arizona Department of Game and Fish, and New Mexico Department of Game and Fish carried out the releases and associated fieldwork.

The final rule governing the reintroduction project (Parsons 1998) and the 1998 Mexican Wolf Interagency Management Plan both require the USFWS to conduct a comprehensive review of the project at the end of the third year (i.e., March 2001). The full evaluation must include recommendations regarding continuation, modification, or cancellation of the reintroduction effort. If appropriate, the evaluation may include recommendations on whether and how to use the White Sands Wolf Recovery Area.

The primary goal of the reintroduction effort is to restore a self-sustaining population of about 100 wild Mexican wolves distributed over 12,950 km² (5,000 mi²) of the BRWRA. Such an objective is consistent with the 1982 Mexican Wolf Recovery Plan (U.S. Fish and Wildlife Service 1982 (EIS)). The 1998 Mexican Wolf Interagency Management Plan projects that about 9 years will be required to achieve this objective. Wolves in the BRWRA are to be managed to reduce negative impacts and maximize positive influences on the lifestyles and economy of local residents.

The USFWS contacted the Conservation Breeding Specialist Group (CBSG) to conduct the specified review. CBSG is ideally suited for the task because of extensive worldwide experience with small population restoration, conservation, and management. On behalf of CBSG, Paul Paquet assembled an expert review team composed of John Vucetich, Michael Philips, and Leah Vucetich. The team review is based on data provided by the USFWS data collected in the first 3 years of the reintroduction project.

2. ISSUES FOR WHICH ASSESSMENTS WERE REQUESTED

Our assessment addresses the following questions as outlined by the 1998 Mexican Wolf Interagency Management Plan.

- ▶ Have wolves successfully established home ranges within the designated wolf recovery area?
- ▶ Have reintroduced wolves reproduced successfully in the wild?
- ▶ Is wolf mortality substantially higher than projected in the EIS?
- ▶ Is population substantially growth lower than projected in the EIS?
- ▶ Are numbers and vulnerability of prey are adequate to support wolves?
- ▶ Is the livestock depredation control program effective?
- ▶ Have documented cases of threats to human safety occurred?

We were not asked to address the following 2 additional questions identified in the 1998 Mexican Wolf Interagency Management Plan:

- ▶ Is effective cooperation occurring with other agencies and the public?
 - ▶ Are combined agency funds and staff adequate to carry out needed management, monitoring and research?
-

3. OUR APPROACH

Although a paucity of data compels us to speculate on many biological issues, we do so using the best available information about wolf ecology. The lack of information reflects the short time the Program has been underway. Where necessary and appropriate we infer from published studies conducted elsewhere, our own experiences, and the experience of other researchers and managers. Throughout the report, we are careful to distinguish fact from inference, speculation, and professional opinion. Our conclusions and recommendations reflect our current knowledge and the fundamental principles of Conservation Biology.

Conclusions and recommendations depend on the likelihood of the assumptions underlying the assessment. Therefore, we reviewed several principles of conservation biology, which apply to restoring and maintaining a viable population of wolves. Some of these principles are established generalizations, some are testable hypotheses, and others are practical guides that we assessed as important in developing our recommendations.

- ▶ The fewer data or more uncertainty involved, the more conservative conclusions must be
- ▶ To be comprehensive, an assessment must be concerned with multiple levels of biological organizations and with many different spatial and temporal scales.
- ▶ Species well distributed across their native range are less susceptible to extinction than species confined to small portions of their range.
- ▶ Large blocks of habitat containing large populations of a target species are superior to small blocks containing small populations.
- ▶ Maintaining viable ecosystems is usually more efficient, economical, and effective than a species by species approach.
- ▶ Viability of wild populations depends on the maintenance of ecological processes.
- ▶ Human disturbances that mimic or simulate natural disturbances are less likely to threaten restoration efforts than disturbances radically different from the natural regime.

We note that how we measure and perceive the success or failure of wolf recovery is contextual. Accordingly, our focus is on wolf ecology and how the quality of management affects the persistence of the reintroduced Mexican wolf population. Specifically, we are concerned with the viability of the population as affected by habitat quality, population size, population isolation, and agency management. Although a viable wolf population could affect people's lives and the economy, we do not consider social and economic issues in this report.

Finally, our protocol for assessment was to:

- ▶ Review pertinent scientific literature
 - ▶ Use available data provided by the Interagency Field Team
 - ▶ Review pertinent regulations, polices, and rules
 - ▶ Evaluate data quality
 - ▶ Identify data gaps
 - ▶ Analyze and interpret data
 - ▶ Compare progress with program goals
 - ▶ Evaluate program success & failures
-

- ▶ Develop data collection, data management & conservation recommendations
-

4. ECOLOGICAL BACKGROUND

a. RESILIENCY

Resilience has been defined as the ability to absorb disturbance and still maintain the same relationship between populations or state variables (Holling 1973) and the degree to which an entity can be changed without altering its minimal structure (Pickett *et al.* 1989). Thus, resilience can be thought of as a property of a system, whereas persistence is the outcome (Weaver *et al.* 1996).

Wolves evolved in environments that included prevailing disturbance regimes with certain ecological characteristics and boundary conditions. Disturbance varied in frequency, duration, extent, and intensity, thereby resulting in different spatio-temporal patterns of change. Behaviors and life history traits conferred resilience that enabled wolves to absorb these intrinsic disturbances and persist. Modern humans, however, have presented new regimes of disturbance that could be considered exotic because they are qualitatively novel or quantitatively atypical.

Three mechanisms of resilience at different hierarchical levels are: individual - plasticity in foraging behavior that ameliorates flux in food availability; population - demographic compensation that mitigates increased exploitation; and metapopulation dispersal - that provides functional connectivity among fragmented populations. Accordingly, flexible food habits, high annual productivity, and dispersal capabilities enable wolves to respond to natural and human-induced disturbances (Weaver *et al.* 1996). However, environmental disturbances at various temporal and spatial scales may exceed the ability of wolves and systems that support them to absorb disturbance (Weaver *et al.* 1996).

Wolves display remarkable behavioral plasticity in using different prey and habitats (Mech 1991). They are able to substitute one resource for another in the face of environmental disturbance (Weaver *et al.* 1996). Specifically, wolves specialize on vulnerable individuals of large prey [elk (*Cervus elaphus*) and moose (*Alces alces*)] yet readily generalize to common prey [usually deer (*Odocoileus sp.*)] (Weaver *et al.* 1996).

Wolf populations are able to compensate demographically for excessive mortality. Under certain circumstances this compensation enables wolves to respond to increased rates of juvenile or adult mortality with increased reproduction and/or survival, thereby mitigating demographic fluctuations (Weaver *et al.* 1996). Dominant wolves are able to reproduce at a very young age and usually reproduce every year thereafter (Weaver *et al.* 1996). Age at reproductive senescence has not been well documented but few females survive to reproduce past age 9 (Mech 1988). Wolves also display remarkable ability to recover from exploitation. For example, during a wolf reduction program in the Yukon, wolves recovered to pre-reduction densities within 5 years (Hayes and Harestad 2000). Wolves immigrated into the study area during early recovery, followed by increases in pack size from reproduction (Hayes *et al.* 2000).

The final mechanism that confers resilience to wolf populations is dispersal. When dispersal is successful, vanishing local populations are rescued from extirpation (Brown and Kodric-Brown 1977) and functional connectivity of metapopulations is established (Hansson 1991). Wolves have tremendous dispersal capabilities and as a result, "connectivity" of populations can be high. Dispersing wolves typically establish territories or join packs within 50-100 km of the pack in which they were born (Fritts and Mech 1981, Fuller 1989, Gese and Mech

1991, Wydeven *et al.* 1995, Boyd *et al.* 1996). Some wolves, however, move longer distances. For example, Fritts (1983) observed a wolf that traveled at least 917 km.

b. THE PERILS OF SMALL POPULATIONS

Small populations, because of random normal variability in demographics, are more likely to become extinct than larger populations (Schonewald-Cox *et al.* 1983). Moreover, these small populations are thought to be vulnerable because of deleterious effects of inbreeding (Wright 1977) and chance environmental disturbances such as forest fires, disease or infestations that affect a species or its prey (Franklin 1980). In theory, the interaction of these factors increases the probability of extinction (Shafer 1987).

Small insular populations may have a restriction of genetic variation because they represent a very small subset of the total population (i.e., a few individuals). As populations become smaller a further reduction in genetic variation results in decreased survival (i.e., increased mortality). Increased mortality leads to additional reduction in genetic variation resulting in an "extinction vortex." Biologists theorize that because of this self-amplifying cycle the rate of extinction for small populations is higher than predicted from the population size alone (cf. Caro and Laurenson 1994).

c. USE OF HABITAT AND PATTERNS OF TRAVEL

Throughout its broad geographical distribution the gray wolf is considered an ecosystem and prey generalist. However, populations are adapted to local conditions and are, therefore, specialized concerning den site use, foraging habitats, and prey selection. In mountain regions, the effects of physiography, weather, prey distribution, and prey abundance combine to concentrate activities of wolves into forested valley bottoms (Paquet 1993, Paquet *et al.* 1996, Paquet *et al.* 1996, Weaver 1994, Singleton 1995, and others).

Elevation can also govern seasonal movements of wolves. In mountainous areas with high snowfall, use of low elevation valleys increases during winter, where frozen rivers and lakes, shorelines, and ridges are preferred because of ease of travel. Ski trails, snowmobile trails, graded roads, and packed roads can artificially enhance the range and efficiency of winter forays (Paquet 1993). Singleton (1995) has suggested that variation in pack size, variation in home range size, and interactions with sympatric predators may influence habitat use and travel patterns. He further speculated that turning frequency or travel route complexity are likely to vary depending on whether an animal is within a patch of concentrated resource availability (e.g., deer winter ranges), moving between known patches, or exploring new areas.

d. INFLUENCE OF WOLVES ON THE BIOLOGICAL COMMUNITY

Generally we understand that the ecology of predators, prey, and scavengers, is intertwined. However, the details of these relationships, and the general role of predation in shaping the structure of ecological communities is poorly understood. Changes in predator-prey relationships may affect species other than wolves and their prey. Disruption of top predators can affect interspecific associations by disrupting relationships within food webs. This, in turn, may cause unanticipated ripple effects in populations of other species (Paine 1966, 1969, 1980; Terborgh and Winter 1980, Frankel and Soulé 1981, Wilcox and Murphy 1985, Wilcove *et al.* 1986, Valone and Brown 1995), which markedly alter the diversity and composition of a

community (Paine 1966). Multi species effects often occur when changes in a third species mediate the effect of one species on a second species (or analogous higher-order interactions). For example, a wolf can affect a grizzly bear (*Ursus arctos*) by reducing the availability of a limiting resource (possibly an ungulate). Also a secondary carnivore such as a coyote (*C. latrans*) can affect the degree to which a herbivore's lifestyle is influenced by a primary carnivore such as a wolf. Ecologists have only begun to develop theory that attempts to explain the coexistence of prey in terms of predator-influenced niches ("enemy-free space").

Terborgh and Winter (1980) noted that we know little about the loss of top carnivores in terrestrial environments, and predicted a wave of extinctions following the loss of any key species. For example, if species interact as competitors, as predator and prey, or as facilitators in successional processes, then the presence of one species may influence the extinction probability of another "linked" species.

Recent evidence suggests the importance of cascading trophic interactions on terrestrial ecosystem function and processes. Research has documented differences within systems from which large predators have been removed or are missing (Glanz 1982, Emmons 1984, Terborgh 1988, Leigh *et al.* 1993, Terborgh *et al.* 1999). Accordingly, the ecosystem impacts of wolves may be more profound than previously expected. For example, on Isle Royale, Michigan wolf predation on moose has been shown to influence positively biomass production in trees of boreal forest (McLaren and Peterson 1994). Growth rates of balsam fir (*Abies balsamea*) were regulated by moose (*Alces alces*) density, which in turn was controlled by wolf predation (McLaren & Peterson 1994). When the wolf population declined for any reason, moose reached high densities and suppressed fir growth. This top-down "trophic cascade" regulation is apparently replaced by bottom-up influences only when stand-replacing disturbances such as fire or large windstorms occur at times when moose density is already low (McLaren & Peterson 1994). This is strong evidence of top-down control of a food chain by wolves (Terborgh *et al.* 1999). Research elsewhere suggests elk (*Cervus elaphus*) populations not regulated by large predators affect negatively the growth of aspen (*Populus tremuloides*) (Kay 1990, Kay and Wagner 1994, White *et al.* 1992, D. Smith pers. comm.), though information remains equivocal (L. Morgantini pers. comm.).

In addition to the obvious interactions between wolves and prey, wolves provide a regular supply of carrion to scavengers. Less obvious community dynamics might include the relationships between different predators, and how wolves influence these relationships. For example, how do wolves modify the relationships between coyotes and foxes?

Interest in the role of wolves in the broader ecosystem is not new. From 1939-1944 Adolf Murie (1944) conducted field studies in Denali Park Alaska to determine "...the ecological picture centering about the wolf of Mount McKinley National Park". Here, he entertained questions about the relationships between park wolves and other wolves, between wolves and their prey, and between wolves and other predators. Few studies, however, are available to yield insights into many of the relationships between wolves and other ecosystem components.

e. RESPONSE OF WOLVES TO HUMAN ACTIVITIES

The seriousness of human disturbance is ultimately a human judgement and, as such, some may consider any alteration of the normal activities of wolves to be undesirable. The ecological issue is how the probability of persistence changes with habitat degradation, small population size,

and population isolation. The management issue is what probability of persistence and environmental quality is compatible with legislation and acceptable to society. Interpretation of the wolf-human interaction is confounded by multiple factors that influence how wolves use the landscape and react to people (Mladenoff *et al.* 1995, L. Boitani pers. comm., L. Carbyn pers. comm., E. Zimen pers. comm.). Because of the wolf's inherent behavioural variability, it is unlikely that all wolves react equally to human induced change. Moreover, many extraneous factors contribute to variance in behaviour of individual wolves. Because we have developed no reasonable expression of those differences, assessments are usually applied at the pack and population levels.

The specific conditions in which wolves are 'disturbed' (i.e., distribution, movements, survival, or fecundity are impaired) are believed to be highly variable. The extent and intensity of disturbance appear to vary with environmental and social context, and the individual animal (L. Boitani pers. comm.). Though wolves are sensitive to human predation and harassment (Thiel 1985, Jensen *et al.* 1986, Mech *et al.* 1988, Fuller 1989, Mech 1989, Purves *et al.* 1992, Fuller *et al.* 1992, Mech 1993, Mech 1995, Thurber *et al.* 1994, Mladenoff *et al.* 1995, Paquet *et al.* 1996), we have limited empirical information on tolerance to indirect human disturbance. Several studies suggest the main factor limiting wolves where they are present and tolerated by humans is adequate prey density (Fuller *et al.* 1992). Although human activities have been shown to influence the distribution (Thiel 1985, Fuller *et al.* 1992, Paquet 1993, Mladenoff *et al.* 1995) and survival of wolves (Mech *et al.* 1995, Mladenoff *et al.* 1995, Paquet 1993, Paquet *et al.* 1996), human-caused mortality is consistently cited as the major cause of displacement (Fuller *et al.* 1992, Mech and Goyal 1993, and others).

Studies that have quantified wolf/human interactions have shown wolves avoid humans or are displaced via human induced mortality (Paquet *et al.* 1996). Avoidance is temporal (Boitani 1982) and spatial (Mladenoff *et al.* 1995, Paquet *et al.* 1996). Several studies that used road densities as an index of human influence concluded that human activities associated with roads affect the survival and behaviour of wolves. Interpretation, however, was confounded because many human activities associated with roads result in the death of wolves. Thus, absence of wolves in an area may not be the result of behavioural avoidance per se. Data from Ontario, Wisconsin, Michigan, and Minnesota suggest that wolf survival is usually assured at road densities below 0.58 and 0.70 km/km² (Thiel 1985, Jensen *et al.* 1986, Mech *et al.* 1988, Fuller 1989, Mech 1989, Fuller *et al.* 1992). A study in Alaska concluded that wolves avoid heavily used roads and areas inhabited by humans, despite low human caused wolf mortality (Thurber *et al.* 1994). Landscape level analysis in Wisconsin found mean road density was much lower in pack territories (0.23 km/km² in 80% use area) than in random non pack areas (0.74) or the region overall (0.71). Few areas of use exceeded a road density of >0.45 km/km² (Mladenoff *et al.* 1995).

Recent reports suggest wolves in Minnesota tolerate higher levels of disturbance than previously thought possible. Wolves, for example, are now occupying ranges formerly assumed to be marginal because of prohibitive road densities and high human populations (Mech 1993, Mech 1995). Legal protection and changing human attitudes are cited as the critical factor in the wolf's ability to use areas that have not been wolf-habitat for decades. If wolves are not killed, they seem able to occupy areas of greater human activity than previously assumed (Mech 1993, Fuller

et al. 1992). Based on these observations, Mech (1995, p. 275) comments that misconceptions about the wolf's inherent ability to tolerate human activity encourage unwarranted protection.

Nonetheless, wolves in Minnesota continue to avoid populated areas, occurring most often where road density and human population are low (Fuller *et al.* 1992).² Moreover, the fact that wolves are using areas of greater human activity suggests dispersers or marginalised individuals are being pushed into suboptimal habitat. More suitable and safe habitat may be saturated by dominant animals or packs. This supports the idea that wolves occupy habitat closer to humans only if necessary. A similar phenomenon has been shown in grizzly bears (D. Mattson *et al.* 1987, Mattson pers. Comm.) and many avian species.

We are aware of only 4 studies that have systematically and explicitly examined human population density and wolf distribution. In all studies, the absence of wolves in human dominated areas may have reflected high levels of human caused mortality, displacement resulting from behavioural avoidance, or some combination of both. All were conducted at a landscape scale and assessed population or pack level responses of wolves to humans. In Wisconsin, human population density was much lower in pack territories than in non pack areas. Wolf pack territories also had more public land, forested areas with at least some evergreens, and lower proportions of agricultural land. Notably, no difference was detected between white-tailed deer (*Odocoileus virginianus*) densities in pack territories and non pack areas. Overall, wolves selected those areas that were most remote from human influence (Mladenoff *et al.* 1995) using areas with fewer than 1.54 humans/km² and less than 0.15 km roads/km². Most wolves in Minnesota (88%) were in townships with <0.70 km roads/km² and <4 humans/km² or with <0.50 km² and <8 humans/km². High human or road densities likely precluded the presence of wolf packs in several localities within contiguous, occupied wolf range (Fuller *et al.* 1992). In Italy, wolf absence was related to human density, road density, urban areas, cultivated areas, and cattle and pig density. However, because human density, road density, and urbanized areas were highly inter correlated no specific human effect was established (Duprè *et al.* in press).

In the Bow River Valley, Alberta the selection or avoidance of particular habitat types was related to human use levels and habitat potential (Paquet *et al.* 1996). Wolves used disturbed habitats less than expected, which suggests the presence of humans altered their behaviour. Very low intensity disturbance (<100 people/month) did not have a significant influence on wolves, nor did it seriously affect the ecological relationships between wolves and their prey. At low to intermediate levels of human activity (100-1,000 people/month) wolves were dislocated from suboptimal habitats. Higher levels of activity resulted in partial displacement but not complete abandonment of preferred habitats. As disturbance increased, wolves avoided using some most favourable habitats. In portions of the Valley where high elk abundance was associated with high

²Wolves from the Midwestern United States have hybridized with coyotes (*Canis latrans*) (Wayne *et al.* 1991, Wayne *et al.* 1992, Lehman *et al.* 1991), be red wolves, or red wolf hybrids (Wilson *et al.* 2001), which may affect their behaviour (Fox 1971) and their relationship with humans. Consequently, extrapolating information from Minnesota, Michigan, Minnesota, and Ontario may be inappropriate for the Rocky Mountains. Wolves in the Rocky Mountains show no introgression of coyote genes (Forbes and Boyd 1996).

road and/or human population density, wolves were completely absent. Overall, habitat alienation resulted in altered predator/prey relationships.

The observed patterns of displacement suggest the presence of humans repulses wolves, although a strong attraction to highly preferred habitats increases a wolf's tolerance for disturbance. As conditions become less favorable, the quality of habitat likely takes on greater importance. Tolerance thresholds are unknown but, as noted, in the Bow River Valley changes in patterns of habitat use were evident when human activity exceeded 100 people/month. Nearly complete alienation of wolves occurred when more than 10,000 people/month used an area.

f. HUMAN INFLUENCE ON HABITAT USE BY WOLVES

The degree of human influence probably varies according to the environmental context. If a particular habitat is highly attractive, wolves appear willing to risk exposure to humans, at least within some limits (Chapman 1977). As levels of disturbance increase, favorableness of habitat likely takes on greater importance. For example, we know that wolves select home sites near intense human activity when denning areas are limited, or where innocuous human activity occurs (Chapman 1977). The presence of artificial food sources (e.g., carrion pits, garbage dumps) also attracts wolves and reduces avoidance of human activity (Chapman 1977, L.D. Mech pers. comm., Paquet 1996, Krizan 1998). In the Bow River Valley, wolves denned within 500 m of the Trans Canada highway when Parks Canada was dumping carrion in the area. Wolves abandoned the home site after Parks stopped dumping of the carrion.

The tension between attraction and repulsion is probably expressed differently by individuals, packs, and populations. Attraction to an area is a complex sum of physiography, security from harassment, positive reinforcement (e.g., easily obtained food), population density, and available choice. Moreover, the response to a particular disturbance seems to depend on disturbance-history (E. Zimen pers. comm.); a critical concept in understanding the behaviour of long-lived animals that learn through social transmission (Curatolo and Murphy 1986, S. Minta pers. comm.).

We can group human influence into effects on wolf habitat and populations. Habitat disturbance can be short or long term and can include direct loss of habitat (i.e., vegetation removal, vegetation change, or isolation and removal of prey). Direct habitat loss does not include the loss of habitat due to temporal or spatial alienation (sensory disturbance) or from fragmentation of habitat. Indirect losses will occur due to habitat alienation, where wolves abandon habitat because of nearby disturbances or are spatially isolated from using them because of impediments to movements. Changes in population can occur directly through alterations in habitat and indirectly because of disturbing activities.

The major impacts of human induced changes are, in order of decreasing importance, physical loss of habitat, loss of prey species, fragmentation of habitat, isolation of habitat, alienation of habitat, alteration of habitat, changes in original ratios of habitat, and changes in juxtaposition of habitats. These effects combine to have local and population level influences by altering the composition of biological communities upon which wolves are dependent, restricting movements, reducing foraging opportunities, and limiting access to prey. Obstructing movements also increases the vulnerability of wolves to other disturbances as they attempt to learn new travel routes.

The degree to which human activities disrupt wildlife reflects the type and extent of disturbance, which interacts with the natural environment to affect environmental quality. In mountainous landscapes wildlife often responds markedly to disturbances that occur at small spatial scales. This is because the topography amplifies the effects of disturbances by concentrating activities of humans and wildlife into valley bottoms. The forced convergence of activities limits spatially the range of options wildlife have for coping with disruption, reducing resilience to anthropogenic disturbance (Weaver *et al.* 1996, Alaska Department of Fish and Game unpublished data).

Indirect human influences can affect an animal's chance to survive and reproduce. As wolves approach their limits of tolerance, they become increasingly susceptible to what would otherwise be minor influences. In the mountainous terrain, natural landforms and the condensed arrangement of habitats make wolves highly susceptible to the adverse effects of human disturbance. Because most development occurs in areas preferred by wolves, human activities unavoidably increase the risk of death and injury for wolves, decrease opportunities for wolves to move freely about, displace or alienates wolves from preferred ranges, and interrupt normal periods of activity. In less physiographically complex environments multiple travel routes link blocks of wolf habitat. Destruction or degradation of one or 2 routes is not usually critical, because safe alternative routes are available. In contrast, wolves living in mountains cannot avoid valley bottoms or use other travel routes without affecting their fitness. Therefore, tolerance of disturbance is probably lower than in other human dominated environments where wolves can avoid disturbed sites without seriously jeopardizing survival.

g. RESPONSE OF WOLVES TO LINEAR DEVELOPMENTS

The security of wolf populations in the many regions may be tenuous, because linear developments heavily dissect wolf ranges (i.e., highways, secondary roads, railways, and power line corridors). Highway mortality has become a primary cause of wolf mortality and there is accumulating evidence of habitat loss, fragmentation, and degradation related to roads (Purves *et al.* 1992, Paquet 1993). Ensured connectivity of quality habitats is important for survival of large carnivores (Beier 1993, Paquet and Hackman 1995, Doak 1995, Noss *et al.* in press), especially for those that face a high risk of mortality from humans or vehicles when travelling across settled landscapes (Noss 1992, Beier 1993).

There are several plausible explanations for the absence of wolves in densely roaded areas. Wolves may behaviourally avoid densely roaded areas depending on the type of use the road receives (Thurber *et al.* 1994). In other instances, their absence may be a direct result of mortality associated with roads (Van Ballenberhe *et al.* 1975, Mech 1977, Berg and Kuehn 1982). Besides fragmenting and consuming critical habitat, linear developments provide access to remote regions, which allows humans to deliberately, accidentally, or incidentally kill wolves (Van Ballenberghe *et al.* 1975, Mech 1977, Berg and Kuehn 1982). Despite legal protection, 80% of known wolf mortality in a Minnesota study was human-caused (30% shot, 12% snared, 11% hit by vehicles, 6% killed by government trappers, and 21% killed by humans in some undetermined manner) (Fuller 1989). Mech (1989) reported 60% of human-caused mortality in a roaded area (even after full protection), whereas human caused mortality was absent in an adjoining region without roads. On the east side of the Central Rockies between 1986 and 1993, human caused

mortality was 95% of known wolf death. Thirty-six percent (36%) of mortality was related to roads (Paquet 1993).

Wolves also experience higher mortality in areas with higher road density. On Prince of Wales Island, Alaska, researchers report a significant jump in wolf mortality (kill/259 km²) in areas where road densities exceeds.25 km/km². While wolf mortality in the category of most densely roaded areas is highest, the variance is also high. The authors suggest that at some threshold of road density or human activity, wolves may abandon an area, resulting in decreased trapping and hunting mortality (Alaska Department of Fish and Game, unpublished data).

Linear developments may also be physical and/or psychological impediments to wolf movement. Road density and human density have been inversely correlated with viable populations of wolves in several areas. Along the Ontario-Michigan border, distribution of breeding packs occurred only in Ontario. Except for Cockburn Island, only lone wolves were found in areas close to the border or in Michigan. In Ontario, the density of roads in areas not occupied by wolves was greater than in areas occupied by wolves. Mean road density in Michigan, where no wolves resided, was also greater than in wolf-occupied areas of Ontario. High human densities, represented by road densities of > 0.6 km/km², were believed to be a barrier to wolf dispersal into Michigan (Jensen *et al.* 1986).

Studies in Wisconsin, Michigan, Ontario, and Minnesota have shown a strong relationship between road density and the absence of wolves (Thiel 1985, Jensen *et al.* 1986, Mech *et al.* 1988, Fuller 1989). Wolves generally are not present where the density of roads exceeds 0.58 km/km² (Thiel 1985 and Jensen *et al.* 1986, cf. Fuller 1989). Landscape level analysis in Wisconsin, Minnesota, and Michigan found mean road density was much lower in pack territories (0.23 km/km² in 80% use area) than in random nonpack areas (0.74) or the region overall (0.71). Road density was the strongest predictor of wolf habitat favorability out of 5 habitat characteristics and 6 indices of landscape complexity (Mladenoff *et al.* 1995). Few areas of use exceeded a road density of >0.45 km/km² (Mladenoff *et al.* 1995). Notably, radio collared packs were not bisected by any major federal or state highway. In Minnesota, densities of roads for the primary range, peripheral range, and disjunct range of wolves were all below a threshold of 0.58 km/km². These results, however, probably do not apply to areas on which public access is restricted. Mech (1989), for example, reported wolves using an area with a road density of 0.76 km/km², but it was next to a large, roadless area. He speculated that excessive mortality experienced by wolves in the roaded area was compensated for by individuals that dispersed from the adjacent roadless area. Wolves on Prince of Wales Island, Alaska currently use areas with road densities greater than 0.58 km/km². Core areas, however, are generally in the least densely roaded areas of the home range, and wolf activity that does occur in densely roaded areas occurs primarily at night. This behavioral response may reflect the limited options wolves have to relocate when they live on islands or insularized landscapes.

The response of wolves to different road types and human presence at the boundaries of Kenai National Wildlife Refuge, Alaska, was examined in a study of radio-collared wolves (Thurber *et al.* 1994). Wolves avoided oilfield access roads open to public use, yet were attracted to a gated pipeline access road and secondary gravel roads with limited human use. Thurber *et al.* speculated that roads with low human activity provide easy travel corridors for wolves. The response of wolves to a major public highway was equivocal. They thought wolf absence from settled areas and some roads were caused by behavioral avoidance rather than direct

attrition resulting from killing of animals. In Montana, Singleton (1995) found that wolves preferred areas 0.5-1 km from open roads for travel routes. He speculated that wolves did not select locations more distant from open roads because of the distribution patterns of wintering ungulates and the barrier provided by the river. Overall, wolves preferred areas with 0.01-2 mi/mi² for travel routes.

5. HAVE WOLVES SUCCESSFULLY ESTABLISHED HOME RANGES WITHIN THE DESIGNATED WOLF RECOVERY AREA?

a. BACKGROUND

Biologists usually define the home range of a wolf as an area within which it can meet all of its annual biological requirements. Seasonal feeding habitat, thermal and security needs, travel, denning, the bearing and raising of young, are all essential life requirements. The manner in which habitats for these requirements are used and distributed influences home range size and local and regional population distributions. Generally, wolves locate their home ranges in areas where adequate prey are available and human disturbance minimal (Mladenoff *et al.* 1995, 1997, Mladenoff and Sickley 1998). Wolves use areas within those home ranges in ways that maximize encounters with prey (Huggard 1993a, b).

Newly colonizing wolf pack might shift home ranges in response to climate, food availability, human disturbance, and other factors. A colonizing pack might have a larger, more fluid, home range than a pack surrounded by other wolf packs (Boyd *et al.* 1996). Some evidence suggests that wolf packs colonize areas that were first “pioneered” by dispersing lone wolves (Ream *et al.* 1991)

In mountainous areas, topographic position influences selection of home ranges and travel routes (Paquet *et al.* 1996). Wolf use of valley bottoms and lower slopes correspond to the presence of wintering ungulate prey and snow depth in these areas (Singer 1979, Jenkins and Wright 1988, Paquet *et al.* 1996). In areas of higher prey density pack sizes increase (Messier 1985) and home range size is closely correlated with pack size (Messier 1985, Peterson *et al.* 1984).

b. DATA SUMMARY

We assessed home ranges using locations from radio-collared animals. Radio-telemetry data (>7000 locations) were provided in an Excel database (Monitor). These data include all telemetry locations from 3 March 1998 to 3 March 2001. Each location was appended by wolf identification, date, time, and pack membership. Although locations were qualitatively ranked for accuracy, no quantitative assessment of telemetry error was available. Thus, we classified locations into 4 categories, which corresponded to the database provided. Class 1, 2, 3, and 4 locations were those within 100 m, 100-250 m, 250-450 m, and greater than 450 m from the true location, respectively. Only class 1 aerial and ground locations were used in the home range analysis.

c. METHODS

Our objective was to quantitatively describe areal distribution of reintroduced Mexican wolves within the recovery region. In a few cases, however, subjective determination of the home range was more appropriate.

Using ArcView Spatial Analyst, we plotted all class 1 locations. We discarded locations deemed to be recording errors, extraterritorial forays, and dispersals. We assumed a wolf dispersed if it permanently left its original pack and formed a new pack or joined an existing one (Messier 1985b).

Locations of individual wolves were grouped by pack affiliation. We defined a pack as 2 or more wolves that traveled together more than 1 month (Messier 1984). For each pack we used one wolf/year to represent the annual home range of the pack. This is a reasonable assumption if a high degree of association exists between pack members (Kolenosky and Johnston 1967, Fuller and Keith 1980, Fritts and Mech 1981, Ciucci *et al.* 1997). We confirmed pack affiliations by examining telemetry locations of wolves believed to be associating and through visual observations of the wolves by the field crew.

We used Home Range and Ranges V[®] software (Kenward and Hodder 1996) to calculate annual (1 Apr–31 Mar) and seasonal 95% minimum convex polygons (Mohr 1947) for individual packs and the entire free ranging wolf population within the primary zone and recovery area (Apache/Gila N.F.). Home range is an extension of ArcView Spatial Analyst. We assumed home ranges were defined when the observation-area curve formed an asymptote (Kenward and Hodder 1996) and locations were obtained throughout the year.

Accuracy of aerial and ground locations for the entire study was estimated to be 250 m, which is the highest mean error of telemetry obtained by researchers on other wolf projects. To account for the 250-m error, we changed the fix resolution from the RangesV[®] software default of 1 m to 250 m. This resolution is used to set the width of the boundary strip that is included in polygon edges and areas (Kenward and Hodder 1996, R. Kenward, pers. comm.). We left the scaling parameter at the software default of 1 m, which means that each coordinate unit was 1 m from the next.

d. RESULTS

From 1998 through 2001, 9 wolf packs were identified by name in the telemetry database. However, the criteria for specifying packs were not always biological. Release sites, geographic locations, and affiliations with other wolves influenced pack designation. Packs, pack compositions, and configurations of home ranges changed as reintroduced wolves encountered other wolves, and established new territories. In addition, the frequent removal and reintroduction of wolves confounded the assignment of individual wolves to specific packs.

The number of recorded aerial and ground locations varied among wolf packs (Figure 1). For the most part, the frequency of locations reflected the time that radio collared wolves were free-ranging, rather than differential effort by the field crew. Time of year, however, affected the number of locations acquired (Figure 2). Discussions with the field team confirmed that for logistic reasons they reduced monitoring activities in winter. We identified some locations that were far outside the reintroduction area. Many of these were recording or data entry errors (Figure 3). Several, however, were from wandering or dispersing wolves.

The proportion of telemetry locations within the primary recovery zone (Apache N.F.) and within the Blue Range wolf recovery area (Apache/Gila N.F.) varied among packs (Figure 4). Temporal trends in the proportion of telemetry locations (pooled across all packs) within the primary zone and within the recovery area also varied (Figure 5). The approximate area occupied by free-ranging Mexican wolf population changed over time as did the density of wolves. This was partially a reflection of periodic releases and recaptures of wolves, and also free-ranging wolves shifting centers of activity as they established pack affiliations and home ranges (Figures 6, 7, 8).

Many individuals and packs showed home range fidelity typical of wolves with established territories (Figure 9). However, frequent social disruption via mortality, recaptures, and re-releases may have altered the natural territorial behavior of packs. Wolves are long-lived social carnivores that transmit information between generations and among individual pack members. In this regard, the establishment, location, and maintenance of home ranges likely depend on a stable pack structure and the persistence of traditional pack knowledge. The home range behavior of reintroduced wolves may be highly susceptible to social disruption because they lack a cognitive map of the area. Moreover, lack of familiarity with the landscape may have a stronger influence on captive reared animals than wild born.

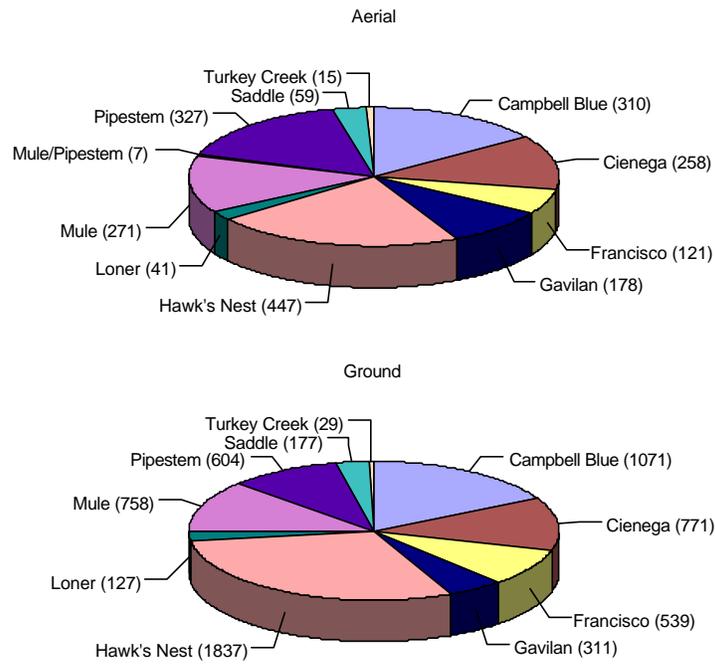


Figure 1. Summary of Mexican wolf radio telemetry data, 1998-2001. Numbers in parentheses are telemetry locations recorded.

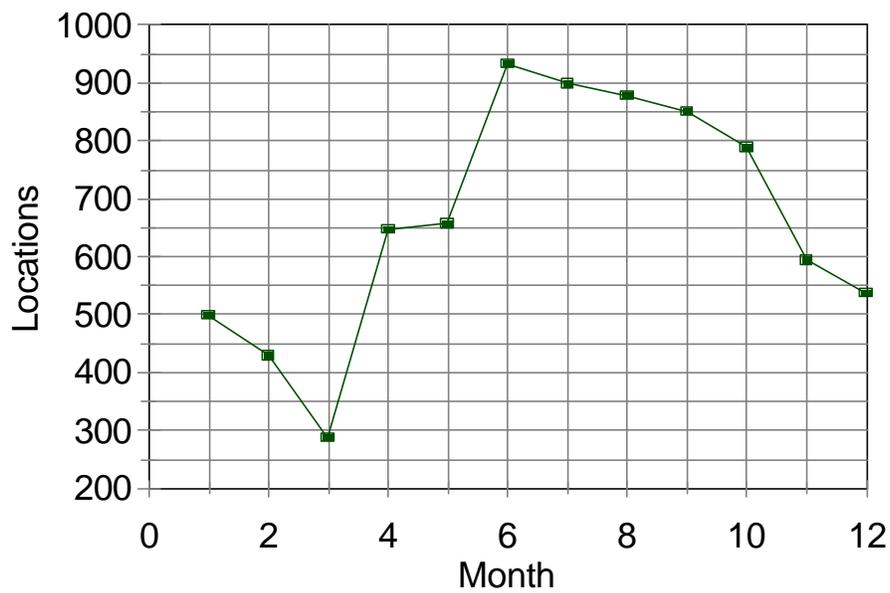


Figure 2. Monthly radio-telemetry locations of reintroduced Mexican wolves, Arizona, 1998-2001.

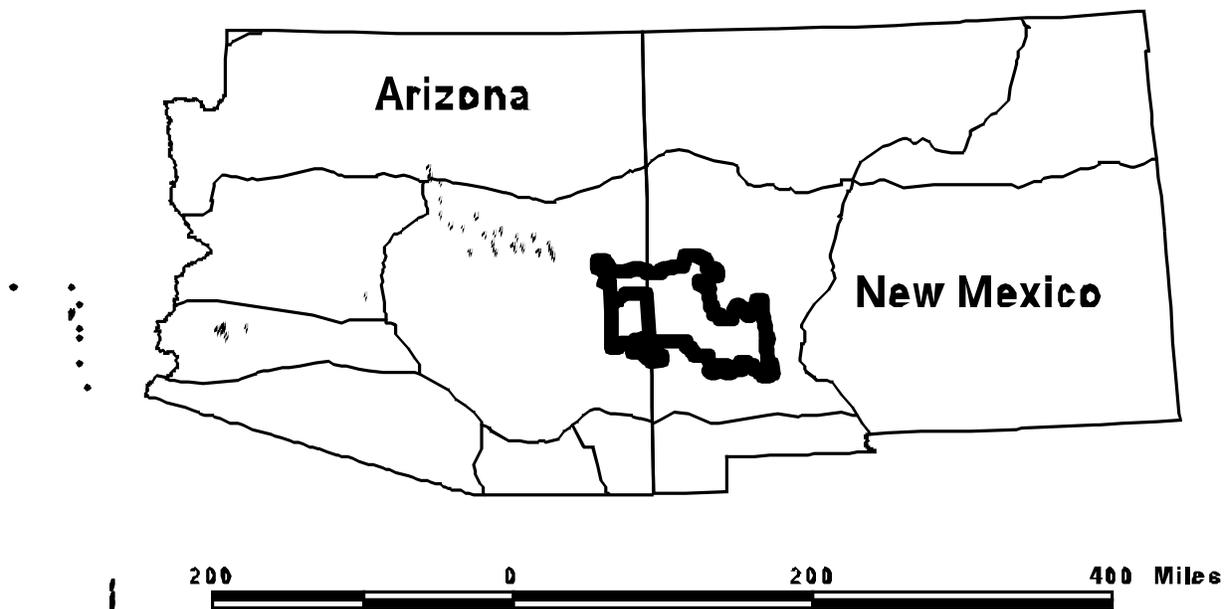


Figure 3. Many telemetry locations resulted from data entry errors. For example, numerous locations were in the state of California and in the Gulf of California.

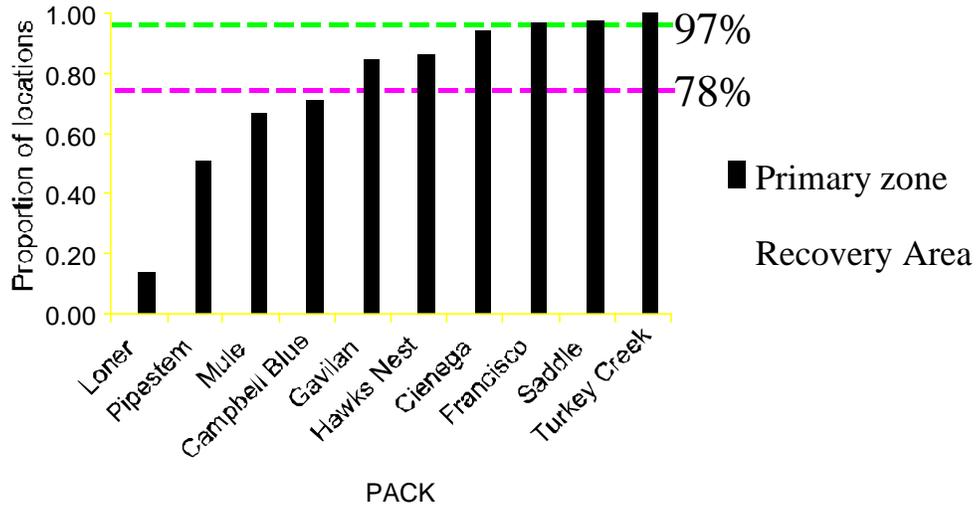


Figure 4. Variation among wolf packs in the proportion of telemetry locations within the primary zone and within the recovery area (Apache/Gila N.F.). These data include all telemetry locations of reintroduced Mexican wolves from 3 March 1998 to 3 March 2001.

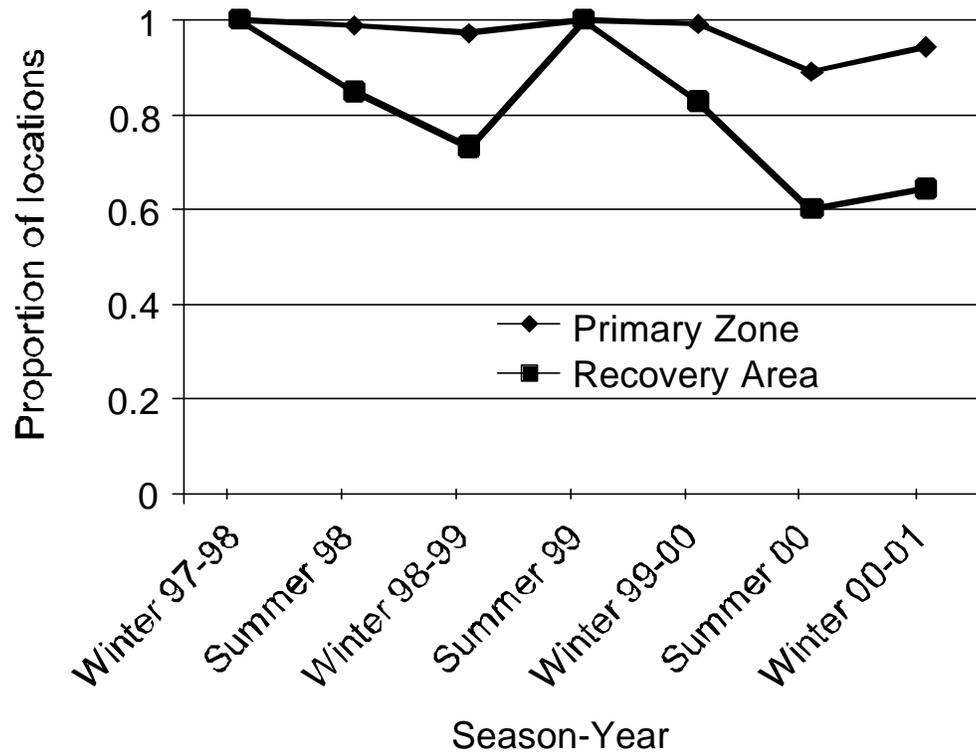


Figure 5. Temporal trends in the proportion of telemetry locations (pooled across all packs) within the primary zone (Apache N.F.) and within the recovery area (Apache/Gila N.F.). These data include all telemetry locations of reintroduced Mexican wolves from 3 March 1998 to 3 March 2001.

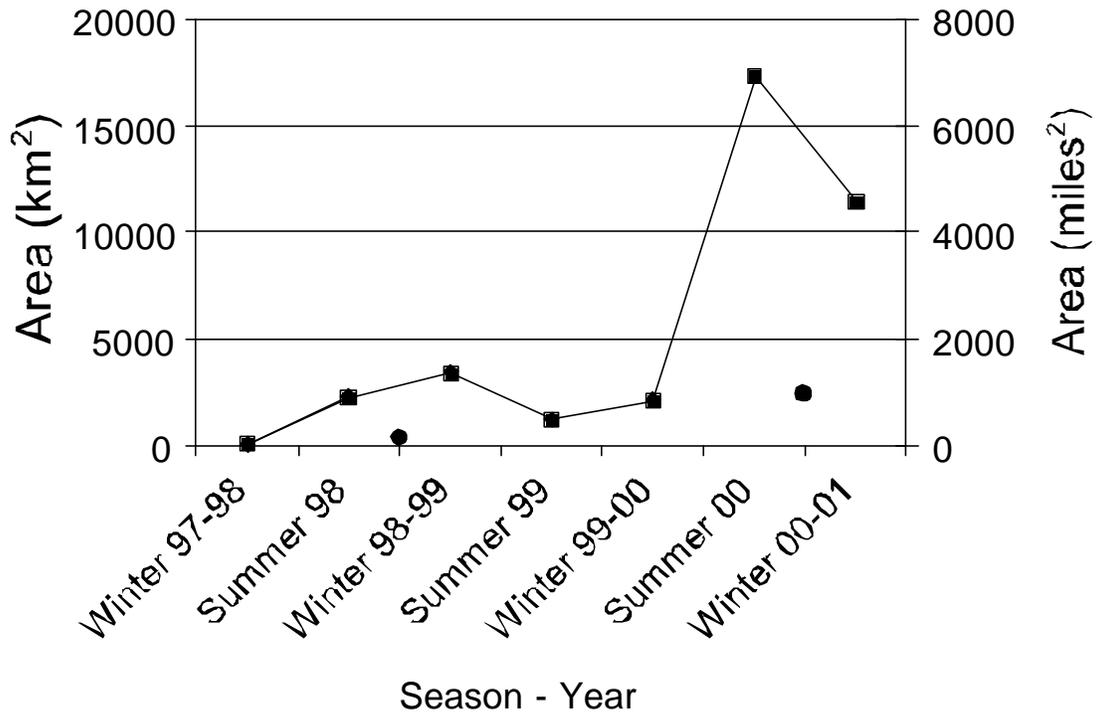


Figure 6. Approximate area occupied by free-ranging Mexican wolf population in Arizona and New Mexico, 1998-2001.

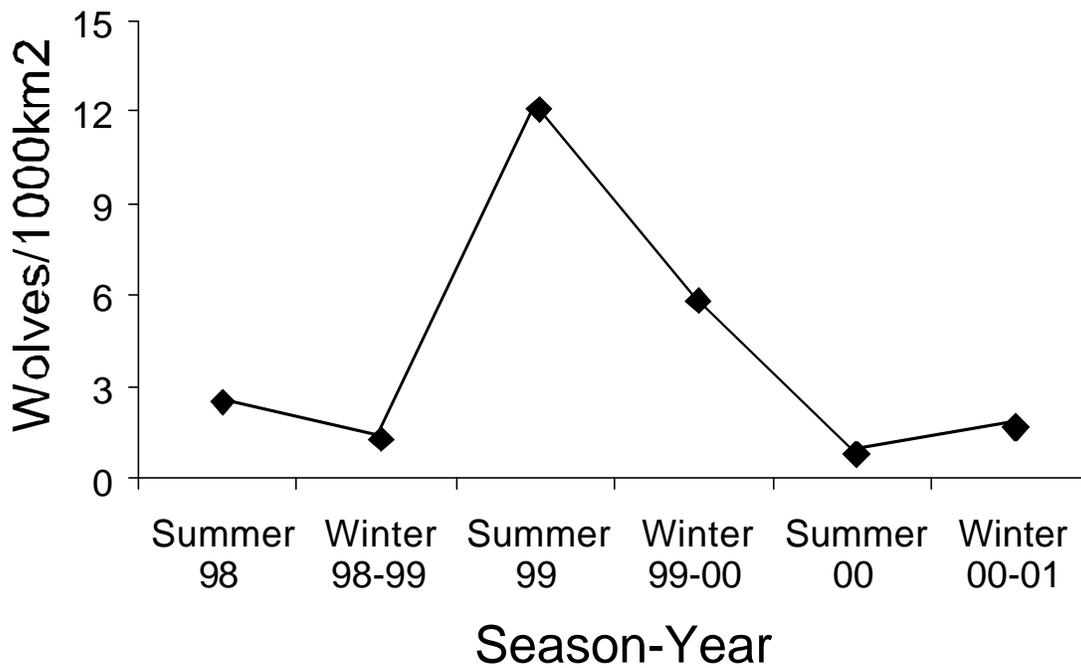


Figure 7. Density of free-ranging Mexican wolf population in Arizona and New Mexico, 1998-2001.

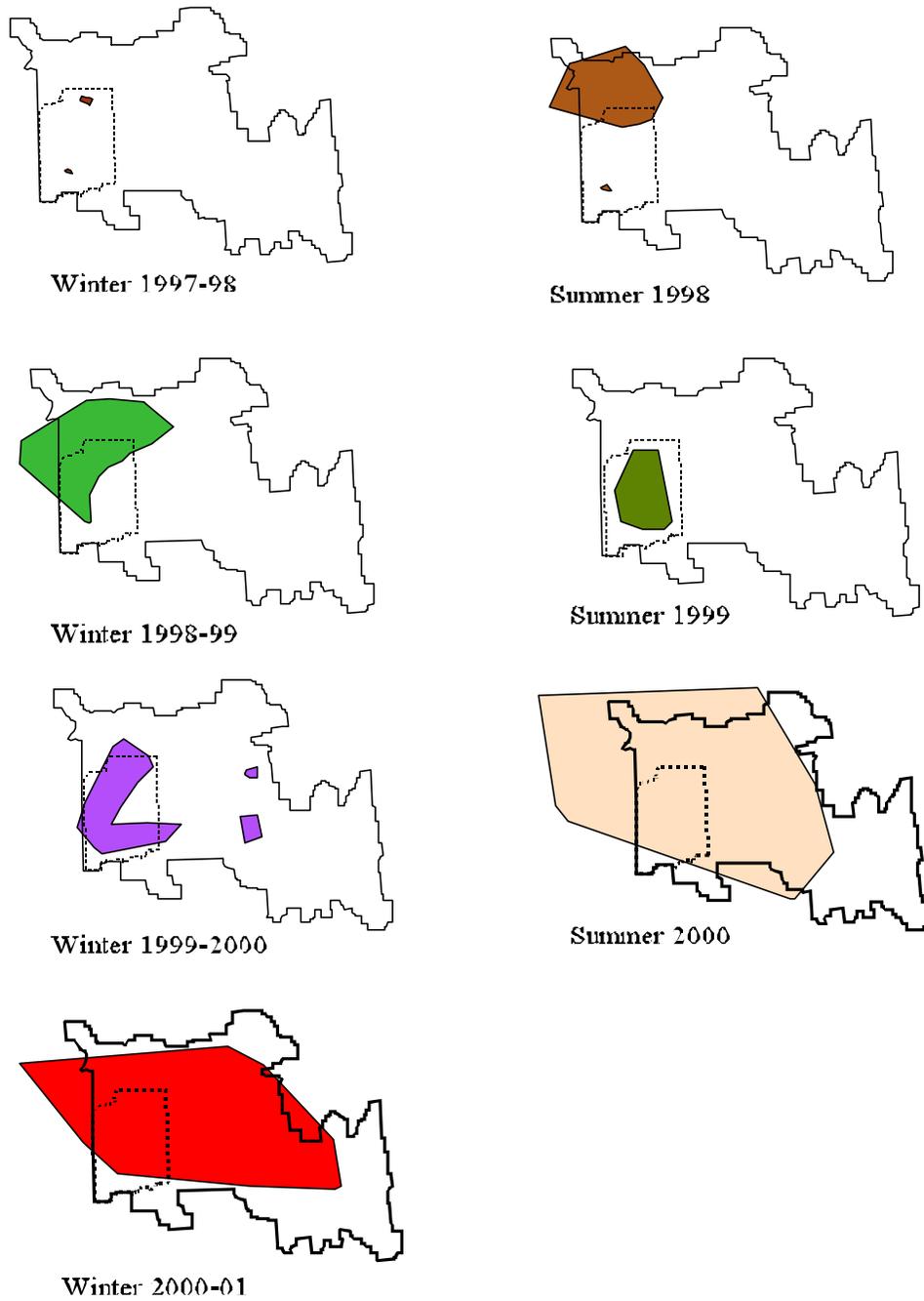


Figure 8. Seasonal distribution of free-ranging Mexican wolf population in Arizona and New Mexico, 1998-2001.

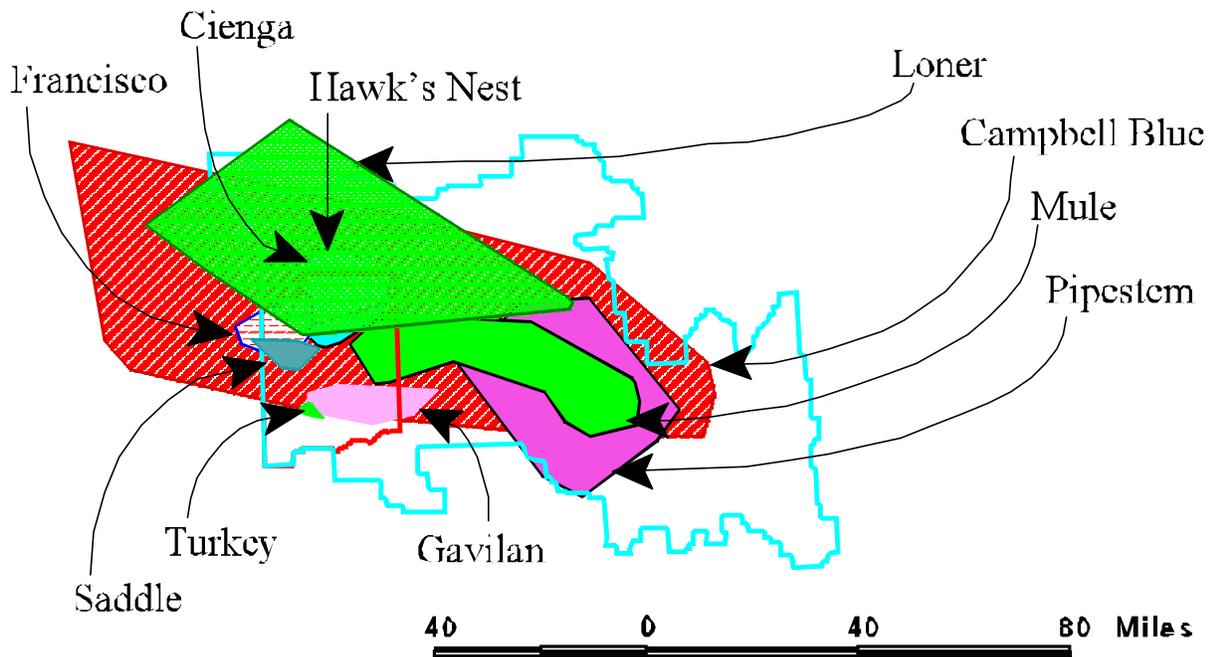


Figure 9. Polygons reflecting the spatial extent of pack home ranges in relation to the primary zone (Apache N.F.) And recovery area (Apache/Gila N.F.). These data include all telemetry locations of reintroduced Mexican wolves from 03 March 1998 to 03 March 2001.

e. CONCLUSIONS

We conclude that some wolves have successfully established home ranges and possibly pack territories within the designated wolf recovery area. We caution, however, that frequent recaptures and re-releases confounded our analysis. These manipulations may also be interfering with pack formation and establishment and maintenance of home ranges. Lastly, individual wolves have shown some indication of dispersing outside the recovery area. This is to be expected and required if the regional population is to be viable.

6. HAVE REINTRODUCED WOLVES REPRODUCED SUCCESSFULLY IN THE WILD?

a. BACKGROUND

i. Births versus recruitment

- (1) Compared with adults, pups have relatively low survival rates during the first year of life.
- (2) In a sense, pups do not really contribute to the viability of a population until they have survived a period of high mortality rate associated with being a pup.
- (3) Although the EIS refers to projected numbers of pups, the projections seem to treat pups as though they have been recruited into the adult population (i.e., with survival rates like adults).

b. DATA SUMMARY

We used information recorded in the telemetry and events databases. Additional information on reproduction was garnered from discussions with the Field Team. Dense vegetation and the secretive nature of wolves precluded regular and accurate visuals of wolves. Consequently, the Interagency Field Team did not routinely observe wolves during spring and summer when pups are easiest to distinguish from adults. We assumed the presence of dens and rendezvous sites when movements became localized in April through July or when lactating females or pups were captured. Sometimes, ground checks confirmed potential denning and rendezvous areas.

c. METHODS

We determined natality directly from field observations of dens, rendezvous sites (pup rearing and resting areas), and packs. We ascertained successful year-specific reproduction using changes in pack size from March to the following December. We assumed unsuccessful reproduction (i.e., no or failed reproduction) when a pack did not display focal activities in the summer. Annual recruitment was derived from winter pack sizes recorded in February.

d. RESULTS

Births have taken place in the wild (Table 1). Births and recruitment rates, however are lower than projected in the EIS (Figures 10 and 11).

Table 1. Known births and recruitments of reintroduced Mexican wolves recorded from 1998-2001. Only 1 litter was conceived in the wild.

PARENTS		ESTIMATED DATE OF BIRTH (M/D/Y)	CONCEIVED IN WILD?	WILD BIRTHS
Female	Male			
174	166	35915	No	Litter of 5 pups (known number due to necropsy report showing 5 placental scars); one survived to ~ 4 months., then disappeared after 174 was killed.
191	208	5/1/99	No	Litter of unknown number (6 confirmed).
482	166	5/1/99	No	Litter of 6 pups (known number due to necropsy report showing 6 placental scars); pups were never documented for this pair by the field team --pair never settled in an area so likely pups were lost immediately.
486	131	5/1/00	Yes	Litter of unknown number (one confirmed).
191	208	5/1/00	No	Litter of unknown number (one confirmed).
189	190	4/15/00	No	Litter of unknown number

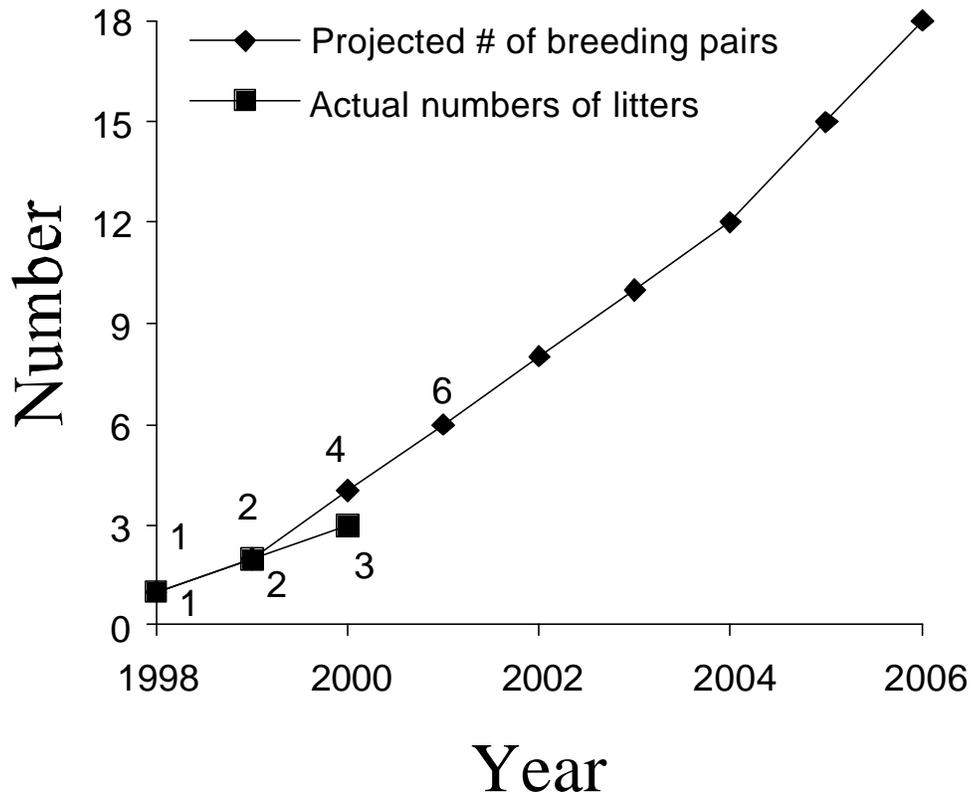


Figure 10. Projected numbers of breeding pairs (in the EIS) and actual numbers of litters for reintroduced Mexican wolves, 1998-2001.

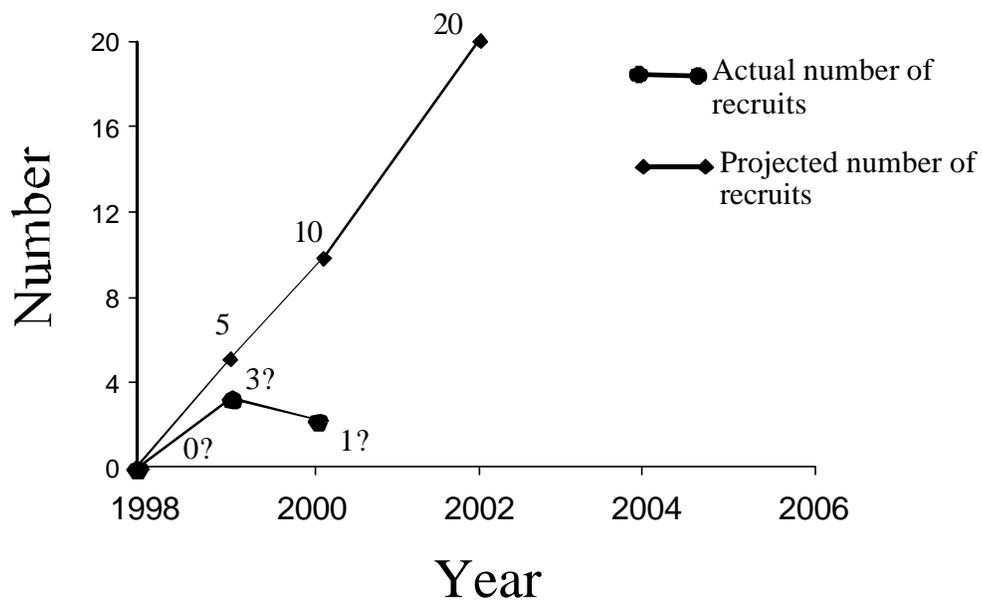


Figure 11. Actual and projected numbers of recruits for reintroduced Mexican wolves, 1998-2001.

e. CONCLUSIONS

The number of free-ranging Mexican wolves at the end of third year is similar to that projected in the EIS. Survival and recruitment rates, however are far too low to ensure population growth or persistence. Without dramatic improvement in these vital rates, the wolf population will fall short of predictions for upcoming years.

7. IS WOLF MORTALITY SUBSTANTIALLY HIGHER THAN PROJECTED IN THE EIS?

a. BACKGROUND

Researchers do not agree on the annual rate of mortality that causes a population decline in wolves. However, Keith (1983) and Fuller (1989) reviewed several wolf studies across North America and concluded that harvests exceeding 28-30% of fall populations resulted in declines. Fuller (1989) further concluded that populations would stabilize with an overall annual mortality rate of 35%. He felt, however, the effects of harvest could vary with time and population structure. Specifically, a population containing many pups could withstand much higher mortality.

Various researchers have suggested different rates of annual mortality they believe control growth of wolf populations. However, the annual rate of mortality that causes a population decline in wolves is unknown. Furthermore, many researchers consider only harvest (hunting or trapping) when they calculate mortality rates that cause wolf population declines. For instance Mech (1970) concluded an annual harvest of 50% or more was necessary to control wolf populations based on pup-adult ratios but did not distinguish between harvest and natural mortality. Keith (1983) reviewed studies of 13 exploited populations and determined that harvests exceeding 30% of fall populations resulted in population declines. Similarly Fuller (1989) found annual rates of wolf increase vary in direct response to rates of mortality and where humans kill wolves, harvests exceeding 28% of autumn or early winter populations might result in a population decline. He concluded a population would stabilize with an overall rate of annual mortality of 0.35 or rate of human-caused mortality of 0.28. Consequently, the exact relationship between the annual rate of mortality from all human causes (harvest, collisions with cars and trains) and population limitation or decline in wolves is uncertain.

In areas where ungulate biomass is low, researchers have noted that starvation and intraspecific aggression are common. For instance, in southwestern Quebec, Messier (1985a) noted wolves with fewer prey available incurred more deaths from natural causes, namely starvation and intraspecific aggression. Similarly, Mech (1977a) noted occurrence of starvation and intraspecific aggression increased as prey availability declined in Minnesota. Disease cannot be linked with certainty to low availability of food but the relationship makes sense intuitively. A population of wolves lacking food should be more vulnerable to disease than one with more food available. Furthermore, food shortage leading to nutritional stress could combine with disease factors to increase the significance of otherwise innocuous or sub-lethal conditions (Brand *et al.* 1995).

In most studies, no disease-related mortality has been reported (VanBallenberghe *et al.* 1975, Mech 1977a, Fritts and Mech 1981, Messier 1985a, Potvin 1987, Ballard *et al.* 1989, Hayes *et al.* 1991, Meier *et al.* 1995, Pletscher *et al.* 1997). In other studies, from 2-21% of wolf mortality has been attributed to disease (Carbyn 1982, Peterson *et al.* 1984, Fuller 1989, Ballard *et al.* 1997). Ballard *et al.* (1997) concluded that occurrence of rabies was a significant factor in a decline of wolves from Alaska. In that study, rabies-caused mortality was 21%.

Quantifying the importance of food in limiting population growth based on cause of death alone is difficult. In the literature, results vary among studies. On Isle Royale, annual mortality from starvation and intraspecific strife (both related to low food availability) ranged from 18-57%

during a 20-year period (Peterson and Page 1988). In populations where some human-caused mortality occurs, and thus compensates for natural mortality (starvation, accidents, disease and intraspecific strife), about 8% of individuals greater than 6 months-of-age can be lost each year (Ballard *et al.* 1987, Fuller 1989). Some researchers have accepted this variability and decided any sign of starvation among adult wolves means food is limiting population growth (Fritts and Mech 1981, Ballard *et al.* 1997, P. Paquet, pers. comm.). This assumption is reasonable given adults typically are the last members of the population affected by food shortage (Eberhardt 1977) and as such, may be the most sensitive indicators of a shortage of food.

Human-caused mortality can also be an important limiting factor (Peterson *et al.* 1984; Ballard *et al.* 1989, 1997). However, quantifying the importance of human-caused mortality as a limiting factor is difficult. These causes include legal harvest (Fuller and Keith 1980, Keith 1983, Gasaway *et al.* 1983, Messier 1985a, Ballard *et al.* 1987, 1997, Peterson *et al.* 1984, Potvin 1987, Bjorge and Gunson 1989, Fuller 1989, Hayes *et al.* 1991, Pletscher *et al.* 1997), illegal harvest (Fritts and Mech 1981, Fuller 1989, Pletscher *et al.* 1997), vehicles on highways (Berg and Kuehn 1982, Potvin 1987, Fuller 1989, Paquet 1993, Parks Canada 1994, Forbes and Theberge 1995, Paquet and Hackman 1995, Thiel and Valen 1995, Bangs and Fritts 1996), and trains (Paquet 1993, Parks Canada 1994, Paquet and Hackman 1995, Paquet *et al.* 1996).

b. DATA SUMMARY

We used information recorded in the telemetry and events databases. Additional information, clarification of events, and interpretation of events was provided by the Interagency Field Team. All free-ranging Mexican wolves were radio-collared from time of release. Moreover, each radio-collared Mexican wolf was and continues to be relocated regularly and frequently via ground and aerial telemetry. Frequent monitoring reveals whether each wolf is alive or dead at the time of relocation

c. METHODS

We were not able to address the question of annual mortality directly because removals and re-releases precluded calculating annual rates of mortality. Thus, we estimated survival rates for the Mexican wolf population and then compared these estimated values with the survival rates projected in the EIS. Survival rate is the chance (or probability) of surviving some specified time. Survival rates are typically expressed as values between zero and one. For example, if the annual survival rate of an individual is 0.82, we would say that individual has an 82% chance of surviving during the next year. Survival is a critical population process and estimating survival rates is an important part of measuring viability of populations. Management of protected wolf populations requires quantitative survival measurements to identify factors that drive population change. From the survival rate one can also understand the mortality rate. The mortality rate of an individual or population is one minus the survival rate.

Using the telemetry data we compiled a table showing the number of wolves that were alive each month, died each month, and recaptured each month. The table provided the foundation for formal analysis of survival rates. We estimated survival rates of radio-collared wolves using the Kaplan-Meier (K-M) product limit estimator (Kaplan EL and Meier 1958). We carried out this analysis using the programs MARK and Minitab (Version 12). Conceptually, the analysis uses the relationships between the number of wolves that die each month and the number monitored

each month. Although estimating a rate of survival for each month is possible, the data show that annual survival rates do not vary substantially across longer periods. Thus, we estimated survival rates using an information-theoretic approach (Burnham and Anderson 1999) that determines the most appropriate time scale (e.g., monthly, seasonally, or annually).

From the perspective of a free-ranging population, returning a wolf to captivity (from now on, recapture event) is equivalent to a mortality event. Thus, we conducted 2 survival analyses. One analysis considered only true biological deaths, and the other treated biological deaths and recapture events as mortality events. In both analyses, we reincluded wolves from time of release until “mortality” or disappearance of the radio-signal occurred.

Sample sizes were too small to use Cox's proportional hazards model and determine the influence of important covariates (such as age and sex) on survival. We did not calculate cause-specific mortality. Mortality was described, however, using percents. We assumed that the proximate cause of death was the ultimate cause of death. We were unable to assess the relative importance of other factors that may have been involved.

The starting date of the survival study was March 1998 and the end date was March 2001. For known deaths we estimated the date of mortality to the nearest day using evidence from the telemetry and events data bases. When information was unavailable, we deemed day of mortality the midpoint of the interval between the last day the wolf was known alive and the day it was discovered dead. The cause of mortality was often identified on site and when possible, confirmed by necropsy (Interagency Field Team pers. comm.)

d. RESULTS

Forty-seven (47) wolves were monitored From March 1998 (when Mexican wolves were first released) to March 2001. Twenty-three (23) wolves are currently being monitored. Four (4) wolves are unaccounted for. Twenty (20) wolves were recaptured following release. Nine (9) of these were re-released and are known to be alive. Two (2) wolves were re-released but contact was lost and their fate is unknown. One of the re-released wolves died. Eight (8) of the recaptured wolves were not re-released and some died in captivity. Seventeen (17) wolves are known to have died, 10 in the wild (Figure 12). Human caused mortality was the most common cause of death. Of the human related deaths, most were caused by gunshots (Figure 13). Wolves also died from distemper and parvovirus. Both these diseases are contracted or originally spread from domestic animals. Death by disease was higher than projected in the EIS.

When recaptures were included as mortalities, survival rates were lower than projected in the EIS (Figure 14). Excluding recaptures as mortalities resulted in survival rates exceeding the EIS projections in 1999 and 2000 (Figure 15). Survival rates from either method, however, were lower than for wolves in the Flathead region of Montana and British Columbia (Pletscher *et al.* 1997), lower than for wolves in the central Canadian Rocky Mountains, lower than a recovering wolf population in the Yukon (Hayes and Harestad 2000), and higher than an exploited population in Alaska (Ballard *et al.* 1987).

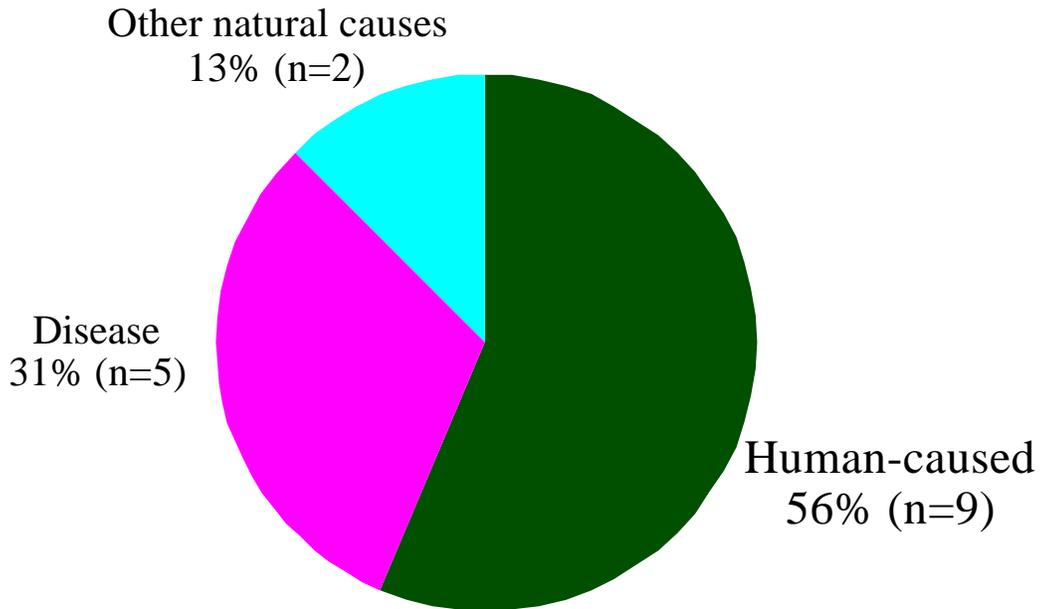


Figure 12. Causes of wolf mortality for Mexican wolves reintroduced to Arizona, 1998-2001.

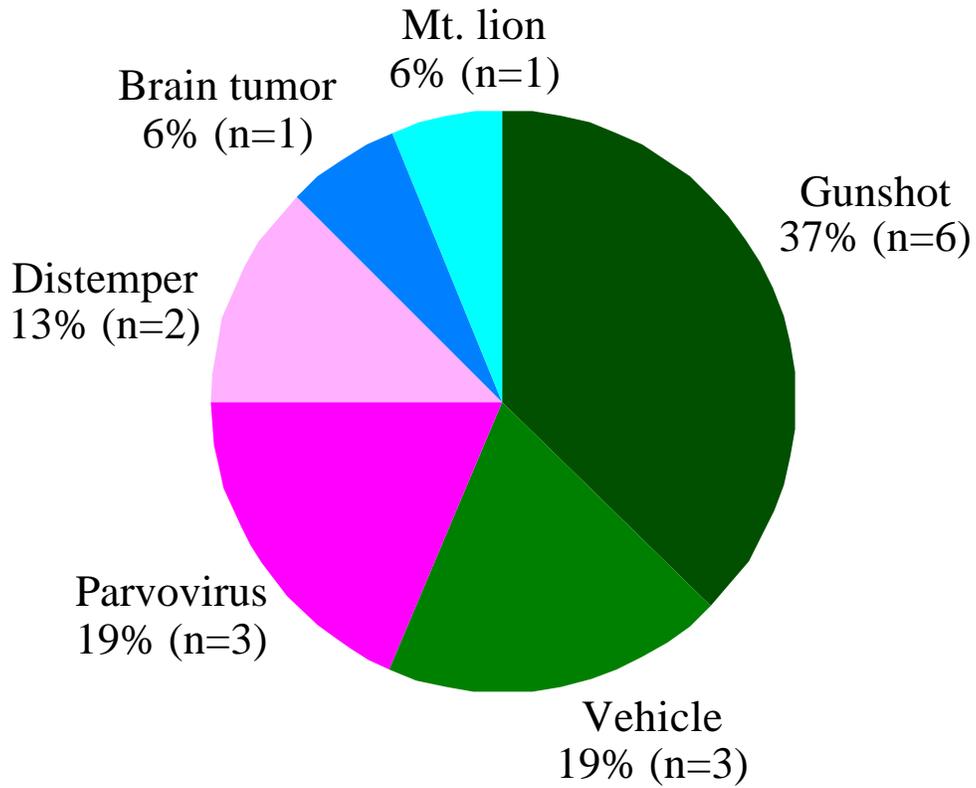


Figure 13. Cause specific wolf mortality for Mexican wolves reintroduced to Arizona, 1998-2001.

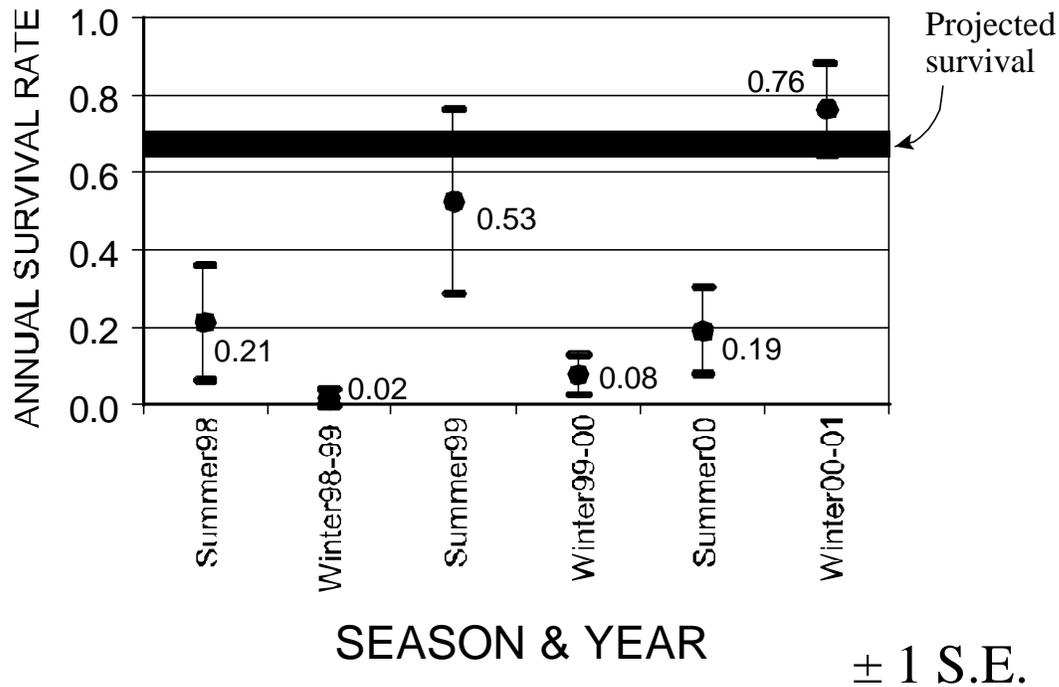


Figure 14. Survival analysis of reintroduced Mexican wolf population assuming that recapture represents a mortality event. Analysis was conducted for the period 1998-2001.

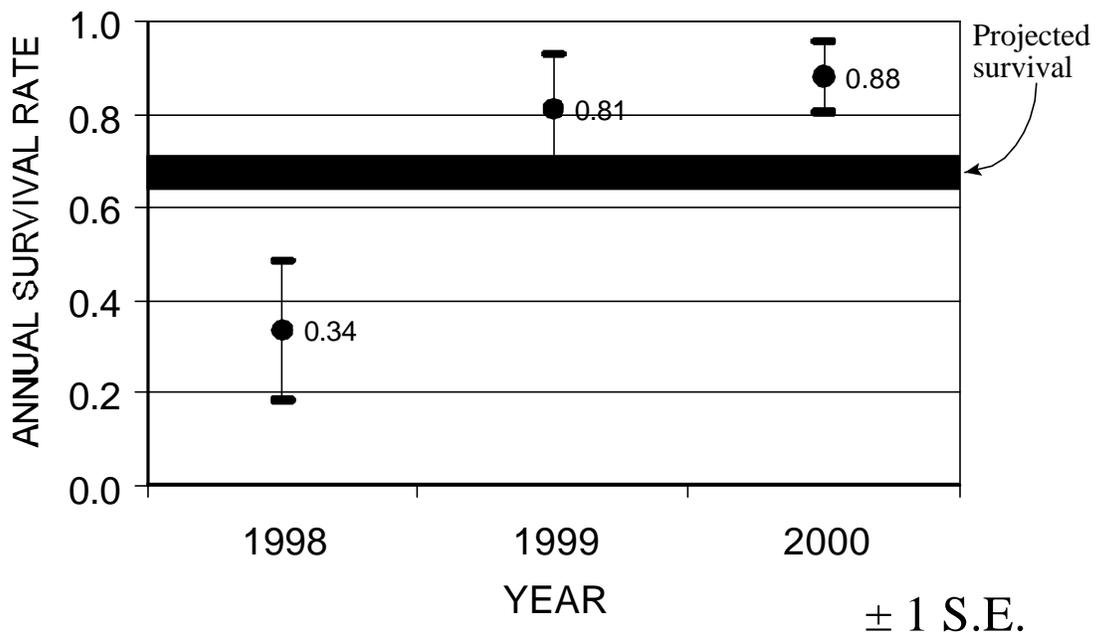


Figure 15. Survival analysis of reintroduced Mexican wolf population assuming that recaptures do not represent a mortality event. Analysis was conducted for the period 1998-2001.

e. CONCLUSIONS

Frequent removals and re-releases of wolves confounded our analysis of rates and causes of mortality. However, if recaptured wolves were at high risk of being killed, then survival is much lower than projected in the EIS. Human-related deaths were the greatest source of mortality for reintroduced Mexican wolves. Shooting was the major source of death. Numerous other studies have reported human-caused deaths as the major cause of wolf mortality (Fuller and Keith 1980, Berg and Kuehn 1982, Boitani 1982, Carbyn 1982, Ballard *et al.* 1987, Fuller 1989, Mech 1989, Pletscher *et al.* 1997, and many others).

8. IS POPULATION GROWTH SUBSTANTIALLY LOWER THAN PROJECTED IN THE EIS?

a. BACKGROUND

Rates of increase in wild wolf populations have varied between 0.93 and 2.40 (Fuller and Keith 1980, Fritts and Mech 1981, Ballard *et al.* 1987, Hayes *et al.* 1991, Messier 1991, Pletscher *et al.* 1997). Several factors limit growth of wolf populations; those reported most commonly include ungulate biomass (Van Ballenberghe *et al.* 1975, Mech 1973, 1977a, 1977b, Fuller and Keith 1980, Packard and Mech 1980, Keith 1983, Messier 1985a, 1987, Peterson and Page 1988) and human-caused mortality (Van Ballenberghe 1981, Gasaway *et al.* 1983, Keith 1983, Peterson *et al.* 1984, Fuller 1989, Paquet *et al.* 1996, Noss *et al.* 1996). Keith calculated the maximum rate of increase for wolves ($r = 0.304$, $\lambda = 1.36$) (1983) based on the highest reproductive and survival rates reported from studies on wild wolves. He corroborated the results by comparing the estimate with data from wolves that colonized Isle Royale National Park, 1952-1959 ($r = 0.304$, $\lambda = 1.39$). These were likely maximum rates of increase because the population was initiated by few individuals with abundant food (Keith 1983). However, both rates are still much lower than a theoretical exponential rate of 0.833 ($\lambda = 2.30$) given maximum reproduction (Rausch 1967), a stable age distribution and no deaths.

Keith (1983) suggested the amount of food available and age structure of the population affect rates of growth of wolf populations. VanBallenberghe (1981), Gasaway *et al.* (1983), Keith (1983), Peterson *et al.* (1984), Ballard *et al.* (1987), and Fuller (1989) found that wolf populations can be limited by harvest levels of 20-40%, but that the lower rate has a more significant effect in an area with low ungulate biomass (Gasaway *et al.* 1983). Another factor to consider is that effects of harvest vary with time and population structure (Peterson *et al.* 1984, Fuller 1989). If productivity is high, and consequently the ratio of pups to adults is high, the population can withstand a higher overall mortality because pups (non-producers) make up a disproportionate amount of the harvest (Fuller 1989). Furthermore, net immigration or emigration may mitigate the effects of harvest (Fuller 1989).

b. DATA SUMMARY

We assessed the density of the wolf population, size of established packs, and population growth using radiotelemetry data and direct observation by the Interagency Field Team. Most of these data are contained in the Monitoring and Events databases.

c. METHODS

We calculated density of wolves/1000 km² by determining intra-pack densities (home range size/number of wolves in pack) of radio-collared wolves and averaging these densities per year (Potvin 1987, Bjorge and Gunson 1989, Okarma *et al.* 1998). The size of packs was based on numbers of wolves observed during midwinter aerial locations (15 Jan-15 Feb). We estimated population growth using finite rates of increase (λ) based on the ratio of successive yearly estimates of density. Mean annual finite rate of increase was calculated by taking the antilogarithm of the mean exponential rate of increase ($\bar{r} = \ln \lambda$) for the population (Fuller 1989).

The fundamental equation of population demography for a closed population is:

$$N_t = N_{t-1} + B_t - D_t$$

where N_t = population size at time t , B_t = number of recruits at time t , D_t = number of deaths at time t ,

For a wild population, removals are similar to mortality and re-releases similar to recruitment. Therefore, the equation that best describes the reintroduced Mexican wolf population is:

$$N_t = N_{t-1} + B_t - D_t + \beta_t - \delta_t$$

where δ_t = (unpredictable) removals of ‘naughty’ wolves, β_t = subsequent re-releases of those ‘naughty’ wolves, $\beta_t \gg B_t$, $\delta_t \gg D_t$

d. RESULTS

From available databases and discussions with the Interagency Field Team, we identified a number of events relevant to assessment of population dynamics (Table 2). Using this information, we calculated population growth rates (Figures 16, 17) and the varying number of free-ranging wolves over time (Figures 18 and 19). Growth rates and numbers of wolves were close to projections, although frequent re-releases and removals obscured comparisons. To provide context for interpreting these results, we also generated mean growth rates for other reintroduced and recovering wolf populations (Figures 20, 21, 22). To date, the growth rate of the reintroduced Mexican wolf population is comparable with similar reintroduction and recovery efforts.

Table 2. Population events recorded for reintroduced Mexican wolf population between 1998 and 2001..

POPULATION EVENT	NUMBER
Recruits	3 - 5
Re-releases	21
Deaths	10 - 16
Removals	31

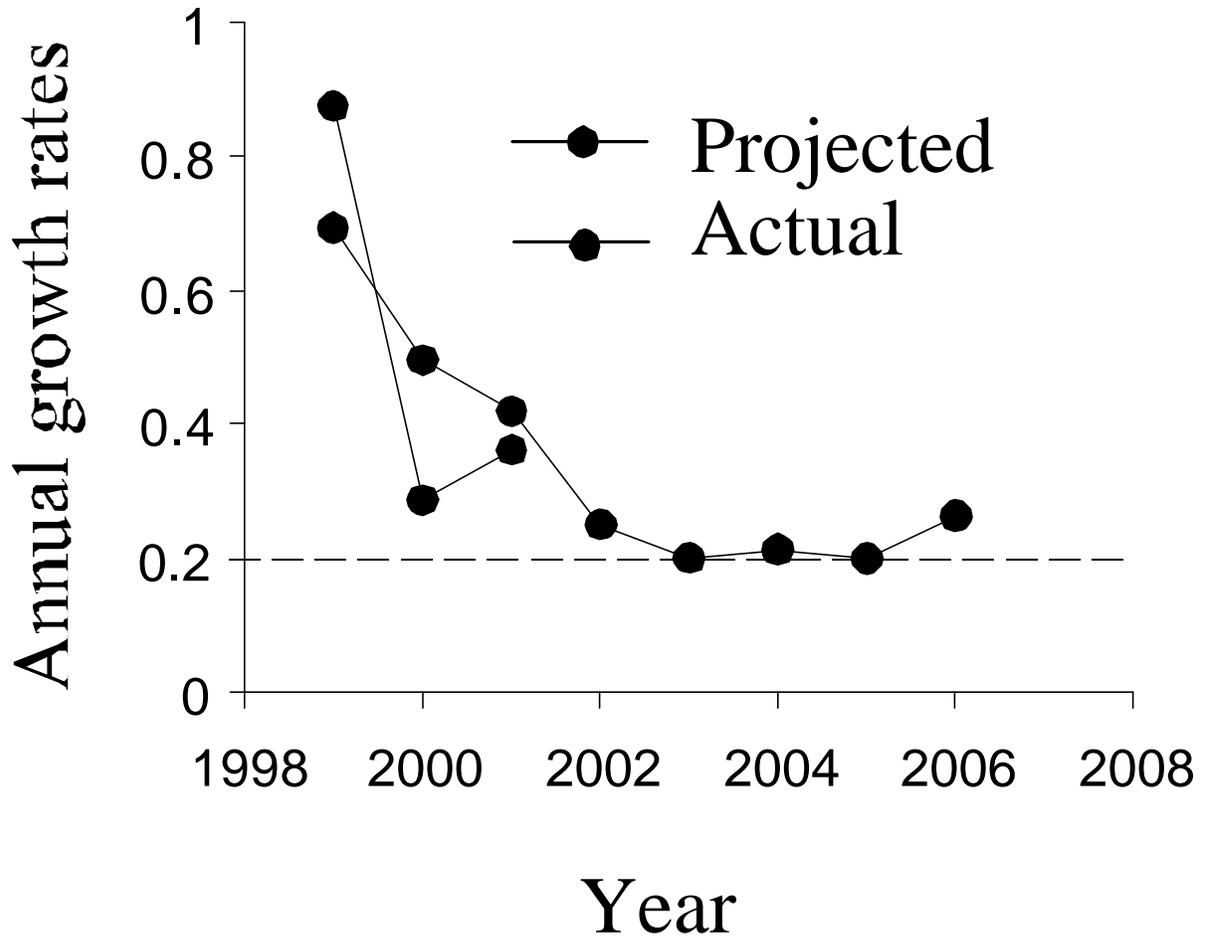


Figure 16. Projected and actual annual growth rates of free-ranging Mexican wolf population. Actual growth rate is strongly influenced by frequent intervention.

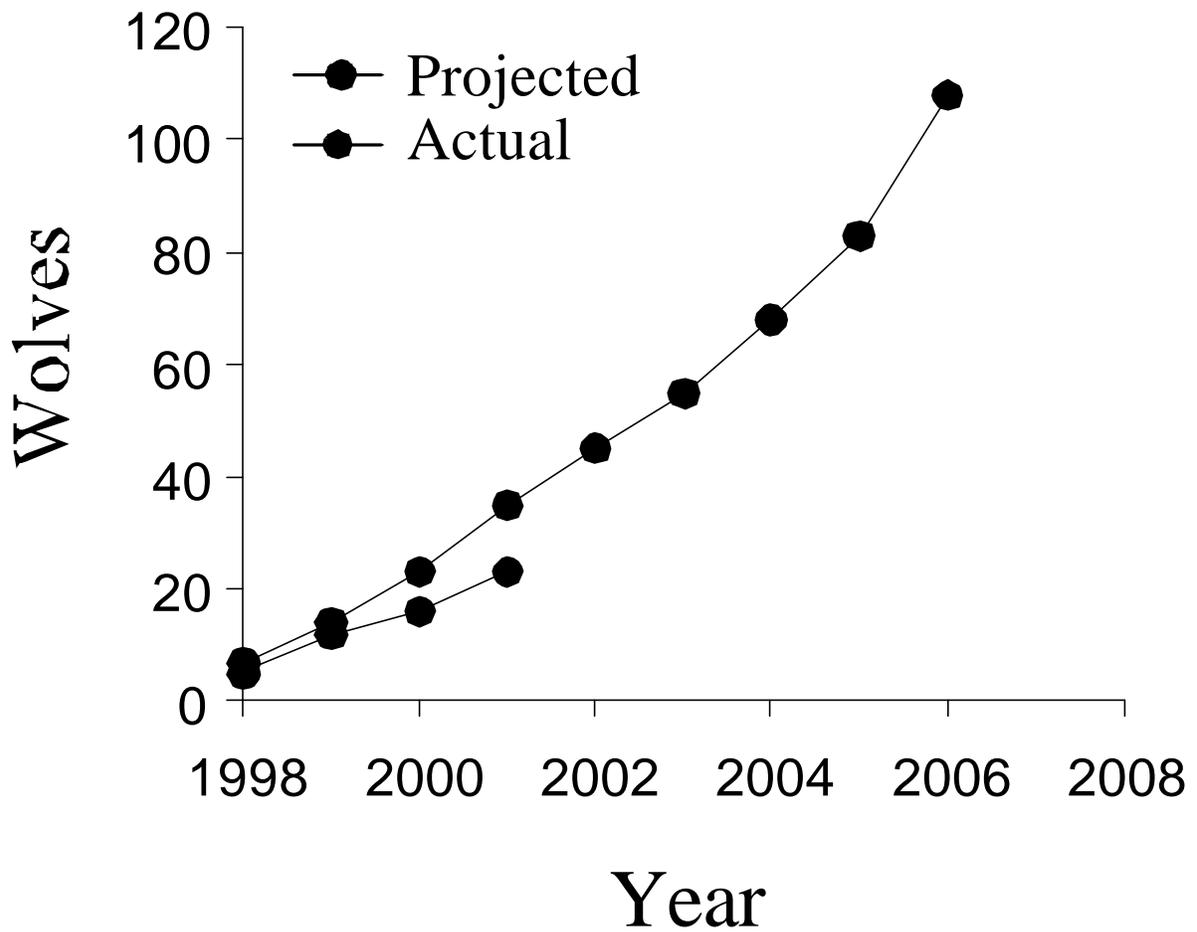


Figure 17. Projected and actual sizes of free-ranging Mexican wolf population, 1998-2001.

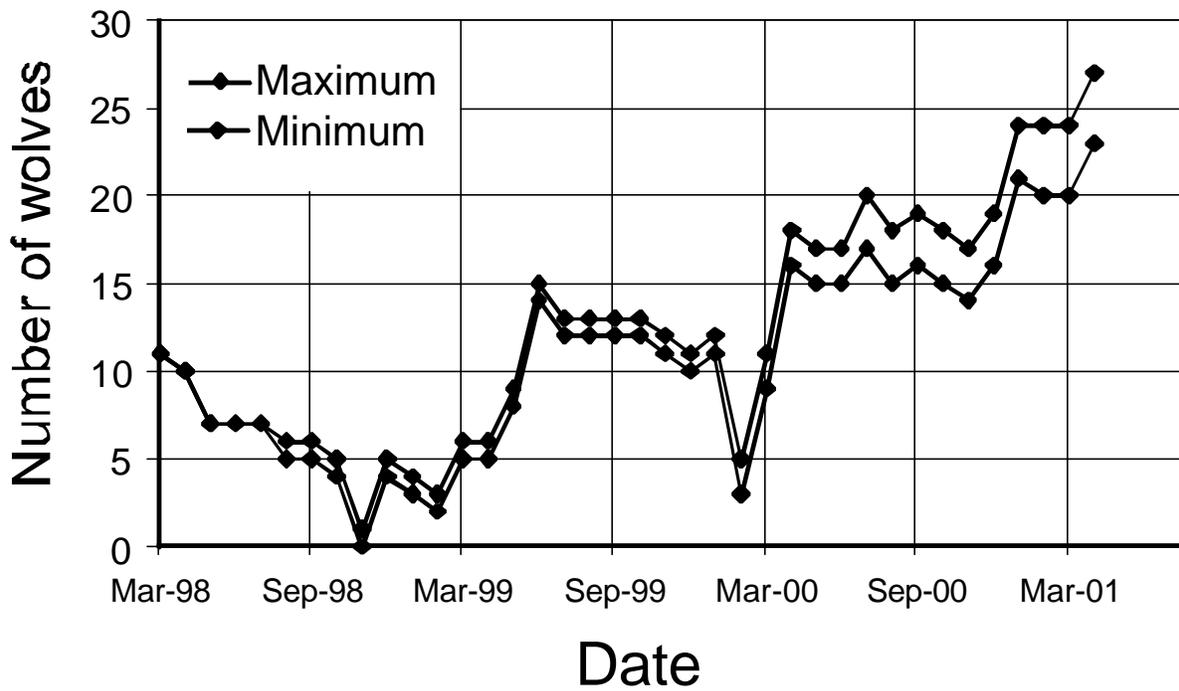


Figure 18. Number of free-ranging radiocollared Mexican wolves, 1998-2001. The difference between the max and min accounts for 4 wolves whose signals were lost, and in one case, a wolf that threw its collar.

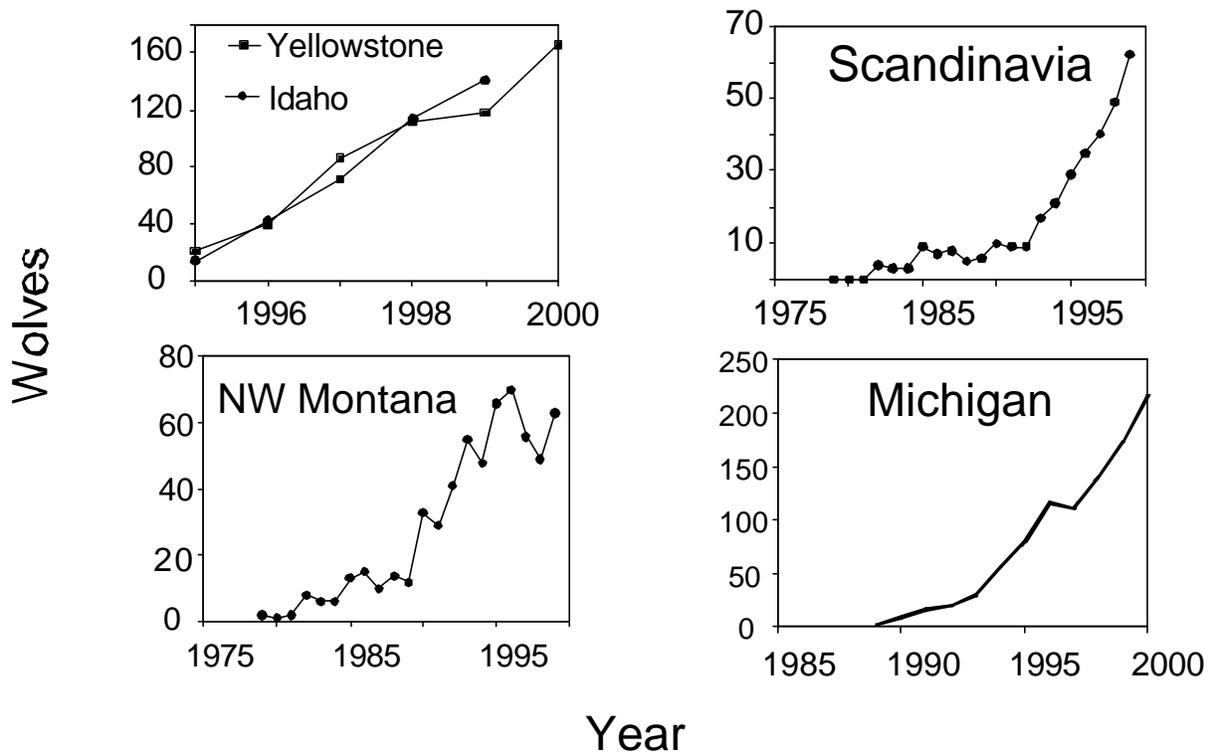


Figure 19. Growth rates of other recovering wolf populations. Sources: <http://www.r6.fws.gov/wolf/annualrpt99/> and unpublished documents from JAV

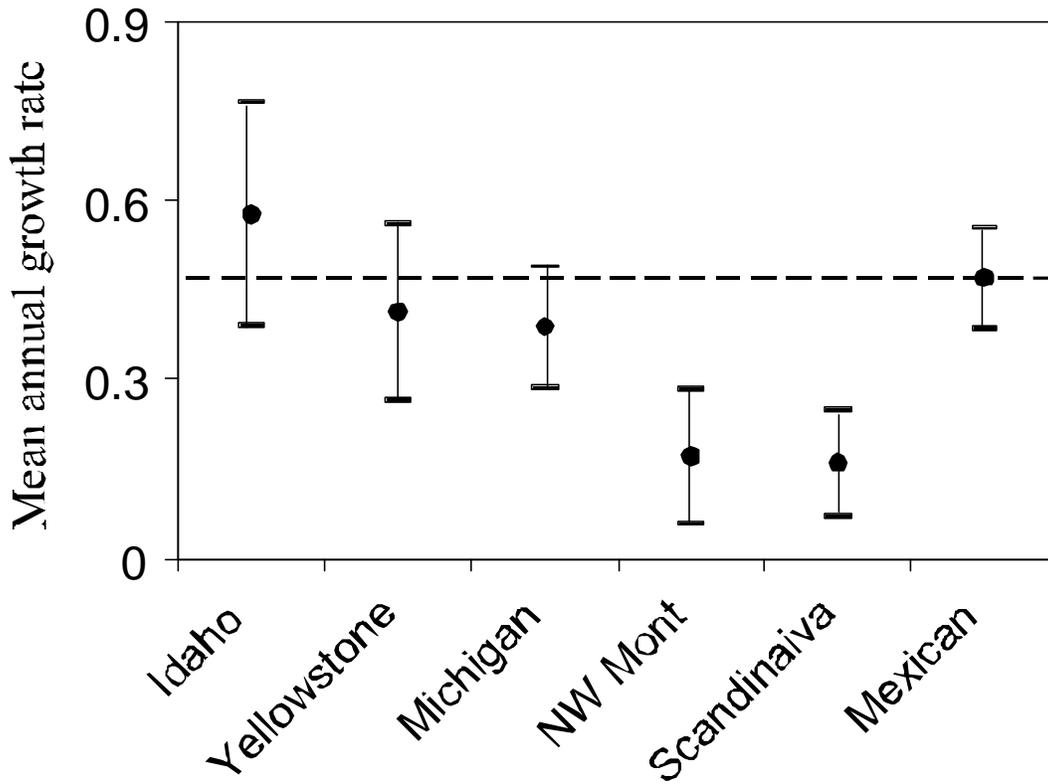


Figure 20. Mean annual growth rate for other recovering populations.

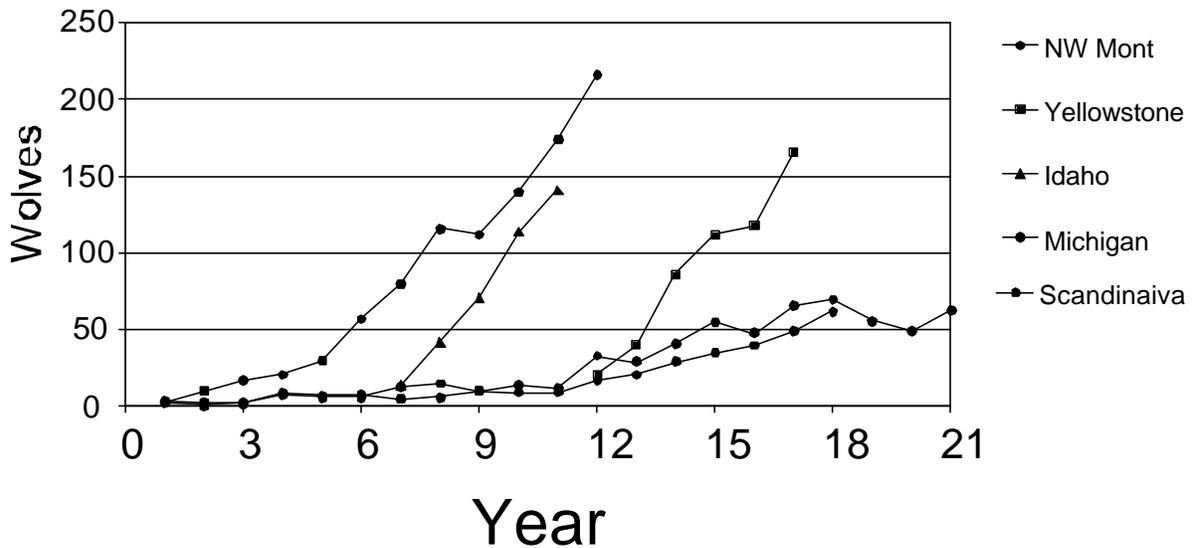


Figure 21. Number of wolves over time in other recovering populations.

Assessing the average growth rate only tells part of the story. Fluctuations in growth rates are also critical. The more fluctuation the greater the extinction risk. In this case, to assess fluctuations, we need to examine the population trajectory on a different time scale.

Using data collected since March 1998, we calculated a 39% chance that the annual growth rate is < 0.0 ; a 43% chance the annual growth rate is ≤ 0.10 ; and a 50% chance the annual growth rate ≤ 0.20 (Figure 22). Using data collected since December 1998, we calculated a 23% chance that the annual growth rate is < 0.0 ; a 26% chance the annual growth rate is ≤ 0.10 ; and a 29% chance annual growth rate ≤ 0.20 (Figure 23).

*A monthly growth rate of 0.083 corresponds to an annual growth rate of 0.1. A monthly growth rate of 0.0166 corresponds to an annual growth rate of ~-0.2.

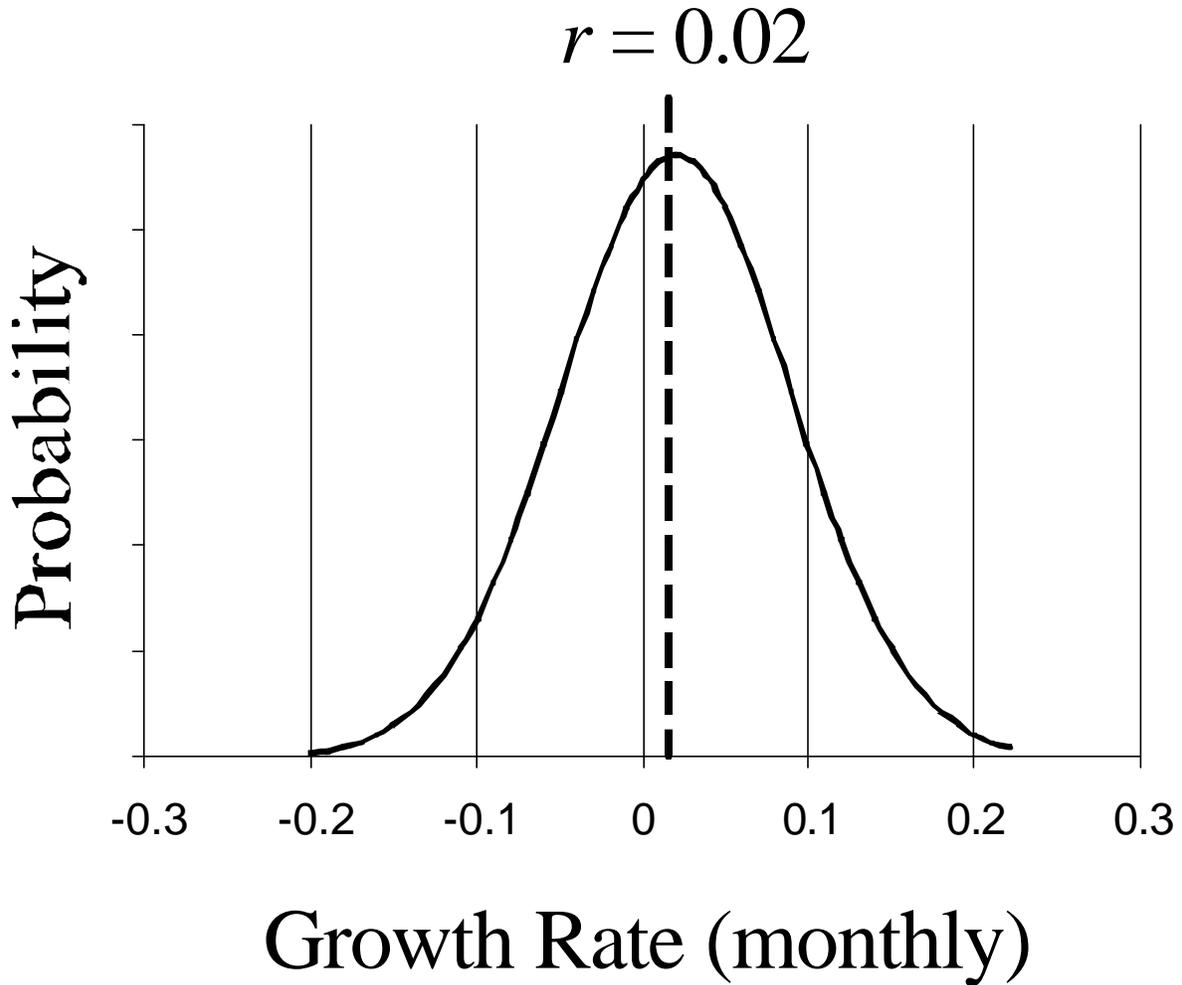


Figure 22. Mean onthly growth rate (r) since March 1998. The expected value of r is 0.02. The standard error is 0.07.*

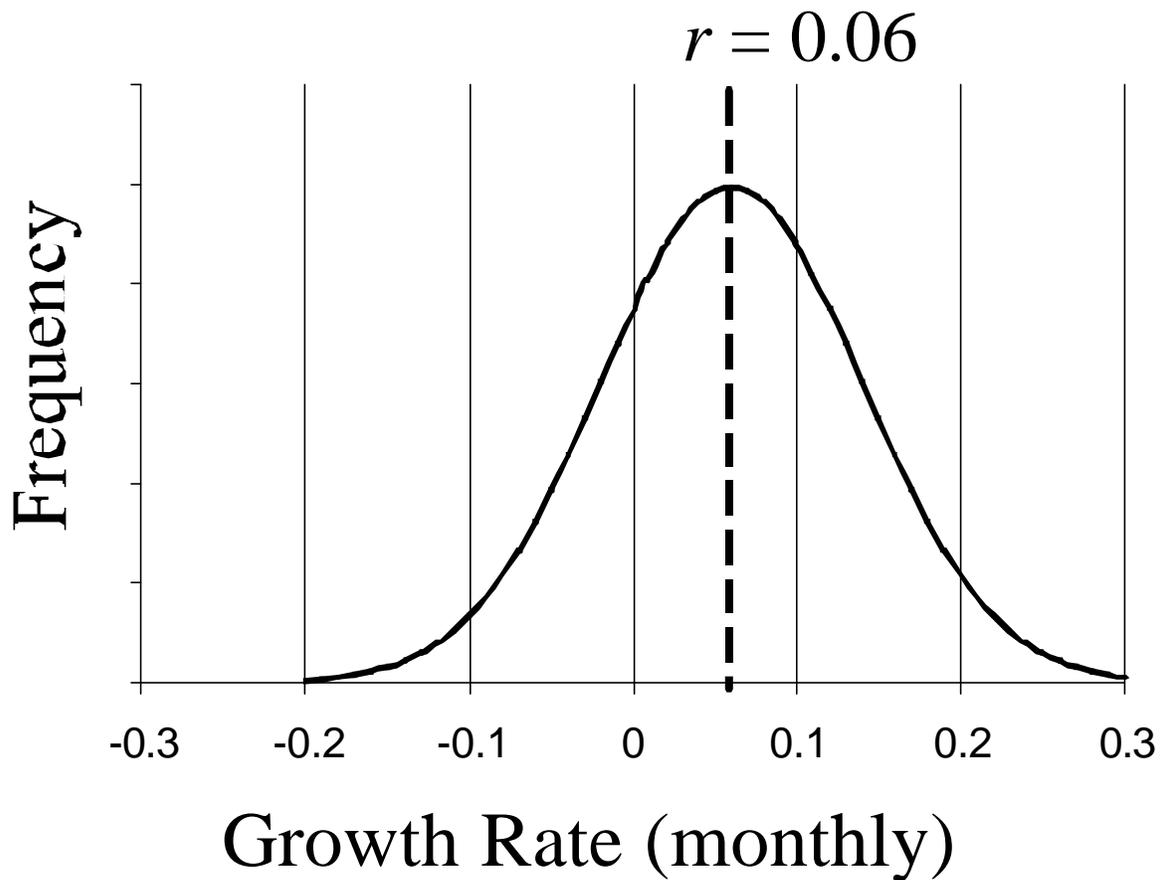


Figure 23. Mean monthly growth rate (r) since December 1998 (when population went temporarily extinct). The expected value of r is 0.06. The standard error is 0.08.

e. CONCLUSIONS

To date, intervention has dominated natural processes. So, determining if the growth rate is lower than predicted in the EIS is not possible. If the current rate of intervention continues, restoration of a population of 100 wolves would require 28 re-releases annually and 41 removals annually. Although the current population size is similar to that projected in the EIS, we suspect that population growth would have fallen far short of expectations without intervention. Clearly, managers must balance future introductions, recaptures, and re-releases with the need to establish and maintain natural population processes (Figure 24).

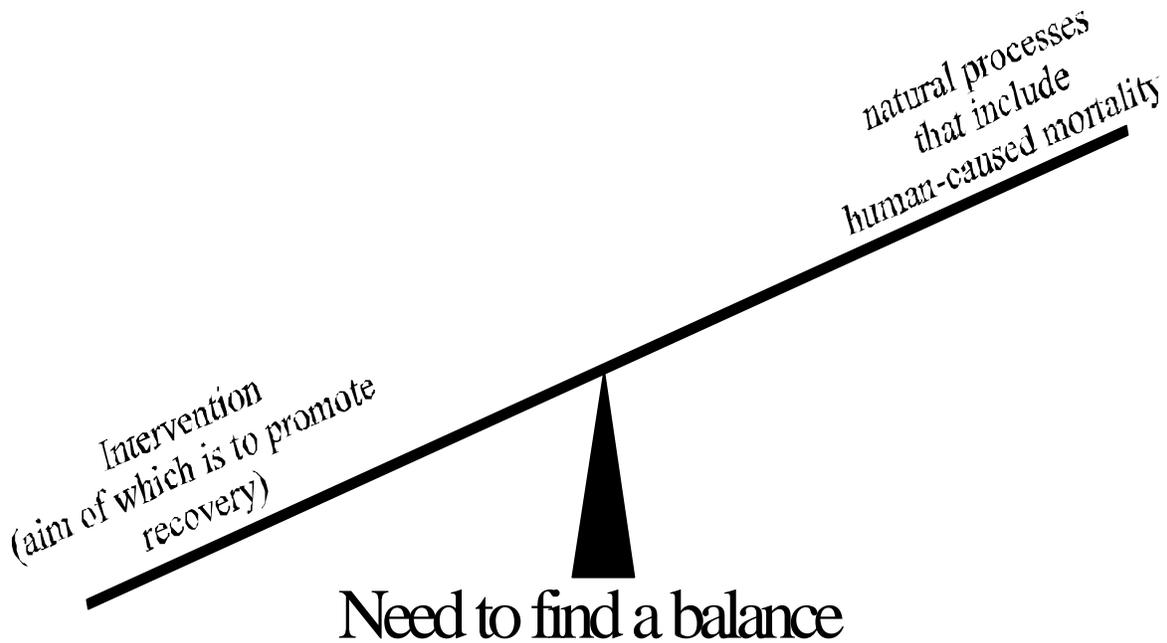


Figure 24. Because of frequent interventions the vital rates we derived (survival and population growth) are unlikely to reflect the population's future viability. A balance between intervention and the effects of natural population processes is needed.

9. ARE NUMBERS AND VULNERABILITY OF PREY ADEQUATE TO SUPPORT WOLVES?

a. BACKGROUND

Without human disturbance, densities reflect the wolf's dependency on ungulate prey species (Keith 1983). Wolf population dynamics are believed to be largely dictated by the per capita amount of prey and its vulnerability to predation, and the degree of human exploitation (Keith 1983; Fuller 1989). The effect of food on wolf demography is mediated by social factors, including pack formation, territorial behavior, exclusive breeding, deferred reproduction, intraspecific aggression, dispersal, and by primary prey shifts (Keith 1983).

Wolf populations are closely linked to population levels of their ungulate prey (Keith 1983, Messier 1985a, Fuller 1989). Maintaining viable, well-distributed wolf populations depends on maintaining an abundant, available, and stable ungulate population. Packard and Mech (1980) concluded that intrinsic social factors and the influence of food supply are interrelated in determining population levels of wolves. In situations where other factors reduce prey populations (e.g., winter weather), predation by wolves can inhibit the recovery of prey populations for long periods (Gasaway *et al.* 1983). In a multiprey system, the stability, or equilibrium, of ungulate prey and wolf populations seems to depend on a variety of factors, including the wolf predation rate, the number of ungulates killed by hunters, the ratio of ungulates to wolves, and the population growth rate of different ungulate species (Carbyn 1982, Huggard 1992, Paquet 1993, Paquet *et al.* 1996, Paquet 1989).

Changes in habitat composition and distribution can have a significant effect on prey densities and distributions, and therefore wolf spatial distribution. Wolf packs may react to changing conditions in varying ways, depending on the location of their territories in relation to other packs and prey distribution. If packs have lower prey densities within their territories, they may exploit territories more intensely.³ This may be achieved by 1) persevering in each attack, 2) using carcasses thoroughly, 3) feeding on alternative and possibly second-choice food resources such as beaver (*Castor canadensis*) (Messier and Crete 1985), and 4) patrolling their territory more intensely (Messier 1985). Messier, in his study area in southeastern Quebec, found daily distances of Low Prey packs were on average either greater (summer) or equal (winter) to daily distances of High Prey packs. The territory size, however, was approximately 35% smaller in the Low Prey area, supporting the fact that wolves were searching each unit area with greater intensity in both seasons.

Many studies emphasize the direct effects (e.g., prey mortality) wolves have on the population dynamics of their ungulate prey (Carbyn 1974, Mech and Karns 1977, Carbyn 1983, Gasaway *et al.* 1983, Messier 1994, Messier and Crete 1985, Peterson *et al.* 1984, Gunson 1983, Ballard *et al.* 1987, Boutin 1992, and others). However, predation can also profoundly affect the behaviour of prey, including use of habitat, time of activity, foraging mode, diet, mating systems,

³ Territory and home range size is more closely correlated with pack size than with prey density (Messier 1985, Peterson *et al.* 1984). In areas of higher prey density pack sizes increase (Messier 1985). Messier's (1985) data indicate that between 0.2 and 0.4 moose/km², territory area per wolf is independent of moose abundance.

and life histories (Sih *et al.* 1985). Accordingly, several studies describe the influence wolves have on movements, distribution, and habitat selection of caribou (*Rangifer tarandus*), moose, and white-tailed deer (Mech 1977, Stephens and Peterson 1987, Ballard *et al.* 1987, Nelson and Mech 1981, Messier and Barrette 1985, Messier 1994). Wolves can increase the rate at which they accrue resources by seeking out areas with dense concentrations of prey (Huggard 1991, Weaver 1994). Prey, in turn, can lower their expected mortality rate by preferentially residing in areas with few or no wolves. Several studies have suggested that ungulate prey seek out predator-free refugia to avoid predation by wolves (Mech 1977, Holt 1987, Paquet 1993). Wolf predation in the Superior National Forest of northern Minnesota was found to affect deer distributions within wolf territories (Mech 1977). Densities were greater along edges of territories where predation was thought to be less.

Unusually mild or severe winter weather can result in ungulate populations that are temporarily higher or lower than predicted habitat capability (which reflects long-term average maximum). Where predation is a factor, ungulates may exist at levels well below carrying capacity for relatively long periods. The interactions of ungulates and their predators (in our case wolves, coyotes, foxes, black bears, and cougars) may, under some circumstances, overshadow habitat capability as a controlling factor for ungulate populations. Ungulate populations may be more strongly influenced by the frequency and depth of population lows, than by habitat capability.

Ungulate biomass can affect rates of population increase and resulting densities of wolves. Building on work of Keith (1983), Fuller (1989) reviewed 25 studies of North American wolf and prey populations and found rates of increase of wolf populations are most affected by relative availability of ungulate biomass (directly influencing survival of pups <6 months old) and human-caused mortality. He concluded that regardless of prey type or stability of wolf populations, average wolf densities are clearly correlated with the biomass of ungulates available per wolf. Furthermore, he found the index of ungulate biomass per wolf is highest for heavily exploited (Ballard *et al.* 1987) or newly protected (Fritts and Mech 1981) wolf populations and lowest for unexploited wolf populations (Oosenbrug and Carbyn 1982, Mech 1986) or those where ungulates are heavily harvested (Kolenosky 1972).

b. DATA SUMMARY

We used information in the carcasses database to assess wolf use of prey species. Prey densities and the weights of prey were derived from Groebner *et al.* (1995).

c. METHODS

We estimated potential wolf numbers using regression equations that relate wolf numbers to ungulate biomass (Keith 1983, Fuller 1989). The equations were modified to reflect prey species available to wolves in Arizona and New Mexico.⁴ Accordingly, biomass was calculated by multiplying population densities of elk, white-tailed deer, and mule deer (*O. hemionus*) by average edible weights of elk, white-tailed deer, and mule deer. We used weights of 159 kg (350 lb.) for elk, 36 kg (80 lb.) for white-tailed deer, and 55 kg (122 lb.) for mule deer (Groebner *et al.*

$${}^4Y = 0.041X$$

where Y = wolf numbers, X = prey biomass

1995). We used prey densities of 1.1 km² for elk, 0.9 km² for white-tailed deer, and 2.8 km² for mule deer (Groebner *et al.* 1995). Assuming that ungulate populations would decline slightly in the presence of wolf predation, prey densities were reduced 10% in our final calculations. We assumed prey were evenly distributed and equally available throughout the primary and secondary release sites. Bighorn Sheep (*Ovis canadensis*), pronghorn (*Antilocapra americana*), javelina (*Tayassu tayacu*), and beaver (*Castor canadensis*) were not included in our analyses because no population data were available.

d. RESULTS

The Interagency Field Team recorded 55 probable wolf kills. Elk constituted 85%, mule deer 7%, and deer of unknown species about 4% of recorded kills. The predominance of elk in the diet was consistent among packs (Figure 25). Based on numbers of prey available and biomass available within the primary release site, elk were used disproportionately. Note, however, that observational bias may skew collection of kill data. Elk are easier to find because they are larger than deer and not consumed as rapidly. In addition, the seasonal movements of wolves and their prey can affect spatial overlap and thus availability. Lack of data and time prevented us from assessing this possibility.

Based on ungulate biomass, the Blue Range Wolf Recovery Area (6,854 mi² or 17,751 km²) can, in theory, support an estimated 468 wolves (range 292-821). The target recovery area of 12,950 km² (5,000 km²) could support between 212 and 599 wolves (Figure 26) (Table 3). We believe these estimates are high because they assume all prey are equal and will be consumed in proportion with availability. Given our experience with multiple prey systems elsewhere this is unlikely to occur. We therefore calculated wolf population estimates for individual prey species. Accordingly, elk in the Blue Range Wolf Recovery Area could support about 213 wolves, and the combined deer species about 255 wolves.

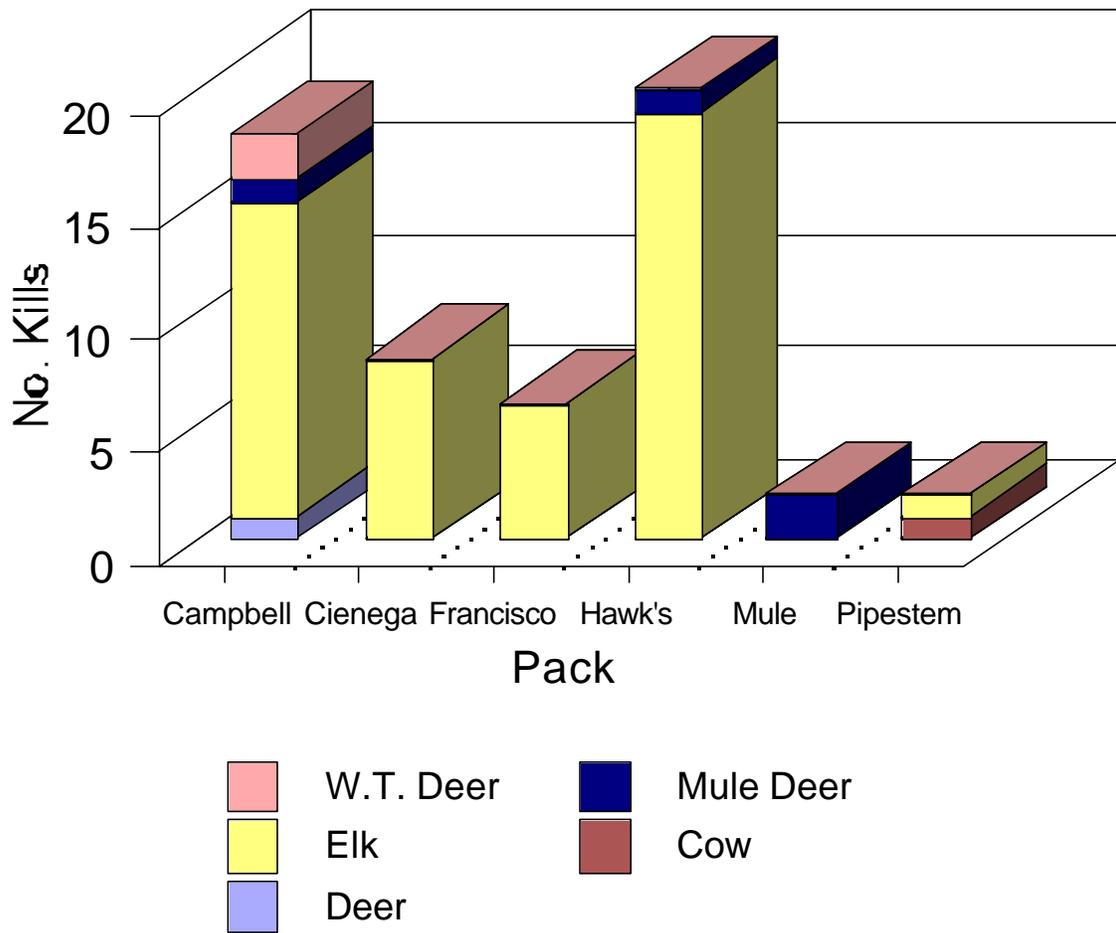


Figure 25. Prey (n = 55) probably killed by reintroduced Mexican wolves, 1998-2001.

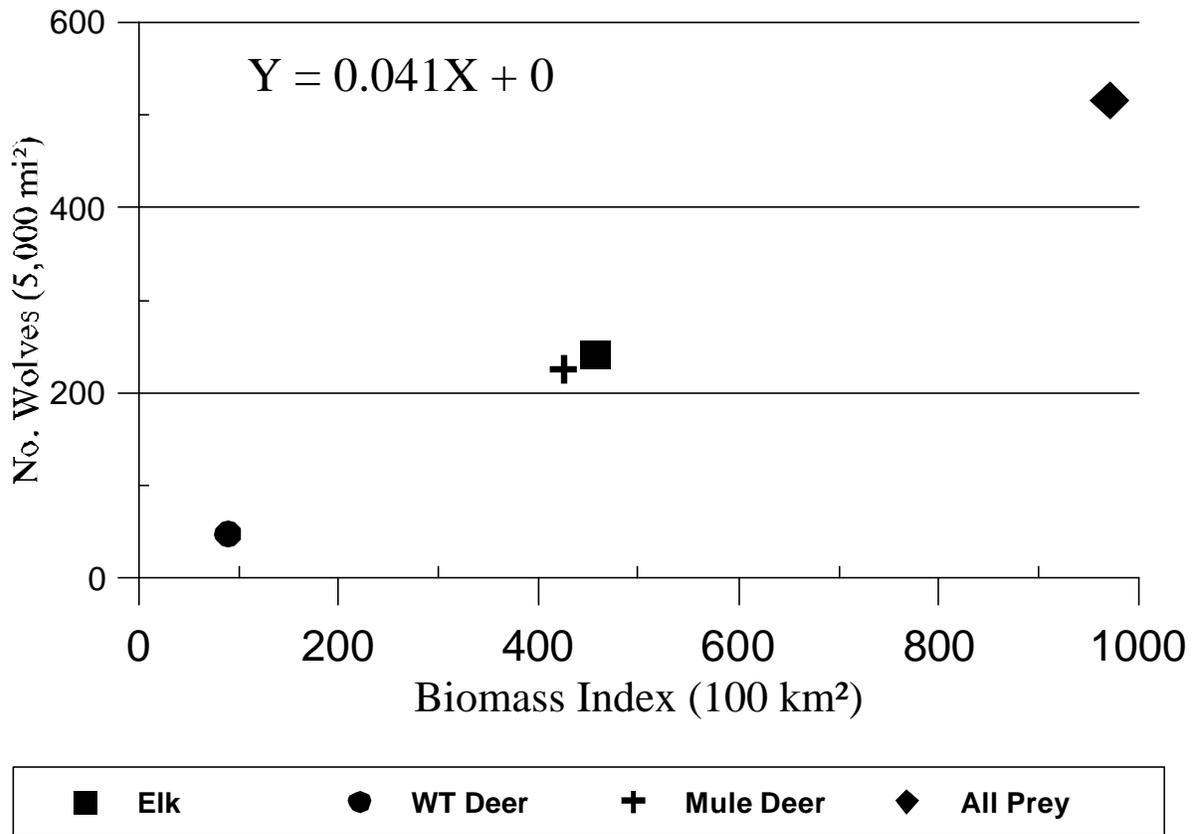


Figure 26. Potential number of wolves that, in theory, could occupy target objective of 12,950 km² (5,000 mi²) within the Blue River Wolf Recovery Area. Estimates are based on prey biomass available to wolves and are maximum numbers. The individual contribution of ungulate prey species is shown for comparison with other studies.

Table 3. Potential wolf numbers (ranges) for recovery areas based on predicted population densities of ungulates 5 years post restoration of Mexican wolf population. We partitioned the table to show the contributions of different ungulate species.

PREY SPECIES	Primary Zone (2,664 km²)	Recovery Objective* (12,950 km²)	BRWRA* Low (17,563 km²)	BRWRA* High (17,563 km²)
White-tailed Deer	10-13			
Mule Deer	46-63			
White-tailed and Mule Deer		118-323	162-245	293-443
Elk	50-67	94-276	129-195	250-378
All Prey	106-143	212-599	292-441	543-821

*For white-tailed and mule deer, we used an average biomass to derive wolf estimates.

e. CONCLUSIONS

Given the current ratio of wolves to ungulate prey, we conclude the reintroduced Mexican wolf population is not limited by food. Adequate prey are available to support and sustain a growing wolf population. Estimated wolf numbers derived from ungulate biomass were similar to numbers projected in the EIS. Because wolves depend primarily on ungulates for food, long-term survival of wolves in the study region depends primarily on protection of habitat for elk and deer.

10. HAS THE LIVESTOCK DEPREDAATION CONTROL PROGRAM BEEN EFFECTIVE?

a. BACKGROUND

Although an effective livestock depredation program is critical for wolf recovery, effective assessment of such a program requires more specific guidance and data than we were provided.

b. DATA SUMMARY AND METHODS

Our analysis is based on interpreting records in the Events and Incidences databases.

c. RESULTS

Forty-two (42) reports of possible wolf-livestock interactions were recorded between March 1998 and March 2001. Of these, the Interagency Field Team concluded that 5 events were accidents, 9 were non-wolf predators [e.g., bear (*Ursus americanus*), lion (*Felis concolor*), coyote (*C. latrans*)], 18 were wolf related, and 10 were probably wolf related. That is, 28 events involved wolves or probably involved wolves. These included uninjured livestock, injured livestock, and killed livestock (Table 4). The Interagency Field Team recorded 10 confirmed livestock-wolf interactions where no injury or death occurred. At a minimum, 55% (26) of all free-ranging wolves have interacted with livestock. Thirty-six percent (17) have interacted with livestock 3 or more times. Approximately 10% have interacted with livestock 5 or more times. Approximately three-quarters of the livestock injuries or deaths occurred on National Forests.

The number of reported livestock-wolf interactions varied seasonally (Figure 27). The interactions reported annually since the first reintroduction of Mexican wolves were; 5 from March 1998 to March 1999, 17 from March 1999 to March 2000, and 6 from Mar 2000 to Mar 2001.

Seventeen (17) reports of wolf interactions with cats or dogs were recorded between March 1998 and March 2001. These 17 reports included uninjured dogs, injured dogs, and killed dogs or cats. Of these, we concluded that; 13 interactions involved wolves; 1 interaction probably involved a wolf, and; 3 interactions cannot be classified using the data provided. The Interagency Field Team recorded 8 dog-wolf interactions where no injury or death occurred. Of the 13 interactions that definitely involved wolves, 5 resulted in the cat or dog being killed or injured (Table 5).

The average response time for all reported domestic animal-wolf interactions was less than 24 hours. The longest response time was 3 days, which occurred once.

Table 4. Numbers of domestic animal injuries and deaths due to wolf depredation. The data are for confirmed, probable and unconfirmed wolf depredations.

SPECIES	OUTCOME OF INTERACTION	
	Injured	Killed
Cow	1	5
Calf	2	8
Bull	1	1
Mini Colt	0	1
Lamb	0	1
Dog	3	1
Cat	0	1
Total	7	18

Table 5. Ownership of property where domestic animal injuries and death due to wolves took place. The data are for confirmed, probable, and unconfirmed wolf depredations.

OWNERSHIP	LIVESTOCK INJURIES OR DEATHS	CAT/DOG INJURIES OR DEATHS
National Forest	14	1
Private	3	2
Other or not recorded	2	2

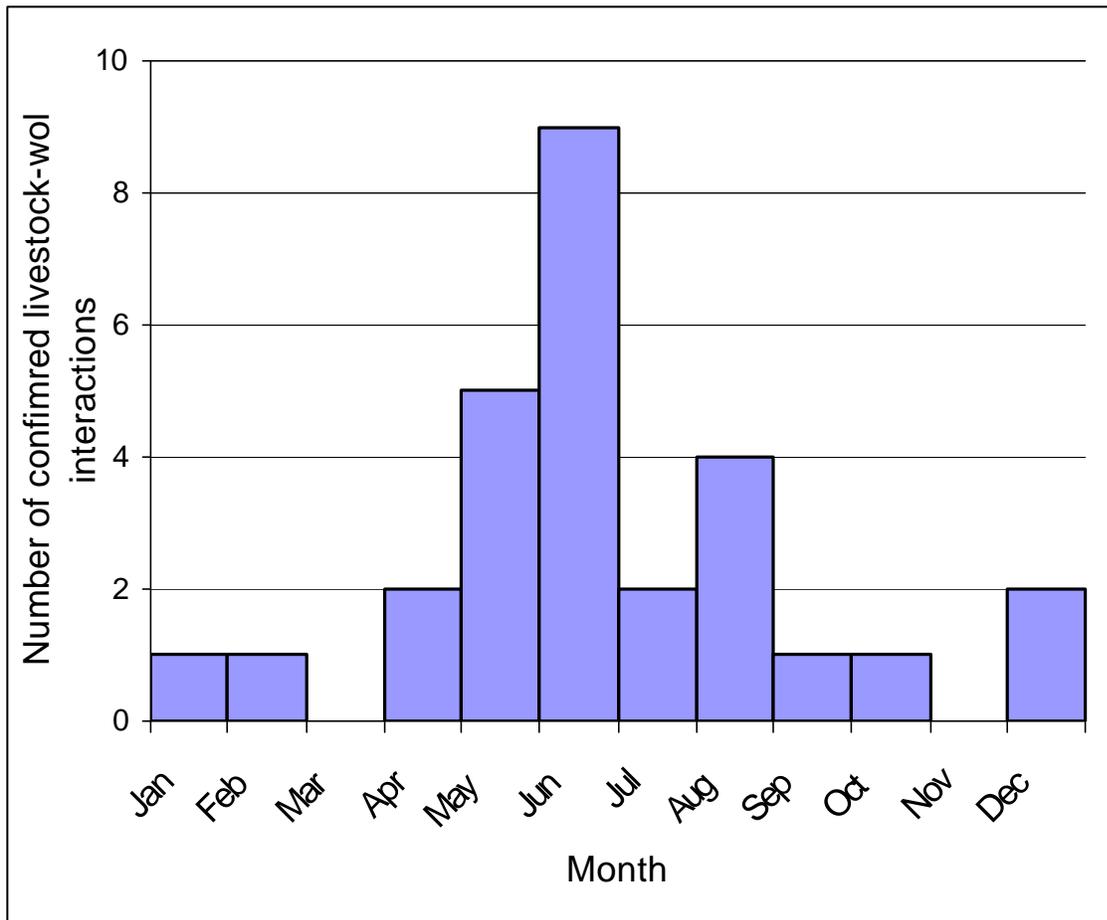


Figure 27. The number of livestock-wolf interactions fluctuated seasonally in the primary recovery zone.

d. CONCLUSIONS

Livestock are omnipresent in the Blue Wolf reintroduction area. Because of the extensive temporal and spatial distribution of livestock, interactions with wolves are unavoidable. From the information made available to us, we believe the Service has been responsive to wolf-livestock and wolf-domestic animal conflicts. An equivalent level of responsive will be necessary in the future. Similarly, livestock producers using public lands can make a substantive contribution to reducing conflicts with wolves through improved husbandry and better management of carcasses.

11. HAVE DOCUMENTED CASES OF THREATS TO HUMAN SAFETY OCCURRED?

Although no injuries or deaths have occurred, several wolf-human interactions have been reported. Consequently, evaluation of these incidences is largely qualitative based on our experiences with wolves in other parts of North America. We note that captive reared wolves released to the wild may behave differently than wild born wolves (Breitenmoser *et al.* in press).

a. DATA SUMMARY AND METHODS

Our analysis of this issue is based on interpreting records in the Events and Incidences database.

b. RESULTS

The Interagency Field Team reported eleven interactions between March 1998 and March 2001 (Table 6). On average, they reported one event every 3 months. However, the rate may be increasing (3 events from Mar 1998 to Mar 1999, 1 event from Mar 1999 to Mar 2000, 7 events from Mar 2000 to Mar 2001). If the rate is increasing, it is probably due to more wolves rather than an increased propensity for wolves to interact with humans. On average, one interaction was reported every 7 weeks from Mar 2000 to Mar 2001. Although data are too few to be certain, interactions do not seem to predominate in any particular time of the year.

Seven (of 11) interactions involved something that would be expected to attract wolves (e.g., dogs, deer carcass, livestock). Specifically, 5 (of these 7) involved dogs. One (of 11) interaction was instigated by the people involved (event #10). In 2 (of 11) events, the people involved *felt* as though their lives were threatened. In 4 (of the 11) events, an official response (i.e., from reintroduction personnel) occurred within 24 hours. In the other 7 events, no response date or time is reported. In 9 (of the 11) events, response involved an inspection of the site.

In 2 events (# 1 and #7), the people involved reported being fearful for their safety. However, experience suggests that because the people of event #7 responded appropriately, they were probably never in danger. In event #1, the wolf was shot. Event #8 is similar to cases in Ontario, British Columbia, and Alaska where wolves have injured people. In these all these cases, the people responded inappropriately to curious wolves or wolves attracted to food.

Twelve (12) different wolves have been involved with human interactions. Approximately 25% of all the wolves that have been released into the wild have been involved in a reported wolf-human interaction. Eight (of these 12) wolves were involved in only a single event. One (of these 12) wolves (i.e., 590) was involved in 4 events. All these events took place in August and September of 2000. Since then, wolf 590 has not been involved in any human interactions. Three (of the 12) wolves (i.e., 587, 509, 511) were involved in 3 events. All 3 events included wolf 590.

The 'immediate' fate of the 12 wolves was: 1 shot, 2 brought into captivity, 1 brought into an acclimation pen, and in 8 cases no attempt was made to capture the wolf. The 'ultimate' fate of the 12 wolves was: 2 shot, 3 permanently brought into captivity, 6 either are still free-ranging or died of natural causes, and for 1 wolf (i.e., #298, the potential data entry error) no data were available.

Table 6. Summary of wolf-human interactions reported for the Mexican wolf reintroduction program, 1998-2001.

E V E N T	DATE	WOLVES INVOLVED	MEMO
1	April 28, 1998	156	Wolf 156 was shot by a camper who feared for his family's safety when the wolf came into their camp and attacked their dog.
2	May 8, 1998	494	494 became a nuisance frequenting the town of Alpine from 5/8/98 through 5/28/98 and was permanently removed from the wild.
3	January 6, 1999	166, 482	Campbell Blue pair jerked down a deer carcass hanging in some archery hunter's camp.
4	January 5, 2000	522	Female 522 hanging around hunters camp interacting with dogs. Trapped and put in acclimation pen to hold through hunting season.
5	April 14, 2000	166, 518	Dean Warren reported very aggressive encounter with Campbell Blue pair with the female, 518 bumping his horse and passing under it. Wolves also attacked one of his dogs. They followed him to cabin and he held up in it until the wolves left.
6	May 16, 2000	298, 191	Renee Dupree jogging with 2 dogs when 2 wolves approached -- wolves clearly interested in dogs. Renee scares wolves away.

- 7 August 20, 2000 511, 509, 587, 590 Don and his cocker spaniel were out in the middle of the meadow behind his trailer when 4 wolves (most likely Francisco) came tearing out of the woods towards them. Don fired 1 hot in front of the wolves but they kept coming ("one with a look of fierce determination"). He fired a second shot as they got closer and they reared away. He was very upset at the situation and felt that they were a danger to both people and animals/pets. Later that week, people camped nearby observed several wolves and pups resting in the shade under and around Don's trailer. At the time, he was inside watching golf with his dog, unaware that the wolves were outside. He was irrate when he learned of the incident, stating that this was not the behavior of wild animals and concerned about what would have happened had he or his dog come out of the trailer.
- 8 August 24, 2000 511, 509, 587, 590 Scott observed Francisco (and Cienega) on multiple occasions during his time camping at Double Cienega. Sometimes they came right through cmp < 5 ft of him taking pictures, although the pups seemed more skittish, other times farther away within the campground or out in the meadow. He also saw them once farther up Double Cienega and "the shaggy one" (yearling male 590) laid down w/in 10 ft and just looked at him while he took pictures.
- 9 September 25, 2000 590 Yearling male 590 hanging around Double Cienega Campground for the majority of the day.
-

- 10 September 29, 2000 511, 509, 587, 590 5-6 people camped in Double Cienega from about 8/21-8/30/00. Throughout the week they interacted with Francisco. On multiple occasions they howled the pack in, chased them on ATVs, left food out, and shot blunt arrows at them. The wolves also chased their horses, mules, and the people in the ATVs. They were informed that this behavior was not acceptable, and we explained that what they were doing may possibly have negative effects on the wolves behavior. On 8/30/00, while speaking with the hunters, N. Sanchez observed the wolves chasing the mules. He then hazed the wolves by running at them and throwing rocks. They ignored him. We first spoke with the group on about 8/23/00. We informed them about the Mexican Wolf Recovery Project, the presence of wolves in the area, and proper behavior with respect to the wolves (ie. Do not leave out food; keep an eye on mules/ horses; if you see the wolves, yell and throw rocks at them.) We also told them to let us know if they had any interactions with the wolves.
- 11 October 1, 2000 Unknown At about 0440 Cole went out the front door on the porch and observed an animal in the driveway. At first he thought it was a German Shepard, then by the color and size he realized it was a wolf. He shewed it away and it headed west down the road. He tried to follow it in his truck but lost track of it. When he got back to the house it was by the back door eating out of the dog dish. He shewed it away again and it ran behind the house between the animal pens and the barn. He checked the dog dish and it was empty. He was not sure if there had been food in it or not. Stark and Grant responded to the call made by Ms. Leona Brown (the landowners sister). We looked at the area where the report was taken and observed large canid tracks in the driveway and yard. (track size=5x3 1/2", in sand and gravel). No other tracks were found in area. Stark and Armistead returned on 10/2 at about 0500.

c. CONCLUSIONS

Wolf-human interactions have been reported consistently and regularly since the beginning of the program. Approximately 25% of the individuals in the free-ranging population have been involved with wolf-human interactions. As the wolf population grows, the Program should be prepared for steadily increasing frequencies of wolf-human interactions. Over time, the frequency of wolf-human interactions (per wolf) may decline with wild-born wolves that are less tolerant of humans. Because wolves can pass information between generations, the attraction to humans may take some time to extinguish. In the Republic of Georgia, for example, captive-born wolves were intensively trained to kill wild prey and to avoid humans before their reintroduction. This release procedure was considered successful after the third generation of wild-born wolves still showed the same behavior as their hand raised parents (J. Badrize pers. comm.).

The Program has responded well to wolf-human interactions, although documentation and data recording have been poor. For example, in the databases USFWS provided us no response dates or times were recorded for 7 events. It is critical that the Interagency Field Team keep comprehensive notes on wolf-human interactions. The Program should continue its practice of responding to all wolf-human interactions with immediate on site inspections and investigations. The Interagency Field Team appears to have made responsible decisions regarding the recapture of wolves involved in human interactions.

12. OVERALL CONCLUSIONS AND RECOMMENDATIONS

a. PREFACE

On 25 April we convened a meeting in Globe, Arizona to present our draft report to the Mexican Wolf Interagency Management Advisory Group (IMAG). We purposefully presented a draft to provide the IMAG a chance to make substantive contributions to our review. Many comments we received during the meeting clarified issues, thus materially improving our review. During the week of 30 April the draft report was, without our knowledge released to the media. During the following weeks several newspaper stories presented the findings of our draft review as final determinations. Moreover, on 12 May the Arizona Game and Fish Commission received a briefing about the reintroduction from representatives from the Arizona Game and Fish Department who also presented our draft findings as final determinations. Draft reports are by definition works in progress. Any discrepancy between the conclusions and recommendations presented in the draft report and those presented here are a result of that simple fact.

Our conclusions and recommendations are based on our analysis of the data. We believe the long term objective is to protect the wolf population and meet human needs by reducing the potential for one to seriously encroach upon the other. Current circumstances demand that wolves be conserved in a human dominated landscape. This requires a systematic and rigorous approach to wolf recovery that integrates the social and economic aspirations of humans with the ecological necessities of wolves.

b. CONCLUSIONS

The ultimate factor determining population viability for wolves is human attitude. Thus, an active and fully enabled Recovery Program comprising private interests, non governmental conservation organizations, local, state, federal, and tribal agencies is essential to ensure success of any restoration. The biology, politics, and sociology of wolf reintroduction in the Blue River Wolf Recovery Area are too complex for recovery to be successful without a fully engaged and participatory Program. Fortunately, the Service has a successful history of reintroducing and effectively managing recovered wolf populations in other parts of the country (Refsnider 2000). Based on this success and the first 3 years of the Mexican wolf reintroduction, we think that expecting a similar outcome in the Blue River Wolf Recovery Area is reasonable.

Overall we are satisfied with the progress of the reintroduction project since its inception in 1998. During May 2001, the Service reported that at least 28 wolves were free-ranging. Most of these animals are in social groups and the Service reports up to 5 litters have been produced in the wild this spring. Monitoring of reintroduced wolves has revealed that captive-born Mexican wolves can adjust to life in the wild by primarily preying on elk. This fact combined with the likely presence of several litters in the wild bodes well for the future. We believe the likelihood is high that continued application of the Service's current practices will result in the restoration of a self-sustaining population of Mexican wolves in the Blue Range Wolf Recovery Area. We believe, however, the Program should continue with some adjustments and modifications.

Not surprisingly, our review revealed room for improvement. Restoration of any wildlife population is fraught with uncertainty and work elsewhere shows that many projects are unsuccessful because of a failure to accommodate new information (Breitenmoser *et al.* in press).

Several factors currently hinder recovery of a self-sustaining and viable wolf population. Those that predominate are:

1. The small areal extent of the primary recovery zone, which greatly hinders the vigor of the reintroduction phase of the reestablishment project
2. The Service's insistence that wolves only inhabit the small Blue Range Recovery area, which is at odds with the naturally extensive movements that characterize gray wolves and current thinking regarding the viability of large carnivore populations (Noss *et al.* 1996).
3. The Service's embrace of a target population of 100 wolves (EIS, page 2) when such a population is not viable over the long term (Shaffer 1987, IUCN 1994, Noss *et al.* 1996, Breitenmoser *et al.* in press).

c. RECOMMENDATIONS

The architects of the Mexican wolf reintroduction program properly accounted for the inevitable uncertainty and difficulty of the project by establishing adaptive management as the overarching operational paradigm. Consequently, our recommendations are largely the inevitable result of the reintroduction project's maturation. In this regard, we predict that the next review will also identify changes that can be made for improving the program..

If the Service adopts the recommendations presented below then the effectiveness of the reintroduction project and prospects for success will improve. Proper adoption of our recommendations will require a long-term and diligent effort by the Service. For many of the recommendations to be effective, biologists involved in the daily matters of the reintroduction effort must embrace them as standard operating procedures.

The current reintroduction project will greatly influence the future of the Mexican wolf recovery program since additional reintroduction projects will be required to remove *Canis lupus baileyi* from the list of endangered and threatened wildlife. Accordingly, we used our review to develop a few recommendations that consider Mexican wolf recovery overall. We also decided to consider programmatic issues that are germane to reintroduction, and issues the Service did not provide data for such as injuries resulting from capture. All of the recommendations below relate directly to the successful restoration of Mexican wolves the BRWRC. We did not elaborate on several biological issues, identified in our recommendations as important, because the reintroduction process is in too early a stage to have accumulated sufficient data.

Biological and Technical Aspects

WE RECOMMEND THAT THE SERVICE:

Continue to develop appropriate opportunities to release (and re-release) wolves for at least 2 years to ensure the restoration of a self-sustaining population.

Begin developing population estimation techniques that are not based exclusively on telemetric monitoring. As the wolf population grows it will become increasingly difficult to maintain telemetric contact with all known or suspected packs. Consequently, the Service needs to

develop non-telemetrically-based methodology (e.g., track station surveys, genetic sampling of hair or fecal material) for assessing the distribution and size of the wolf population.

Develop data collection forms and data collection and management procedures similar to those used by the red wolf restoration program in North Carolina.

Require biologist to promptly and carefully enter field data into a computer program for storage and analysis. The Service should require biologists to record data on a per wolf and per day basis. Data checking should be improved to eliminate data entry errors. In this regard, picklists and auto filling fields can simplify data entry and improve accuracy. Lastly, the Service should require that data files be proofed at least once before they conduct analyses. We remind field biologist working on the project that generally 1 hour of productive time in the field requires 2 hours in the office for data management and initial analyses.

Make all data available for research and peer review.

Carefully consider using a modified #3 soft-catch trap for capturing Mexican wolves rather than the McBride #7. We are concerned that the #7 might cause unacceptably frequent and serious foot injuries. The Service might find that a modified #3 soft-catch trap is more appropriate for capturing wolves that have a high probability of being re-released or that are fairly small (e.g., smallish adults or pups). Modified soft-catch traps have been used to capture hundreds of red wolves that are similar in size to Mexican wolves and larger gray wolves (Quebec) with no serious foot injuries (M. Phillips unpublished data, P. Paquet unpublished data). However, careful consideration of all aspects of capturing wolves with leghold traps will lead to a proper decision about the use of a modified trap for capturing Mexican wolves.

Encourage research that will help to inform future Program evaluations and adjustments.

The research we suggest is beyond the scope of the current Mexican wolf program because of resource limitations (personnel and fiscal) and the need to focus on the central mission of reintroducing wolves. However, research partnerships with universities and other organizations should be developed. Increasing the capacity of the Mexican wolf recovery Program, should be a principle charge of the Recovery Team. The following areas are of contemporary conservation and academic interest and should be research priorities:

1. Population modeling (PVA and metapopulation model) and sensitivity analysis of short- and long-term demography and distribution
 1. Assessment of new threats to population including new guild structure, disease, and human activity.
 2. Habitat viability analyses of the release area and projected population range (environment, resources, carrying capacity, spatial characteristics, etc.)
 3. Development of guidelines for decision-making in conflict situations
 4. Reassessment of policies for intervention in the release phase
 5. Assessment of monitoring programs
 6. Evaluation and design of long-term management program, including
-

- a. Evaluation design of long-term monitoring program
 - 1. demography and population range
 - 2. genetic surveillance
 - 3. health surveillance
 - 4. long-term adaptation of individuals and population to ecosystem
 - 5. effects on ecosystem (predation, displacement)
- 7. The interaction of Mexican wolves with other carnivores in the reintroduction area. Reintroduction or recolonization of wolves influences the behavior, abundance, and distribution of other carnivore species. For example, wolf recovery in the Rocky Mountains has resulted in interference and exploitation competition among wolves, bears, coyotes, and cougars, causing changes in the composition and structure of the carnivore guild.

Develop a contemporary definition of a biologically successful wolf reintroduction and the criteria needed to measure success. The latter includes methods and time scales. Specific issues that need to be considered are:

- 1. How many wolves and how many breeding pairs will result in a demographically and genetically viable population?
- 2. How do metapopulation dynamics affect the viability of Mexican wolves?
- 3. How broad a geographic area would such a population inhabit?
- 4. What affect will a viable population have on elk, deer, cattle, etc.?
- 5. What target population size will lead to long-term demographic viability?
- 6. What target population size will lead to long-term genetic viability?

We propose the application of the IUCN Red List Categories (IUCN 1994) to assess success and failure at 5 and 10 years following completion of the release phase (Table 7). The classification is based on an assessment using 5 criteria; population reduction, area of occurrence and occupancy, 2 criteria for population density, and a quantitative analysis of the extinction probability. If the population is assessed as “critically endangered” after 10 years the project should be considered a failure because there is a very high risk of extinction in the wild in the future. The minimum standard for success should be vulnerable or better. Vulnerable populations still face a high risk of extinction in the medium-term future and require ongoing management.

Table 7. Biological criteria for measuring project success of Mexican wolf reintroduction at about 5 and 10 years following completion of reintroduction phase. If the evaluation falls between failure and success, the viability of the population should be classified as uncertain. These guidelines follow the Red List Categories (IUCN 1994: www.iucn.org/themes/ssc/redlists/ssc-rl-c.htm)

CRITERIA	FAILURE	SUCCESS
Population reduction of x%, projected or suspected within the next 10 years.	> 80%	< 20%

Extent of occurrence estimated to be x km ² or area of occupancy estimated to be y km ² , and estimates indicating 2 of the following: (1) severely fragmented or known to exist in only one location; (2) projected decline or extreme fluctuations in extent of occurrence, area of occupancy, habitat area or quality, number of locations or subpopulations, or number of mature individuals; (3) continuous, observed, inferred or projected decline in area, extent or quality of habitat.	x < 100 y < 10	x ≥ 5,000 y ≥ 500
Population estimated to number x mature individuals and projected continuous decline in number of mature individuals, and population severely fragmented or all individuals in a single population	x < 250	x ≥ 250
Population estimated to number x mature individuals.	x = 50	x ≥ 250
Probability of extinction is x within ye years or z generations, whichever is longer.	x ≥ 50% y = 10, z = 3	x < 20% y = 20, z = 5

Valuational and Organizational Aspects

WE RECOMMEND THAT THE SERVICE:

Modify the recovery team by inviting an appropriate individual other than the recovery coordinator to serve as the team leader. While ultimate responsibility for Mexican wolf recovery would still reside with the recovery coordinator, enlisting another individual to serve as team leader would increase the capacity of the recovery program. Other recover program use this administrative structure and it works well (e.g., the California condor recovery program).

Instruct the modified recovery team to revise by June 2002 the 1982 recovery plan. A revision of the recovery plan is long overdue for several reasons. First, the current plan does not contain any standards for removing *C. l. baileyi* from the endangered species list. Second, since the plan was approved great advances have been made in the science of conservation biology; such advances would greatly instruct revision of the recovery plan. Finally, due to work with red wolves in the southeast, gray wolves in the Great Lakes states and the northern Rockies, and Mexican wolves in the Blue River Wolf Recovery Area we have a much greater understanding of wolf reintroductions and management; such understanding would greatly inform revision of the Mexican wolf recovery plan.

Immediately engage the services of the modified recovery team. The challenges of wolf restoration are many and varied. Meeting such challenges requires a restoration effort that is itself diverse and capable. The current reintroduction project and Mexican wolf recovery in general would benefit substantially from the efforts of a fully engaged recovery team.

Immediately modify the final rule (Parsons 1998) and develop the authority to conduct initial releases into the Gila National Forest. Several releases conducted during the first 3 years of the reintroduction project resulted in wolves settling much of the primary recovery zone in the Blue River Wolf Recovery Area. As work elsewhere (Phillips unpublished data) has revealed, wolves should not be released in areas that support resident animals. Over time, it will become harder for the Service to find suitable release sites in the primary recovery zone. The Service can best address this problem by obtaining the authority to conduct initial release in the secondary recovery zone, most notably the Gila National Forest. This recommendation was first made to the Service by a panel of experts (including Phillips) enlisted by the Service to review the reintroduction program in January 1999. Despite the Service's approval of the recommendation, they have taken no implementation action. This is by far the most important and simplest change the Service can make to the existing reintroduction project. The Gila National Forest is approximately 75% of the 4.4 million acre Blue River Wolf Recovery Area. The Gila Forest includes about 700,000 acres that are roadless and free of livestock. Several high-quality release sites are available in the area. Using them is the best way for improving the cost-effectiveness and certainty of the reintroduction project. Accordingly, we strongly recommend that the Service immediately take whatever action is necessary to conduct initial releases of captive-born (and wild-born if appropriate) Mexican wolves to the Gila National Forest.

Immediately modify the final rule to allow wolves that are not management problems to establish territories outside the Blue River Wolf Recovery Area. For specific language and instruction for this modification we strongly recommend that the Mexican wolf recovery program review the final rule promulgated for the gray wolf recovery in the northern Rockies (Bangs 1994). During the first 3 years of the reintroduction the Service recaptured some Mexican wolves simply because they left the Blue River Wolf Recovery Area. As the wolf population grows, more animals will disperse from the Blue River Wolf Recovery Area. Retrieving animals because they wander outside the primary recovery area is inappropriate because it is:

1. inconsistent with the Service's approach to recover wolves in the southeast, Great Lakes states, and the northern Rockies;
2. will lead to serious logistical and credibility problems as the wolf population grows and more wolves disperse from the area; and
3. needlessly excludes habitat that could substantially contribute to recovery of *Canis lupus baileyi*.

Before the current Mexican wolf reintroduction project was initiated, the red wolf recovery program adopted a similar approach (Henry 1995) with dire consequences (Phillips and Smith 1998). Extensive tracts of public land and some private land outside the Blue River Wolf Recovery Area are suitable for wolves. Consequently, we strongly recommend that the Service develop the appropriate flexibility to allow wolves to occupy lands outside the Blue River Wolf

Recovery Area. We believe that obtaining the requisite flexibility will require that the Service modify the final rule currently governing the reintroduction project.

We recognize that the statements above as they relate to private land may cause controversy so we offer the following remarks. Allowing Mexican wolves to inhabit suitable tracts of private land (e.g., large holdings) in the absence of problems, would bring the reintroduction project into compliance with Service-led efforts to recover wolves elsewhere. Allowing wolves to inhabit private property in the absence of a problem should not be construed to mean that the Service would begin to actively target private lands as wolf habitat that needs to be settled. Quite the contrary, and note that nowhere is the Service effecting management of private land to promote wolf conservation. However, throughout the U.S (except in the Blue River Wolf Recovery Area) if a wolf wanders onto private property and does not cause a definable problem, and its mere presence is not a definable problem, then the Service is not required to remove the animal even if the landowner demands such action.

Such an approach to wolf recovery is consistent with the determination in the United States that the public owns wildlife, rather than private landowners. Within limits, landowners can manage their property in a way that promotes or hinders the welfare of wildlife. However, through laws enforced by state and federal officials, citizens decide under what circumstances wildlife can be captured and moved or killed from public and private land. Such decisions are not the prerogatives of the landowner, regardless of whether the animal(s) in question are naturally occurring or present because of a reintroduction program.

In sharp contrast with the Service's approach elsewhere, the Mexican wolf project developed a rule that requires wolves to be removed from public and private land outside the Blue River Wolf Recovery Area, even in the absence of a problem (Parsons 1998). Such regulations are inappropriate for at least 2 reasons: 1) they are nearly impossible to effectively carry out as the wolf population grows because of the difficulties of managing an ever-increasing number of wide-ranging dispersing animals, and 2) they establish a precedent that could be effectively used to argue for the removal of other endangered species inhabiting certain tracts of public or private land.

Certainly local opposition to the Mexican wolf reintroduction program affected the development of such a rule. Indeed, the recovery program coordinator assumed from personal knowledge of local politics and sentiments that a more restrictive rule would have significantly hindered and possibly caused the termination of the project (D. R. Parsons personal communication 1996). Maybe this was a valid assumption. Opinion polls, however, suggest widespread and persistent local support for wolf recovery in the southwest (Duda and Young 1995, Pate *et al.* 1996, Meadows 2001). Regardless, noting that wolf recovery elsewhere has faced substantial opposition is instructive, but the Service did not promulgate similarly onerous rules (e.g., see Bangs 1994, Henry 1995). And to date, recovery efforts elsewhere have been quite successful (Refsnider 2000).

Resist any opportunity to reintroduce Mexican wolves in the White Sands Wolf Recovery Area (WSWRA). Two independent assessments suggest that the WSWRA could support only 20 to 30 wolves (Bednarz 1989, Green-Hammond 1994); such a population is not viable (Shaffer 1987). The inability of the WSWRA to support a viable population of wolves is due to the area's relative smallness (about 10,311 km² or 4,028 mi²) and its distance from other suitable habitat.

For example, the WSWRA is about 100 km (62 miles) from the extreme eastern edge of the BRWRA. While wolves can easily traverse such a distance, the “dispersal area” comprises very poor wolf habitat, supports the town of Truth or Consequences, New Mexico in its core, and is bisected by the heavily traveled federal Interstate 25. Accordingly, the USFWS should not expend resources on reintroducing wolves to the WSRWA.

Provide biologists with opportunities to visit other wolf projects to gain training with capturing and handling free-ranging and captive wolves.

Station the field coordinator in the Blue River Wolf Recovery Area (e.g., in Glenwood or Silver City, New Mexico or Alpine, Arizona) and insist that this person be intimately involved with all aspects of fieldwork (wolf management; public relations; data collection, management, analysis, report preparation; etc.). We think it would be a serious mistake to station the field coordinator in the Regional Office in Albuquerque. Such a decision would add a level of complexity that is entirely unwarranted.

Put forth a concerted effort to develop realistic expectations for the project. Restoration is an imprecise process that is by definition “heavy-handed”. The Service needs to constantly remind the public and the media of this fact. It is certain that the Service will have to overcome great challenges in the future. Such challenges will mean that intervention will be required, that wolves will disappear, and that some animals will die. But just as certainly, meeting the challenges will ensure the restoration of a self-sustaining population of Mexican wolves in the Blue River Wolf Recovery Area.

Initiate programs to educate people about wolf behavior. In most events involving humans, wolves are interested in dogs or food (e.g., carcasses, dog food, etc.). Members of the program expected to respond to wolf-human interactions should be well educated on the nature and variety of reports from Algonquin provincial park, Alaska, and British Columbia. The Program should contact other western communities and agencies that have dealt with large carnivore-human interactions (e.g., mountain lions, bears, wolves). The Program should also actively warn people that dogs, deer/elk carcasses, and livestock carcasses may attract wolves. Although the danger is not the same, hunters should be advised to behave as though they are in grizzly bear country.

Require livestock operators on public land to take some responsibility for carcass management/disposal to reduce the likelihood that wolves become habituated to feeding on livestock. Currently livestock grazing is permitted on about 66% of the Blue River Wolf Recovery Area. At least 3 packs were removed from the wild because they scavenged on dead livestock left on national forest lands. Such scavenging may predispose wolves to eventually prey on livestock. Accordingly, reducing the wolves’ access to carcasses will greatly facilitate coexistence between ranchers and wolves in this portion of the recovery area carcasses.

While some predation on livestock is inevitable, reasonable means of reducing the frequency of occurrence will enhance wolf recovery so that is respectful of the needs and concerns of livestock producers. Consequently, livestock producers using public land in occupied Mexican

wolf range should be required to exercise reasonable diligence in finding livestock that have died to either dispose of the carcass or enable the Service to do so. Such diligence will probably reduce predation on livestock, which in turn will improve the cost-effectiveness and certainty of the reintroduction project.

When writing or lecturing about the project, the Service should emphasize a community approach to understanding the wolf reintroduction project and its effect on other species and ecological processes. Conservation policy is shifting away from the preservation of single species toward preservation and management of interactive networks and large-scale ecosystems on which species depend. It is extremely important that the Service view the wolf reintroduction program in this context.

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