Compiled Subject Matter Expert Questionnaires

Technical Input regarding options for bringing wolves to Isle Royale National Park (IRNP)

Overview: As part of the National Park Service (NPS) evaluative process for alternatives and approaches for determining whether and how to bring wolves to Isle Royale National Park (IRNP), a team of eight Subject Matter Experts (SMEs) were tasked with completing a NPS-approved questionnaire (dated May 16 2016, version 4). The questionnaires were developed by a Lead Coordinating SME with significant input and approval from the NPS.

The Lead Coordinating SME was charged with distributing the questionnaire to the SMEs and compiling their results. This document is the compiled product. In compiling the answers, the Lead Coordinating SME individually examined each completed questionnaire and inserted the answer to each question into the final document. On occasion this was complicated by the formatting approach used by an individual SME, who may have combined responses to particular questions or discussed broader issues related to a subset of questions or a particular alternative. Minor formatting, typographic and grammatical editing was conducted. [redacted]

The compiled answers to each question are delineated by the abbreviated name of each SME:

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Subject Matter Expert Questionnaire
16 May 2016

The NPS will evaluate alternative approaches for bringing wolves to Isle Royale, as well as the alternative of not bringing wolves to Isle Royale (the no-action alternative), which remains a viable option. The preliminary range of alternatives for an estimated 20 year operational period includes:

Alternative A: (No action alternative) The NPS would not intervene and would continue current management. Wolves may come and go through natural migration, although the current population of wolves will probably die out.

Alternative B: The NPS would bring wolves to Isle Royale as a one-time event over a defined period of time (i.e. over a 36 month period) to increase the longevity of the wolf population on the island.

Alternative C: The NPS would bring wolves to Isle Royale as often as needed in order to maintain a population of wolves on the island for at least the next 20 years, which is the anticipated life of the plan. The wolf population range and number of breeding pairs to be maintained on the island would be determined based on best available science and professional judgment.

Alternative D: The NPS would not take immediate action and would continue current management, allowing natural processes to continue. One or more resource indicators and thresholds would be developed, which could include moose or vegetation-based parameters. Once a threshold is met, wolves would be translocated to Isle Royale [redacted] through multiple introductions (per alternative C).

When answering the questions below please provide rationale for your answers and supporting literature references where appropriate. When providing your professional opinions please indicate as such. Each alternative is distinct and should be reviewed in their unique context

1. Questions Regarding Actions Common to All Alternatives

1.1. Scientific Research and Monitoring: Regardless of actions taken as a result of the NPS's current planning process regarding wolf reintroduction, research and monitoring of Isle Royale's ecosystem will be necessary to inform park managers on ecosystem health. Answer the following questions to exclude research and monitoring activities associated with wolf reintroduction alternatives as these will be addressed later in this document.

1.1.1. What research and monitoring activities should be conducted, excluding wolf introduction, with what goals, and how should these research and monitoring protocols be undertaken? Discuss the pros and cons of the suggestions you provide. Topics or subject matter under this question could include: moose population demography, distribution and abundance; herbivory and associated ecosystem impacts; climate change; or any salient research and monitoring activities that, in your opinion, is critical to understanding the island's ecosystem.

BP: The National Park Service is mandated to "preserve resources unimpaired" which suggests promoting ecological integrity. According to Parks Canada, "..ecosystems have integrity when they have their native components intact, including: abiotic components (the physical elements, e.g. water, rocks), biodiversity (the composition and abundance of species and communities in an ecosystem, e.g. tundra, rainforest and grasslands represent landscape diversity; black bears, brook trout and black spruce represent species diversity) and ecosystem processes (the engines that makes ecosystem work; e.g. fire, flooding, predation; http://www.pc.gc.ca/eng/progs/np-pn/ie-ei.aspx). As such, research and monitoring on Isle Royale should strive to determine and monitor ecosystem function, particularly with respect to identifying and quantifying deviations from naturally expected ecosystem functioning, components and structure. This would include monitoring of representative species from all trophic levels, and in particular the interactions among levels.

Ecosystem-wide monitoring offers the benefit of being comprehensive and informing on many species and processes simultaneously, and also generally is conducted at large (representative) spatial extents, but the obvious tradeoff is labor intensiveness and cost. On IRNP such a program should monitor the abundance and dynamics of the islands major herbivore species (moose, beaver, snowshoe hare) and the trophic levels above and below these.

RP: In 2008, an initiative by the Superintendent of ISRO led to an island visit by a distinguished group of ecologists, who reviewed the ongoing research at Isle Royale and responded with comprehensive recommendations for science at ISRO (Schlesinger et al. 2009). The committee provided 10 research questions that could frame new research and synthesize previous work. The first priority question was, **"What explains the spatial and temporal dynamics of vegetation on Isle Royale?"** The committee also provided 13 recommendations for science management at ISRO: the first priority recommendation was **to "maintain and extend critical external relationships to networks and university researchers to draw on expertise that is currently not available at the Park."** Their next priority recommendation was to **"maintain financial support for and expansion of ongoing studies of moose-wolf dynamics at Isle Royale."** I think it is accurate to say that few of the committee's recommendations or ideas have been acted upon or implemented. I cannot improve on the work of this committee, except to update with specific suggestions relevant to the subsequent collapse of the wolf population. First, I offer a few general comments – I have listed, without any effort to prioritize, components of a comprehensive ecologically-based research and monitoring program for the Park in the context of extinction and possible restoration of the primary apex carnivore, the gray wolf. I have excluded social science parameters such as visitor objectives and satisfaction, which are outside my expertise but nonetheless important. There are common "pros" and "cons" associated with these suggestions: positive benefits include ability to understand and interpret ecological status and change in this ecosystem, increasing the scientific value of this NPS unit; negative aspects of these suggestions include the need for an increased budget and research/monitoring capacity (people and funding). Additionally, there might be negative aspects to specific activities, such as impacts of research methods on visitor experience and wilderness values. Some of the following suggestions are already underway, in one form or another with varying levels of long-term assurance, while others have never been attained in this NPS unit.

1) Moose population size and age structure, as currently done through aerial census and population reconstruction (only the latter provides age structure).
2) Moose population cause-specific annual mortality and annual reproduction rate.
3) Moose health parameters, specifically those available from non-invasive monitoring in winter: urinary nitrogen:creatinine (UNC), pregnancy rate (fecal progesterone), winter food habits (fecal microhistology), urinary glucuronic acid:creatinine (responds to plant secondary chemicals), foraging path analysis (availability and use of winter forage

plants), urinary CTXI (index of bone remodeling), urinary CTXII (index of cartilage turnover), individual ID by fecal DNA analysis.

4) Moose body size (metatarsus length of 9-month calves dying of wolf predation and other natural causes), bone marrow fat level of moose dying of wolf predation and other natural causes, calf moose body size determined by remote cameras. Peterson et al. 2011 showed that, consistent with theory, moose body size shrank in the first half of the 20th century in the absence of wolf predation. Vucetich and Peterson (unpubl. dat, from length of metatarsal bones) have determined that since the arrival of wolves the body size of moose on Isle Royale has increased. The rate of increase appears to be much slower than the decrease in body size, taking place on the scale of decades.

5) Beaver population parameters, with order of priority indicated: First, biennial aerial counts of active beaver colonies; second, the number of beavers per active colony; third, foraging path analysis (foraging distance from water and preference).

6) Intensity and impact of moose and beaver herbivory in aquatic systems, particularly beaver impoundments (Bergman and Bump 2015). This may include impacts on distribution and abundance of native fish species.

7) Forest inventory five-year interval, as begun by NPS Inventory and Monitoring program (budget constraints have pushed the re-sampling time to nine-years, and a second sampling has not yet been done after initial sampling in ca. 2010).

8) Initiate a system to monitor status of specific plant species that are both important in the diet and moose and particularly vulnerable to moose impacts (e.g. regeneration and height growth of balsam fir in the western portion of the island).

9) On a decadal scale, plan and initiate construction of moose and beaver exclosures (terrestrial and aquatic) for both research and interpretation. ISRO already contains the oldest continuously-maintained herbivore exclosures in North America (McInnes et al. 1992, Risenhoover and Maass 1987, Krefting 1974)

10) Rebuild a capacity for Geographic Information System (GIS) analysis within the Natural Resources division of Isle Royale National Park.

11) Maintain a comprehensive system of monitoring weather parameters (especially temperature and precipitation) at shoreline and interior sites, with data posted and summarized on a public internet site. Plan and initiate a wind monitoring system (direction and windspeed).

12) Maintain annual indices of abundance for key scavengers of dead moose, such as red fox and raven.

13) Maintain an annual index of abundance for snowshoe hares, a key herbivore in boreal ecosystems (Krebs et al. 2001).

14) Ensure long-term Inventory and Monitoring as currently conducted by NPS, which emphasizes water quality in interior lakes. This should include monitoring and mitigation for invasive species in interior lakes as well as Lake Superior waters within the Park.

DP: I believe it would be beneficial to study the moose population and the vegetation beginning as soon as possible. A priority should be continuing lines of research that have been conducted on IRNP through the course of the wolf/moose studies. Of particular interest would be moose demographic and population dynamics, especially if wolves are not introduced right away. A century ago, several biologists (including Aldo Leopold) wrote about population “irruptions” of ungulates following the cessation of market hunting and the elimination of many species of predator. Questions addressed then are quite different than questions that could be addressed now in the absence of predation by both human and other predators.

TR: Key monitoring activities should include: (a) annual recruitment of tree seedlings, mainly balsam fir, white spruce, sugar maple, and trembling aspen into size classes (< 10 cm tall; 10-

29 cm tall; 30-99 cm tall, and > 100 cm tall). This should include a few plots in which individual seedlings are followed through time to get estimates of growth rates and size class recruitment, and several plots with spot counts of seedlings in each size class. (b) Percent moose browsing on each of size classes, and how this is changing through time. (c) The North Atlantic Oscillation and snow depth, and how these relate to growth rates and annual recruitment of seedlings. (d) Number of days per year with fire danger levels rated as high, very high, and extreme. (e) Number and extent of fires per year.

Pros/rationale. Monitoring the abundance and recruitment dynamics of common tree species will indicate whether sustained recruitment gaps are occurring (Beschta and Ripple 2009). This can be coupled with data on moose browsing, multiannual climate trends, and summer drought/fire conditions to discern possible causes. One disadvantage of this monitoring protocol is it requires both expertise and prior experience in plant demography studies—specifically the ability to anticipate losses of individuals due to missing tags, missing plants, (and occasionally) missing plots. Sample sizes need to be large in order to overcome these drawbacks, and large sample sizes require adequate resources. Monitoring the NAO, snow depth, and fire conditions is easier and cheaper, but it needs to be linked to plant demography (and future forest dynamics) to be meaningful.

TV: Research and monitoring are not ends in themselves and decisions for what research and monitoring to undertake should be determined by the management needs of the park. The most important threat to the IRNP ecosystem over the long term is likely to be a warming climate. Over the shorter term, managing human impacts is important. With that in mind here are a few ideas.

a) A genetic evaluation of connectedness of the IRNP moose population and the moose of Lake Superior's northern shore. Under a warming climate, moose range may shift northward effectively isolating the IRNP moose population. Modeling should be used to project the impacts of additional isolation given moose demography and numbers. This research would be relatively inexpensive but could enable managers to plan a scenario where the viability of the moose population might be an issue.
b) Effects of wolves and moose on plant community structure. How would vegetation respond to the loss of one or both of these large mammals? Would loss of ungulate herbivory cause ecosystem disruption or change some essential character of the park? How does this compare with visitor expectations?
c) Alternative monitoring. The iconic research done by Allen/Mech/Peterson/Vucitich and collaborators has yielded great understanding in terms of ecosystem dynamics but aerial surveys, trapping and telemetry, etc. may not be the most cost-efficient way to monitor mammal populations. Camera trapping and new analytical techniques that do not require marked animals may be a better option for long-term low-intensity monitoring (Royle and Nichols 2003, MacKenzie et al. 2006). Similarly, refined genetic techniques based on collected scat or hair can be used to non-invasively study diet, population genetics, and to identify individuals (which gives access to a highly developed suite of statistical tools for understanding demography).
d) Dynamics and inventory of rare plant and animal species and their communities. Research should focus on how vulnerability changes with a changing climate (including changing ice cover) and flux in population of large mammals.
e) Continued monitoring to maintain long-term databases on wolf and moose population trend and balsam fir browsing.

JV: Important monitoring activities that could be carried out are outlined in section 1 of this [*MG: See the SME's questionnaire*] document. Important guidance with respect to scientific research is two-fold.

First, the best science tends to be science exposed to critical and competitive peer-review. The most critical and competitive peer-review process in the United State for proposed research is conducted by the U. S. National Science Foundation (NSF). The NSF has reviewed and funded research proposals pertaining to Isle Royale. Critically important guidance for the best scientific research would rise from those research proposals.

Second, Isle Royale National Park (IRNP) sponsored a blue-ribbon panel comprised of nine distinguished scientists. The panel's purpose was to review scientific research in ISRO and to offer a strategic plan for future scientific research in IRNP. The result of that panel's effort is a 2009 document, entitled Strategic Plan for Scientific Research in Isle Royale National Park (Schlesinger et al 2009). While that document is now seven years old, its stated scope is "the next few decades." And, much of the advice offered there remains pertinent. Critically important guidance for the best scientific research would rise from that document.

RW: Three primary issues dominate current and future research concerns.

First, the absence of wolves may cause top-down affects such that moose populations expand, overgraze and cause reduced tree growth and recruitment. Such relationships are well documented in a variety of ecosystems, including Isle Royale. Consequently, wolves, moose and tree abundance, as well as recruitment and population growth should be monitored to further understand the quantitative and condition-dependent relationship between these plant and animal communities and allow for predictions of the effects under all 4 scenarios. Climate change needs to be incorporated in such models.

Second, inbreeding and genetic homozygosity may contribute to the population decline of the wolf and moose. Current baselines need to be established, and associated with indicators of population health. Should wolves be reintroduced, the effect on these two genetic factors on survivorship and fecundity need to be established and followed overtime (e.g. Riley et al. 2014). This will allow more precise modeling and estimation of the genetic effects on fitness of specific reintroduction and breeding scenarios, as well as facilitate modeling how long such effects will persist and the need for future augmentation.

Third, the possibility of natural migration needs to be better considered. This research might include climate predictions and lake conditions that might result in natural ice-bridges with some estimation of frequency and location. This research would consider the proximity and abundance of mainland wolf populations to possible locations of an ice bridge and use probabilistic models incorporating climate data to predict the likelihood and location of natural migrants. If the possibility is very unlikely, especially given climate warming, then arguments for reintroduction gain support.

Pros- Answers to all three questions would enhance the decision making process and future population management. They would provide an assessment of possible harm that might come from inaction or limited action.

Cons- Even aggressive management and reintroduction might not restore the wolf population for the long-term, and would be futile and expensive. Similarly, research can be expensive and inconclusive. Specific goal-related research with frequent evaluation and oversight is needed.

AW: Population monitoring should continue for moose (*Alces alces*) and beaver (*Castor canadensis*) as the two keystone large herbivores on Isle Royale, and are likely to have the most noticeable impact on vegetation that will be detected if wolves are extirpated and loss influence. Moose surveys should be surveyed annually in a similar fashion as described by Peterson (1977), and Peterson and Page (1993). Beaver should be surveyed at least every two years following methods of Shelton (1966) with modifications suggested by Romanski (2010). Surveys should also be conducted on important browse species such as balsam fir (*Abies balsamea*), aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), mountain maple (*Acer* *spicatum*), yew (*Taxus canadensis*), mountain ash (*Sorbus decora*), as well as trees/shrubs that are generally poor moose browse that may become more abundant including white spruce (*Picea glauca*) and alder (*Alnus crispa*) (Pastor et al. 1993). Surveys of herbaceous plants that could be impacted by over grazing should also conducted periodically, especially looking at decline of native forest understory, spread of exotic species, and spread of savanna-like conditions (Rotter 2014).

Wolf (*Canis lupus*) monitoring should be continued until the last individuals dies off and monitoring for potential dispersers should be done each winter, probably in conjunction with moose surveys.

The goals of these surveys should be to determine impact of moose and beaver feeding on the vegetation of Isle Royale and examine herbivore impact without predator influence, as well as examine potential for bottom-up ecological influences for stabilizing or limiting herbivores. The goal of surveys will also be to determine the ecological impact these herbivores have on the Isle Royale ecosystems, especially herbivore communities lacking apex predators and controlled by climate, density dependence, disease and competition.

The pro for such surveys are benefits to developing better understanding of herbivore ecological impacts, and point to value of predators or active management of herbivore community.

The con of any surveys are costs and time commitment from managers and researchers. The commitment to any one survey always means time and funds will not be available to other surveys. Another con will be that surveys will likely demonstrate major negative impacts to ecosystems, if wolf reintroduction or other management actions are not planned, greater efforts will be placed on doing some corrective actions.

1.1.2. Other Suggestions or recommendations?

RP: None.

DP: In addition to monitoring the numbers and age/sex composition of moose, you may have an opportunity to use genomics to determine genetic characteristics of moose that survive a population crash versus those that don't. If/when wolves are reintroduced onto the island, traits of moose that are killed by predators could be similarly compared to those that survive a set time period. This is not my field of expertise, but I understand that very small samples for genetic work can be stored at very low temperatures almost indefinitely. Traits of moose that survive a population crash are probably quite different than traits of moose that remain successful in the presence of a predator like the wolf.

TR: Invasive plants and animals should also be monitored, using early detection systems to identify (and hopefully prevent) newly established species from spreading, and impact monitoring associated with invasives that cannot be controlled, such as emerald ash borer.

TV: I think IRNP should be transparent and thoughtful about its goals for park management. The overriding question of wolf restoration is often couched in terms of population or community ecology with words like “viability”, “persistence”, and “genetic diversity.” These terms often carry with them a lurking assumption of some sort of equilibrium condition. In fact, the number of wolves likely to live on Isle Royale is too small to function as a population in the conventional senses that population biologists use. Nonetheless, we are probably forced to use the term “population” to describe the collection of wolves that may live on Isle Royale because another suitable term (collection? aggregation? …) would sound too contrived. In effect, history demonstrates and managers should expect no real equilibrium condition (Vucetich and Peterson 2009). Hence – having wolves on the Island might be accomplished with a one-time release or it might require and ongoing program of occasional releases. I think it's perfectly acceptable to state the goals for restoration in very simple terms like: restoration is important because park visitors expect there to be wolves, or because of the ongoing and important scientific research, or to help contain or reduce overabundant moose (although this latter goal also make an assumption about wolf effects).

RW: 1. Culling of moose according to age and viability mimicking wolf predation might be considered. This would leave open the possibility of natural wolf migration but mitigate the effects of herbivory on native vegetation.

2. Artificial insemination of remaining female wolves with semen from outbred wolf populations.

3. Modeling of the effects of specific wolf reintroduction scenarios on genetic variation and population increase including factors such as wolf number, age composition, sex ratio, and past reproductive history.

4. Careful analysis of past genomes in the wolves that have been sampled to determine genomic changes that might explain decline and lead to better modeling under reintroduction scenarios.

AW: As moose and beaver populations grow and fluctuate over time being regulated by non-predatory forces such as vegetation, climate, disease, parasites and competition, broad impacts to the islands ecosystems and biodiversity are likely to occur and will need to be carefully monitored. With changing climate, new diseases and new exotics or change in ecology of existing exotics is likely to occur. These will need to be carefully monitored to assess their impact on moose, beaver, biodiversity and ecosystems, and thresholds may need to be established for determining levels of management needed to mitigate the negative impact of disease and exotics.

1.1.3. The life of this EIS is intended to cover about a 20 year operational period, what is the range of changes to habitat and the ecosystem that might occur.

RP: Vegetation communities are very slow to develop even though they can be degraded or changed very quickly. Within a 20-year time frame, the primary dynamic influences are likely to be herbivory and fire. The combination of high herbivory and fire, in particular, have potential to produce vegetation patterns with no historical analog, for example, a spruce savannah (Peterson et al. 2003, Frelich et al. 2012). More specifically, within 20 years the fate of balsam fir at the west end of Isle Royale will be determined, an immediate consequence of whatever level of moose browsing will prevail (Peterson et al. 2014). The outcomes include: either sufficient saplings and trees will escape herbivory to reach seed-producing size, thus maintaining this signature tree of the boreal forest that sustains moose in winter, or herbivory by a run-away moose population will halt regeneration sufficiently to eliminate the species as established stems die out over a period of several decades.

DP: Moose have had a tremendous impact on the vegetation of IRNP and this has been fairly well documented through time. Any existing, long-term studies of the impacts of herbivory by moose should be continued.

I'm sure you are already monitoring the frequency with which ice bridges to the mainland occur, and this should continue. Monitoring the moose population for evidence of immigrants (through genetics) would also be of value.

Twenty years is plenty of time to see a moose population crash and the start of a recovery.

TR: Key monitoring activities should include: (a) recruitment tree seedlings into larger size classes will be observable. (b) Percent moose browsing could increase, decrease, or show no trend through time. (c) The North Atlantic Oscillation should behave as it has in the past, although annual snow depths might increase, decrease, or remain unchanged over time. (d) The number of days per year with fire danger levels rated as high, very high, and extreme may increase or stay the same. It would be surprising if they decreased. (e) Number and extent of fires per year may increase or remain the same. Also, we can anticipate new introductions of invasive species over the 20-year time window.

TV: Twenty years is a relatively short time period for boreal wolf-moose system. The range of conditions for wolves (assuming a 1-time release of some small number leading to a breeding pair) would range from extirpation to a historically high number of wolves. Recolonizing wolves can achieve an annual growth rate of around 30% per year (Hayes and Harestad 2000, Wabakkan et al. 2000, Van Deelen 2009) which could mean a population of >50 wolves in as few as 13 years. On the other hand, growth of small populations of wolves may be inhibited by an Allee effect (Hurford et al. 2006, Stenglein and Van Deelen 2016) wherein annual growth fails to increase or even becomes negative (risking extinction) until a critical threshold density is achieved (Wabakkan et al. 2000, Hurford et al. 2006, USFWS et al. 2007, Stenglein and Van Deelen 2016). Presumably wolves on Isle Royal would reach an Allee threshold sooner because the hypothetical mechanism (dispersers unable to find mates; Hurford et al. 2006, Stenglein and Van Deelen 2016) is constrained on an island.

Other ecological effects of wolf restoration (assuming a one-time or on-going release) are just too uncertain to predict (Vucetich and Peterson 2009). I would speculate that given an established wolf population, wolves would dampen the tendency for moose populations to erupt and thereby over-browse island plant communities.

RW: Moose populations could overgraze trees in that period of time, as suggested by past studies, and initiate large-scale habitat transformation. Without the population sorting caused by wolves, the overall genetic health of the moose population may decline, and predatory responses lost. If the latter occurs, new wolves may increase rapidly given naïve prey, but decline as moose become more scarce and wary of predators. More stable population cycles could be initiated by an introduction of moose experienced wolves and with number close to the carrying capacity of the population. This strategy would also maximize the starting genetic diversity of wolves and the persistence of diversity into the future.

AW: As moose and beaver populations rise with few limitations on population growth, impacts are likely on vegetation, soil fertility and hydrology on Isle Royale (McInnes et al. 1992, Pastor et al. 1993, McLaren 1996). Moose likely will cause major declines in balsam fir, deciduous trees and shrubs and promote a forest more dominated by white spruce. Forest areas are likely to become more savanna-like in appearance which may also impact breeding bird population diversity and abundance as well as other aspects of biodiversity. Beaver populations will probably be limited by habitat/ hydrological conditions but will probably reduce abundance of aspen and willows and promote more coniferous forest or wetland meadows (Naiman et al. 1988, Naiman et al. 1994, Baker and Hill 2003). If no new wolves appear on the landscape, eventually there likely will be some major crashes in both beaver and moose populations, but timing will depend on climatic, disease/parasites, vegetation, competition and other factors, and it is not clear if such a crash would occur within the 20 year operation period.

2. **Questions Common to All Action Alternatives Reintroducing Wolves (Alternatives B-D)**

2.1. Obtaining wolves for reintroduction

2.1.1 Where (geographic location) should the source wolves be obtained? If wolves are added over time, should NPS use multiple source populations?

BP: I see no reason why “local” wolves (i.e. from the Western Great Lakes States or NW Ontario) wouldn’t be used. These would be representative of the wolves that have naturally colonized the Island and preyed on its moose and beaver on several occasions during the past century. I discuss pros and cons of different source populations in more detail below.

RP: As close to Isle Royale as possible. Note there is documented Isle Royale ancestry in an immigrant wolf that arrived in 1997 (Adams et al. 2011), suggesting (to me, at least) that there may have been genetic transfer both directions. The most important consideration for evaluating source population is that the wolves are experienced at preying on moose. Multiple source populations within a radius of a couple hundred km would be appropriate, but not necessary. I would defer to geneticists’ opinion, but I would think, e.g., 10 wolves captured at random from several packs even within an area spanning tens of km would comprise an adequate level of genetic diversity, a level that probably exceeds the diversity in the small number of founders that probably arrived in the late 1940s.

DP: Wolves should be obtained from the geographically closest packs of wolves where ice bridges have historically occurred, which I assume would be in Ontario. If wolves are added over time, they should still come from these same limited areas to most closely mimic what would bridges occurring more frequently, IRNP went a long time before moose showed up and before wolves showed up (and caribou occurred before that). So there may have been variable periods of time when wolves would have died out due to disease or inbreeding before new wolves used an ice bridge or other means to recolonize the island in the absence of climate change. But the wolves would most likely come from areas near ice bridges.

In addition, use what you know of past immigrant wolves, if possible, to inform future reintroductions (should they occur).

TR: Wolves should come from Ontario, Minnesota, Michigan, or Wisconsin.

TV: The principle for deciding on source population for reintroducing wolves to IRNP should begin with selecting for a locally adapted genotype (Woodroffe and Ginsberg 1999) subject to the logistical constraints imposed by management and governmental jurisdiction. Given that the ecological conditions on Isle Royale most closely match those of Lakes Superior’s northern shoreline, north-central Ontario would be the most logical geographical source. One would expect that Ontario wolves, in addition to having an appropriate western Great Lakes genotype, would also have some experience with moose as prey. Wolves in Minnesota and Michigan/Wisconsin (especially) likely have less experience with moose as prey although obtaining wolves from these states do not have the complication of needing to move wolves across and international border. Hence, I view north central Ontario as first choice, Minnesota as second choice, Michigan/Wisconsin as a third choice.

Given the likely population size and the relative isolation of the Island from the mainland, managers should consider that loss or even rapid loss of genetic diversity is simply inevitable (inbreeding) and plan accordingly. This issue will likely become more and more important as human development increases on Lake Superior’s north shore and climate change reduces the frequency of ice cover between the mainland and the island. Hence, my opinion is that it is not worth the added complication of selecting founders from different populations (which may risk outbreeding depression). I would select founders from various packs in north central Ontario which would be expected to represent an appropriate genotype and embark on a program that includes monitoring for genetic anomalies (Räikkönen et al. 2009) and provisions for occasional releases of additional wolves as warranted (Adams et al. 2011).

JV: My sense is that wolves should be brought from somewhere in the Great Lakes region. I also think it does not matter whether the wolves come from one location or several locations. I would also quickly defer to the SMEs who have more knowledge on this aspect of wolf genetics.

RW: More critical than the specific population(s) used as founder source is the number of founding wolves. As in Yellowstone, it is key that large genetically diverse founding population be established (they used two distinct founding populations). To maximize variation, this founding population should be near carrying capacity and be demographically similar to the age structure found in a natural non-harvested wolf population. A large founding population would delay inbreeding problems beyond the EIS period. A second consideration is the genetic composition of founders. Previous genetic analysis of the Isle Royale population found evidence of admixture with coyotes, and based on an analysis of nuclear markers about 25% of the genome is derived from coyote-like canids, and the mitochondrial genome is coyote derived (vonHoldt et al. 2011; and in press). There is no reason to maintain this percentage that I can think of, and given that hybrids are of uncertain status under the Endangered Species Act (Wayne and Shaffer, 2016) and the possibility that the legality of protection of hybrids may be questioned, pure gray wolves from the closest land population should be used. Perspective founders should be genetically tested to assess purity and levels of genetic variation and inbreeding (e.g. runs of homozygosity). A pure gray wolf might also be larger in body size and perhaps more effective predators on moose.

AW: Wolves should be obtained from adjacent areas of Minnesota, and probably any wolves within a few hundred km of Isle Royale would be suitable. Adjacent areas of Ontario would also be suitable but add the complexity of international permits and protocols which may reduce flexibility of relying on these wolves. Ideally wolves for reintroduction would be from mainland populations that have some familiarity with hunting moose. With declining moose populations in Minnesota, wolves with experience with moose may become more difficult to locate in the future.

The region of northeastern Minnesota probably provides an adequate population of wolves with appropriate genetic health to produce a genetically diverse population on Isle Royale and there is no need to go outside this region for source populations, but removals should be spread throughout the region to avoid capture of closely related individuals and to avoid impacts on any local wolf populations.

Wolves or wolf packs that have been involved in pet or livestock depredation are probably less desirable for reintroduction.

Wolves in northeast Minnesota that had previously colonized Isle Royale were initially considered a subspecies of gray wolves and designated as the eastern timber wolf (*Canis lupus lycaon*) by Goldman (1944). Nowak (1995) reclassified wolves in the region to plains wolf (*Canis lupus nubilus*). More recently wolves in the region have been referred to as “Great Lakes wolves”, which are considered a form or ecotype of gray wolves with some coyote (*Canis latrans*) hybridization by some (Koblmüller et al. 2009, vonHolt et. al. 2011), while others consider Great Lakes wolves to be admixed populations of eastern wolves (*Canis lycaon*) and gray wolves (*C. lupus*) with very limited coyote introgression (Wheeldon 2009, Fain et al. 2010, Chambers et al. 2012, Rutledge et al. 2012, and Rutledge et al. 2015). The latter group of researchers also consider the eastern wolf (*C. lycaon*) as a distinct species (Kyle et al. 2006), but the former group considers the eastern wolf as a subspecies of gray wolves that have been heavily hybridized by coyotes. There is some confusion also between Great Lakes wolves and eastern wolves, but Rutledge et al. (2015) provide strong genetic evidence that eastern wolves are a separate species from gray wolves, and the Great Lakes wolves form an admixed population of both species. Relative pure versions of eastern wolves occur only in and around Algonquin Provincial Park in southeastern Ontario and adjacent areas of southwest Quebec (Thiel and Wydeven 2009, Rutledge et al. 2012, 2015).

While early genetic research suggested that some Great Lakes wolves may be breeding with coyotes (Lehman et al. 1991), more recent research demonstrates such interbreeding is likely very rare in existing wolves in the region (Wheeldon et al. 2010). Mech (2011) found no evidence of ongoing interbreeding in the Great Lakes region. Thus concerns of translocating wolf/coyote hybrids onto Isle Royale are not of great concern from wolves captured in northeast Minnesota.

There are some differences of opinion as to occurrence of eastern, gray and eastern/gray wolf hybrids in the Great Lakes wolf population. Fain et al (2010) considered the Western Great Lakes wolves to include individuals that were mostly gray wolves, mostly eastern wolves, and hybrids of both. On the other hand, others have considered the Great Lakes wolf population as a completely admixed population that can no longer be separated into these three categories (Wheeldon 2009, Wheeldon et al. 2010, Rutledge et al. 2015). Mech and Paul (2008) did find that wolves in northeastern Minnesota were smaller than those further west in Minnesota, and suggested that the wolves to the east were perhaps more closely related to eastern wolves. Because eastern wolves are probably less efficient moose hunters and larger wolves are more effective predators on large ungulates (MacNulty et al. 2009a), selection of wolves to move to Isle Royale should focus on larger Great Lakes wolves.

2.1.2. What pre-release care or treatment should wolves receive?

BP: On one hand it would be tempting to de-worm the wolves and vaccinate them from common viral diseases such canine parvovirus and canine distemper. However, the wolves that have naturally colonized the island in the past, and which have successfully fulfilled the role of top predator, “came as they were” suggesting that a hard release and no prior treatment might be successful/ acceptable. Regardless of the potential success of simply catching intact packs of wolves and depositing them on the island, maximizing the future health of the reintroduced wolves and the success of the overall relocation program would probably be furthered if the wolves were released in good health; i.e. in a fully disease and parasite free state.

RP: Of primary importance is elimination through vaccination or drug therapy diseases and parasites. Note Isle Royale wolves currently lack *Taennia krabbei* and mange, and it would very important to not inadvertently introduce problematical diseases and parasites.

DP: All injuries or infections sustained in capture operations should be addressed. Wolves should also receive immunizations to disease where that could increase the probability that the reintroduction will be successful. While in captivity prior to release, captured wolves should have their time with humans minimized.

TR: This is outside my area of expertise, although it seems animals should be screened for canine parvovirus and canine distemper. Animals with one or both of these diseases should be excluded from translocation.

TV: I discussed this with my consulting veterinarian (Joe Bodewes DVM, All Creatures Veterinary, Hazelhurst, WI). He has extensive experience with wild bears, zoo wolves, and sled dogs. Pre-release, wolves should be well fed and hydrated, trap-related injuries (feet, toes, teeth) should be treated by a competent veterinarian. Dr. Bodewes would also recommend a broad-spectrum anti-parasite injection (Ivermectin) and a canine distemper/rabies vaccination.

JV: A wildlife vet would more readily answer this question than I.

RW: Only healthy animals should be used, and they should be minimally handled. Vaccination for common canine disease, such as distemper may be considered to maximize the success for reintroduction.

AW: All captured wolves should be treated for any injuries and vaccinated for common canid disease. Holding pens should be adequate to allow wolves to move about and in area where wolves are not exposed to humans including the smells and noises associated with human activity. In general captive holding facilities should be for as short a period as possible. Efforts should be made to avoid conditioning wolves to humans. Food should be natural prey species, preferably road killed animals. General guidance for holding wild mammals in captivity should be followed (Sike et al 2016).

2.1.3. Genetics

a). What are the pros and cons of various genetic mixes of reintroduced wolves?

BP: Body size is positively associated with ability to subdue due large prey like moose (MacNulty et al. 2009a). Large unhybridized gray wolves from western Canada or the Western US might best perform the role of moose predator, but the Great lakes wolves that have naturally colonized IR in the past are slightly smaller, being a *Canis lupus * lycaon* hybrid (Wheeldon et al. 2010). Nonetheless, much evidence suggests that these animals successfully fulfilled the role of top predator on the island for decades. Furthermore, predation rate (% prey population removed/ unit time) is the product of the *per capita* kill rate (measured as the functional response) and the density of predators (measured as the numerical response). Territory size among wolves and coyotes is partially a function of body size (B. Patterson, unpublished data) and larger gray wolves may desire/ require larger territory sizes (e.g. Kittle et al. 2015) and thus resolve into fewer packs than the smaller Great Lakes wolves which typically have smaller territory sizes (e.g. Potvin et al. 2005). This could have important implications for the overall predatory impact of the reintroduced wolf population on IRNP.

RP: There would be some risk of outbreeding depression if wolves from widely separated regions were used (e.g. Yellowstone and Minnesota). Repeating from 2.1 above, I would defer to geneticists' opinion, but I would think, e.g., 10 wolves captured at random from several packs even within an area spanning tens of km would comprise an adequate level of genetic diversity, a level that probably exceeds the diversity in the small number of founders that probably arrived in the late 1940s.

DP: Genetic variability should increase the time before inbreeding becomes a problem again…but it **will** become a problem again at some point given the low population of wolves possible on the island and the difficulty of "natural" immigration. If possible, you should consider existing wolf pairs from the mainland (assuming they are not also from a small, isolated population). Given the distances with which wild wolves disperse, those alpha individuals will often not be closely related

TR: This is outside my area of expertise. Key to understanding the various genetic mixes of reintroduced wolves is the fact that in the absence of gene flow (or in the increase in time between gene flow events—Hedrick et al. 2014), the Park may be too small to support a genetically-viable population over the long term. During periods of population decline, it is possible that the genetic load (deleterious alleles in the population) can be removed or purged through the deaths of non-breeding carriers of these genes. However, in an isolated population, alleles that are identical by descent (IBD) will slowly increase over time, thereby increasing the inbreeding coefficient of individuals within the population over time. Maximizing genetic differences of translocated animals and colonists can slow but not eliminate this process. The potential con is outbreeding depression—having animals not well-adapted to the Isle Royale ecosystem.

TV: In theory, the pros of using “various genetic mixes of reintroduced wolves” would be enhanced genetic diversity with which to counteract inbreeding depression and provide material for natural selection. While the cons would be out-breeding depression and the loss of locally adapted genotypes such as a large-bodied genotype that may be an advantage for preying on large ungulates like moose. The persistence of a relatively pure strain of the large bodied *Canis lupus nubilis* along Lake Superior’s northern shore may be a signature of adaptation for hunting moose (Nowak 2009).

JV: I can address these items, but given the short time that we were granted to answer these questions (see footnote 1, page 1 [MG: see SME’s questionnaire]), I defer an answer at this time to SMEs who focus more on genetic issues.

RW: See above discussion of hybrids, otherwise, wolves living in close proximity but in similar habitats with similar prey might be considered. Previous research has suggested wolves sort according to similarity in prey base and habitat, and thus selecting wolves from the same “ecotype” but not too geographically distance would be best (Carmichael et al. 2007; Pilot et al. 2012; Schweizer et al. 2016). A similar strategy was used in Yellowstone. In this regard, given high rates of gene flow and low levels of differentiation, multiple populations could be used if logistically and financially they are more appealing. However, sampling from a single genetically diverse population living in similar habitat to the island and similar prey base may be the most efficacious approach.

AW: As stated above, the logical population from where to select wolves would likely be Great Lakes wolves (*Canis lupus x C. lycaon*) from adjacent areas of northeastern Minnesota or adjacent areas of Ontario. While coyote-wolf hybrids would be highly unlikely, if such animals were collected they likely would be less effective predators on moose. Wolves that genetically are closer to eastern wolves, might not be as effective predators on moose, though even in Algonquin Park eastern wolves can be regular predators on moose (Theberge and Theberge 2004). Wolves that genetically are closer to gray wolves would probably be more effective predators on moose. But in general there would be little reason to be concerned about any regional Great Lakes wolves not being suitable candidates for reintroduction to Isle Royale. Outside of low risk of hybrids with coyotes or dogs (*Canis familiaris*) (Fain et al. 2010), the main genetic concerns would be making sure individuals considered for translocation to Isle Royale are not closely related individuals, as well overall health of wolves and avoiding physically small individuals that may be less effective predators on large ungulate (MacNulty et al. 2009a).

b). Provide an assessment of genome variation and deleterious variants and our awareness and ability to track them.

BP: I’ll defer to Bob for this.

RP: I have no advice here.

DP: This is far outside my expertise.

TR: This is outside my area of expertise. Until the whole genome of *Canis lupus* is sequenced, we have a limited ability to detect and track genome-wide deleterious traits.

TV: The genomic and genetic variation of Great Lakes Wolves is high and classification is unsettled (Nowak 2009). I will leave the question of “ability to track them” to others who have the expertise.

RW: Levels of variation are about half that on the mainland, there is clear evidence for inbreeding depression (Wayne et al. 1991; Hedrick et al. 2014). A full genome analysis to identify deleterious variation is underway (e.g. Robinson et al. 2016; Robinson et al. in prep).

AW: In general heterozygosity He levels between 0.66-0.68 similar to mainland populations would be desirable (Fain et al. 2010, Adams et al 2011). Heterozygosity averaged as low as 0.49 in late 1990s on Isle Royale prior to a short-term genetic rescue by new male wolf entering the island in 1997, but rose to 0.59 after this animal started breeding (Adams et al. 2011).

Breeding coefficient *f* had risen as high as 0.81 on Isle Royale in the 1990s but dropped to 0.09 shortly after a migrant entered the population in 1997 (Adams et al. 2011). But without additional genetic rescues increased back to 0.22 after 5 years (Adams et al. 2011). Maintaining a wolf population with heterozygosity above He 0.60 similar to the mainland and inbreeding coefficient *f* below 0.10 would be desirable for maintaining genetic diversity and avoiding development of deleterious gene variants in the Isle Royale wolf population.

c) What level of genetic dissimilarity between prospective mates should be considered and used to select among founders?

BP: Relatedness coefficients are presented by Hedrick and Lacy (2015).

RP: I have no advice here.

DP: Same.

TR: This is outside my area of expertise. If we had a list of candidate wolves, along with their genetic sequences, we could optimize matches, but it would be hard for me to develop quick rules-of-thumb.

TV: I would avoid full siblings and parent offspring pairs in selecting for founders. Given the inevitability of inbreeding, I don’t think it’s worth making heroic efforts to select and screen for some optimal mix of genetic diversity. Just trap individuals from separate packs.

RW: As in the red wolf program, mated pairs are best, so if possible, entire packs that have successfully reproduced should be used, with the caveat that the alpha male and female should show no evidence of kinship. As we are uncertain about the relationship between specific values of dissimilarity of parents and the fitness of offspring (with the exception of highly related individuals), a specific value or cutoff is difficult to justify.

AW: Founders should be selected with inbreeding coefficients *f* of less than 0.1. Fain et al. (2010) found mean values of 0.06 for Great Lakes wolves.

d) If the current population of wolves on Isle Royale persists to the time of reintroducing new wolves, are there concerns with these wolves passing on deleterious traits (e.g., spinal malformations) to the introduced population? Should members of the current resident population of wolves be removed from the island before the introduction of new wolves due to their poor genetic health? What are the pros and cons of retaining these wolves or removing these wolves?

BP: Potentially, but it seems unlikely that any of the current residents would have the opportunity to breed if intact pack(s) were introduced to the island. Removing these wolves prior to reintroduction would be a further deviation from the "let natural processes and events govern" paradigm, and would represent an even greater degree of meddling and invasiveness (con) but might enhance the chances of successful recolonization (pro). Leaving these animals, and allowing the possibility that they might breed would promote future genetic diversity and potentially delay the time until inbreeding becomes problematic again (pro).

RP: I have no such concerns. If the spinal problems arise from deleterious recessive genes, outbreeding with new wolves from the mainland should mask the recessive genes. I would argue strongly against removing native wolves before introducing new wolves. Removing resident wolves would be logistically extremely difficult, would be a public-relations disaster, and would run counter to scientific benefits of learning about genetic rescue. Allowing new wolves to mix with resident wolves would allow transfer of much local knowledge to the new wolves.

DP: I have not heard anything about reproduction this year of the existing wolves on Isle Royale but know that those adults are relatively old and related. Assuming that they did not successfully reproduce this year, I would still wait until they died prior to a reintroduction. I believe it would be very likely that the first thing the new Isle Royale wolves would do would be to kill the existing wolves, but from historical and biological standpoints, how long the current colonization that first occurred about 70 years ago lasts is of interest.

TR: This is outside my area of expertise. However, without a priori knowledge of whether those deleterious traits are due to a single locus or are multi-locus (quantitative) traits, I cannot reasonably assess the risks. The pro side is that you would eliminate deleterious alleles from the population. The con is it might not be necessary to remove these alleles if new individuals are being introduced.

TV: I think the likelihood of passing on deleterious genes from extant Isle Royale wolves to a re-introduced population is minor because one would expect the deleterious traits to be recessive and maladaptive and to be swamped by new founders. Killing the resident wolves would be a public relations disaster for the park and should be avoided. If park managers want to prevent residents from breeding, a more benign technique would be to capture the few residents and sterilize them (Bromley and Gese 2001).

RW: The pros are that the new wolves may genetically rescue (reverse) the expression of deleterious variation, and allow the retention of island-experienced wolves. The cons are that with limited breeding, the phenotypic effects of recessive deleterious variants may in the future reappear as homozygosity increases. Given that there are only about two wolves remaining, perhaps starting with a healthy and genetically diverse large founder population would be a strategy more likely to reduce long-term genetic problems and enhance the probability of success throughout the 20 year EIS period. The remaining wolves could be removed or sterilized and kept in place.

AW: The best strategy might be to wait until the current population has died off before considering any reintroduction. Releasing wolves with existing highly inbreed wolves on the island may risk maintaining deleterious genes in the wolf population. While outbreeding by new wolves may reduce fixation of deleterious alleles, maintaining the alleles in the population may risk future manifestations as the wolf population goes through new stresses. Because the Isle Royale population is likely to remain relatively small (average about 22 wolves), the population is never far away from potential effects of inbreeding, and thus keeping known deleterious genes out of the population would be highly desirable.

2.1.4. Do the source wolves need to have experience killing the moose, found on Isle Royale? Explain

BP: Not necessarily, for example the wolves reintroduced to Yellowstone NP quickly learned to successfully exploit a range of novel prey (Smith 2005), however effective use of the large and dangerous bison took several years suggesting learning and tradition was involved (Smith et al. 2000). Similarly, in NE Minnesota wolves were traditionally thought to prey primarily on white-tailed deer and only rarely exploit moose (Mech and Fieberg 2014) and its only more recently that they have been identified as significant predators of moose, particularly in areas where deer numbers have been depressed.

RP: Preferably, introduced wolves would have experience killing moose, but obviously their experience does not have to be on Isle Royale. If native wolves remained, their knowledge of the island would be valuable for newly-arrived wolves. Wolves that do not have experience killing moose can learn to do so, but there would be a higher expectation of failure (mortality) for such wolves. It would not be necessary for all wolves to have moose experience, if for example a small number of wolves from a different location such as Upper Michigan were used for release (particularly when enough moose-killing wolves had been released that success was guaranteed.

DP: No – wolves are quite adaptable, but I suspect most of the wolves found on the mainland closest to Isle Royale have had experience with moose.

TR: This is outside my area of expertise. However, research by Smith et al. (2000) suggests that wolves do not need experience to kill novel prey, provided of course that individual prey within the population exhibit a range of conditions from excellent to poor.

TV: Ideally, yes. Hunting is a complex behavior and involves social collaboration, learning and experience (Peterson and Ciucci 2003). To optimize the likelihood of a successful re-introduction, I would recommend obtaining source individuals from an area where moose are a primary prey item.

JV: Reintroductions do not always work on the first attempt. As such, it may be prudent to give some preference to bringing wolves that have experience killing moose. However, wolves are capable of learning to kill novel prey.

RW: This is preferable if the prime objective is to enhance the likelihood of success of the wolf introduction.

AW: While some experience with moose might be useful, any wolves obtained from moose range in Minnesota or Ontario should be able to adapt to moose hunting fairly quickly. Even the smaller eastern wolves in Algonquin Provincial Park have become fairly effective moose hunters when other ungulates are not available (Theberge and Theberge 2004). Individuals that are unusually small might be avoided because smaller wolves are likely to be less effective hunters of large ungulates (MacNulty et al. 2009a).

2.1.5. Suggest a strategy for handling the animals during capture and holding prior to release.

BP: Helicopter netgunning (during late autumn/ early winter for reasons discussed below) is the best way to ensure multiple members of an intact pack are captured on the same day. Wolves should be blinded and administered a long acting tranquilizer such as Clopixol-Acuphase, then released as a pack on the island as soon as possible.

RP: I favor NOT holding wolves between capture and release on Isle Royale, as it greatly increases stress for the animal and potential for injury (e.g., breaking teeth on chain-link fencing). Also, holding wolves would necessitate having a holding facility on the mainland, feeding the wolves, etc. A major reason for holding the animals would be to allow time (weeks would be necessary) for genetic screening, but I feel the disadvantages outweigh this single advantage. Similar to the release in the Northern Rockies, I recommend that wolves be tranquilized with drugs during the initial capture, placed in a crate with smooth interior (no chance to break teeth), and transported without delay to be released on Isle Royale, ideally within two days and preferably less (this is a technical point that should receive scrutiny by NPS veterinarians).

DP: Follow the protocol used on the central Idaho and Yellowstone reintroductions of 2 decades ago. This was very successful.

TR: This is outside my area of expertise. I have never participated in capture or handling of animals larger than woodrats. In Idaho, both hard and soft release procedures were successful in establishing breeding wolf populations (Fritts et al. 2001).

TV: Wolves are most commonly caught with foot-hold traps, hence capture wolves should be evaluated (and treated if necessary) by a competent veterinarian prior to release. Wolves should be held in secure pens with ad-libitum food and water and minimal disturbance for as short a time as is practical. Protocol should be designed with a team that includes researchers with experience trapping wolves and a wildlife veterinarian and should comply with relevant federal, state, and provincial laws governing the capture and handling of wild animals.

JV: The most important issue is to minimize opportunities for wolves to associate with people or to associate people with food. USFWS staff from the Mexican wolf program and red wolf program have considerable experience housing wolves that are later released into the wild. Consulting with those staff persons would be quite valuable.

RW: Minimize handling and exposure to humans. Minimize acclimatization cage time on the island, follow the Yellowstone protocol and lessons learned from that successful reintroduction.

AW: Protocol for capturing, handling and release should follow the American Society of Mammalogists Animal Care and Use Committee guidance (Sikes et al. 2016). Attempts should be made to capture 3 unrelated (coefficient of inbreeding < 0.1) males and 3 unrelated females from across wolf range in northeastern Minnesota. Capture should be with modified foothold traps to reduce injury (Kuehn et al. 1986), and tranquilized with mixture of Ketamine/ Xylazine (Kreeger 2003). Ideal trapping time would be late summer or early fall, for fall release onto the

Island. After capture, potential candidates for reintroduction should be transferred to holding facilities after taking measurements, collecting blood for disease testing and genetics, and imbedding microchip into each animal.

Wolves should be held in captive facilities on the mainland while DNA assessments and disease testing can be completed. Holding pens should be kept isolated from people as much as possible, and wolves fed natural prey such as moose, deer, beaver, generally from road killed animals or trapper captured animals (beaver). Holding pens should be ~20-25 m2 or larger for several weeks of holding. Once disease tests show wolves are in healthy condition and have not recently hybridized with coyotes or dogs, the wolves can be transported to Isle Royale.

Wolves can be removed from holding pens by again using drug mixtures similar at the time of capture. At this time the wolves can be fitted with satellite radio collars prior to placement into holding crates. The sedated wolves can then be transported by boat to the island. Placement of a moose carcass near a planned release site may help habituate the wolf to the release site and encourage the wolf to return to the general area. While temporary holding pens might be useful to encourage wolves to initially localize movements, eventually most wolves will probably explore much of the island before developing a home range/territory in a portion of the island.

Ideally single unrelated male and females can be released at sites on west side, middle and east side of the island, for total of 3 males and 3 females released on the island to attempt to create 3 territories and maximize genetic diversity. The two genders at each site do not need to be released together, but should be released within a few weeks of each other.

Fall releases would probably be optimum to prevent wolves from walking back inland on ice, and provide opportunity for pair bonding before breeding seasons. Capture of wolves on the mainland in late summer or early fall would be less disruptive to existing packs than removal during pup caretaking period in spring and early summer. Summer and fall captures also eliminates risk of freezing toes in foothold traps.

2.1.6.   If the source wolves are pairs should they show evidence of having bred and raised pups successfully?

BP: This shouldn’t be necessary. Successful reproduction is the norm for breeding age wolves as evidenced by the rapid and successful colonization of the western great lakes region (MI, MN and WI) following legal protection. Accepting only wolves demonstrating evidence of breeding will result in release of older wolves than necessary which will have a reduced life expectancy and lifetime reproductive output relative to a younger pair of wolves for which there would be no reason to doubt that successful breeding will occur. However, this is a moot point if the breeding pair and one or pups from an intact pack are captured and moved simultaneously.

RP: This would be desirable but not necessary. Almost all adults have the capacity to reproduce successfully (Mech et al. 2016).

DP: I do not think that is necessary, especially if the wolves come from packs. Wolves usually serve time as helpers in raising their younger siblings for a year (and sometimes longer). They also instinctively know what to do relative to reproduction.

TR: This is outside my area of expertise. However, this seems to be a reasonable criterion.

TV: Ideally, yes. If pairs are selected, it would be best to somehow select pairs that are known to have bred successfully. That said, selection may be difficult and verifying reproduction may be difficult or overly-invasive. I would not let this preclude a re-introduction effort.

JV: Generally, no. But it might depend on the time of year (e.g., a lactating female should not be separated from her pups).

RW: Yes, see above.

AW: It is probably not critical for young or middle age healthy individuals within a pair to have bred. As long as animals are held for the period of genetic and disease testing, poorly fit animals can be eliminated. But in general it might be useful just to start with three single males, and three single females and allow them to bond on the island. A pair, especially if they have had pups, may be more tenacious in trying to return to their home territory once the lake freezes over. Wolves have also been known to swim as far as 13 km to islands in coastal British Columbia (Darimont and Paquet 2002) and some might initially attempt to return to shore from Isle Royale.

2.1.7. Based on current knowledge, is there an approximate demographic profile (age and sex) that should be developed as the source wolves are assembled into a population?

BP: Rather than trying to assemble a population with a specific demographic profile, I would focus on releasing intact packs. A wolf pack is a family and the stress of the introduction should be minimized by moving most or all of entire pack(s) (family). If less than the entire pack is moved care must be taken to include both members of the breeding pair (typically largest male, but the female would need to demonstrate evidence of breeding, contradicting my answer above!).

RP: The wolves themselves will “assemble” the population, make no mistake. I recommend young to middle-aged adults, 50:50 sex ratio. Last year when I asked Doug Smith (Yellowstone Wolf Project Leader) about the composition of wolves that might be brought to Isle Royale, he favored (but not strongly) one family group that could immediately start preying effectively on moose and claim a territory without delay, plus an assortment of “other” unrelated wolves that would, in time, provide additional mating pairs and potential mates for maturing animals from the primary pack.

DP: Relatively young breeding-aged ($\geq$ 2 years) wolves would maximize the longevity of the initially reintroduced wolves. A relatively balanced sex ratio would provide future breeders, especially important for a successful reintroduction if one or more of the initial breeders die not long after the reintroduction.

TR: This is outside my area of expertise, but capturing and releasing existing packs seems reasonable (Fritts et al. 2001).

TV: Any mature wolf is a potential breeder and the formation of a new pack involves the interplay of finding a mate and finding a territory (Mech and Boitani 2003) and obviously you need a mix if males and females. However, adult wolves that are translocated show a strong homing tendency (Fritts et al. 1984, Bradley et al. 2005). Hence a strategy based on capturing and releasing a mated pair may fail if the pair decides to leave the island if ice conditions permit. It may make sense to capture and release subadult and young adult wolves (both sexes) with subordinate status because they may have reduced territorial bonds and may be actively seeking a mate (Fritts et al. 1984, Mech and Boitani 2003).

JV: Young adults or prime-aged adults would be best.

RW: Mimic that seen in healthy populations, potentially sample entire packs (adults and offspring) and place 4-5 packs on the island. Again, mimic the Yellowstone success.

AW: The demographic profile of wolves to be used for reintroduction should be males and females of age levels that would likely be dispersing but old enough to have some experience in hunting. The age range of 1 to 3 years old of male and female would probably be most suitable. Most wolves in the Great Lakes region disperse between 1-3 years of age, and 39 to 78% of dispersers are yearling (Gese and Mech 1991, Treves et al. 2009). Generally older individuals are more successful in establishing new territories (Gese and Mech 1991, Treves et al. 2009). Wolves peak at predatory ability on large ungulates at 2-3 years of age, and abilities decline thereafter (MacNulty et al. 2009b). Thus between age 1 to 3 years old wolves should be very capable hunters and have high likeliness of establishing territories.

2.1.8. Comment on the pros and cons of the best time of year to obtain source wolves.

RP: I think there is considerable flexibility here, especially during the open water season (May-October), when there are abundant moose calves and beaver available. Ideally, perhaps late summer would be the best time, as there would be zero probability of escaping across an ice bridge, and moose would be entering the rut when possibilities for predation probably increase and beaver will be entering fall-cutting period. The wolves would also have plenty of time to sort out potential mates before winter. A winter release would face the immediacy of breeding or another year would pass before the next breeding season. Winter would also bring the possibility of escape over an ice bridge, should one form, and also considerably more challenging logistical issues in terms of moving animals to the island (there would not be a boat option, and the availability of ski planes and appropriate winter weather for travel to Isle Royale can be very problematical.

DP: I believe early winter is the best time to obtain source wolves. The down side of this is that it makes logistics of capture and provision of wolves moderately more difficult. However, pups are traveling with their packs at that time and packs are relatively cohesive. Food is abundant (especially during and right after hunting seasons and in late winter), and wolves are usually in good condition.

TR: This is outside my area of expertise. However, it seems that the worst time to obtain wolves would be during the denning season, when animals are most stressed and/or vulnerable. The nomadic hunting season (fall, winter) would be best, I think, but my confidence in my own answer here is low.

TV: I will defer on this question to subject matter experts with relevant experience.

JV: My thoughts about the best time of year are associated with answers to other questions in this section.

RW: Body condition should be a prime concern, which may be maximal after winter if there is evidence of winter kill.

AW: Late summer and fall would be good times for capturing wolves. Some pros would include, adult wolves less critical to pup survival by late summer. In spring and early summer presence of adults in a pack will improve pup survival. While the parents are most critical, other adults can also contribute to pup survival. Late summer and early fall wolves are still using rendezvous sites, which should improve trapping success to later in fall as the pack becomes more nomadic. Trapping efforts that include foot-hold traps have less chance of freezing toes. Weather generally is mild by late summer reducing chances of overheating, but with little risk of hypothermia. Temperatures for transporting tranquilized wolves are fairly mild in late summer and early fall. Conditions for transporting by water remain good during fall.

Cons for trapping and collaring in late summer/early fall include: start of hunting seasons, including bird hunting with dogs that may be captured by wolf traps and create disturbance around potential trapping sites; greater mobility and naïve nature of pups may result in high capture rates of non-target individuals that may need to be tranquilized and removed from traps; as wolves abandon rendezvous sites and become more nomadic in early fall, locating wolves for capture will become more difficult.

2.2. What is minimum number of wolves and of wolf packs desired for IRNP? Why?

BP: I recommend 2 intact packs because the impact of 2 packs on the islands moose population will be greater than if a single pack is released, but given the relatively small size of the island it is unclear if >2 packs can successfully be sustained in the long run. If so, the wolves should decide this not people (we can't anyway as discussed below).

RP: The long-term average ratio of moose to wolves is 30:1 at Isle Royale, so presently there is probably sufficient food for >30 wolves. In order to get wolf predation restored without further delay (I suggest this as a goal), I'd recommend introducing a minimum of 10 wolves. If a cohesive pack were introduced, 10 additional wolves would not be too many. There should be ample room for at least three wolf packs, which historically has been the most common number of breeding packs. If 10 wolves were introduced, they would do considerable sorting amongst themselves, and it should be expected that some will fail (die or be killed by other wolves) and some will reproduce. I don't think it would be useful to try to engineer the specific outcomes in terms of individual wolves. Make sure there is an ample number of healthy adults introduced, male and female, and then stand back and let the wolves figure it out.

TR: This is hard to define without other parameters, such as: what is the moose population? A population of 50 wolves distributed among 5 packs would be wonderful, provided there were sufficient moose to support a population at that level. The ideal minimum number wolves will be a function of both the number of moose that can support the wolves, the total area of the island, the genetic composition of the wolf population, and the frequency of gene flow to the island. A reasonable minimum number of wolves would be 25-30 distributed among 3-6 packs.

TV: The minimum number of desired wolf pack for Isle Royale is probably 1. A single wolf pack on the island could potentially provide the ecological and human social goals (unstated) that the park has for wolf re-introduction.

JV: If this question refers to the number of wolves that should be on the island in general, that question is misplaced for reasons that are implied by the ideas expressed in section 1 of this document.

If the question is about how many wolves should be released on the island, then the answer may be found by understanding the overarching purpose of bringing wolves to Isle Royale. If the purpose includes minimizing the risk of long-term damage by over-browsing, then it may be prudent to restore predation as an unimpaired force in a prompt manner. Prompt restoration would entail releasing more rather than fewer wolves. Between 8 and 12 wolves would result in relatively prompt restoration of predation shortly after they were released.

RW: I think previous evidence would suggest five packs of 4-6 individuals could be sustained (refs) but observed relationship between wolf and prey density on the mainland can also be used to estimate carrying capacity.

AW: Establishment of at least 2 packs from 4 unrelated individuals would probably be a minimum level for stocking IRNP. Historical two packs have regularly been on the island but often as many as three packs existed. Establishment of as many as 3 packs from 6 unrelated individuals might be more optimal and provide opportunity to maximize genetic diversity. More than 3 packs are more likely to create conflict amongst established wolves without much opportunity for the additional wolves to establish.

2.2.1. What number of source wolves would facilitate reaching the minimum threshold of wolves and wolf packs?

BP: Once the wolves are released they will reproduce annually and organize themselves socially in ways that are difficult to predict. For example, consider the history of changing number of packs and pack sizes on IR during the past 75 years. Importantly this means that the Parks Service will have no control on the ultimate number of wolves or packs that result from a reintroduction of any size. I recommend starting with two intact but small (3-5) packs as these should be socially stable and able to effectively establish and defend territories quickly after their initial release. I recommend 2 intact packs because the impact of 2 packs on the islands moose population will be greater than if a single pack is released but given the relatively small size of the island it is unclear if >2 packs can successfully be sustained in the long run. Small packs of 3-4 should still be able to effectively prey on moose in their first year (Thurber and Peterson 1993).

RP: The long-term average ratio of moose to wolves is 30:1 at Isle Royale, so presently there is probably sufficient food for >30 wolves. In order to get wolf predation restored without further delay (I suggest this as a goal), I'd recommend introducing a minimum of 5 and preferably 10 wolves. If a cohesive pack were introduced, 10 additional wolves would not be too many. There should be ample room for at least three wolf packs, which historically has been the most common number of breeding packs. If 10 wolves were introduced, they would do considerable sorting amongst themselves, and it should be expected that some will fail (die or be killed by other wolves) and some will reproduce. I don't think it would be useful to try to engineer the specific outcomes in terms of individual wolves. Make sure there is an ample number of healthy adults introduced, male and female, and then stand back and let the wolves figure it out.

DP: I assume that the moose population will be quite high by the time wolves are reintroduced onto IRNP, so prey quantity should not be a problem. Initial reproduction has a high probability of being successful. I believe that 4-5 wolves in each of 2 reintroduction sites on the island would be sufficient. Moose are large and dangerous, so smaller group sizes could be problematic in terms of killing prey. Two packs have commonly inhabited the island (sometimes more), but "natural" immigration to the island would be small. In about 2 years the island would have close to the average wolf numbers it has seen over the years. Wolf populations will probably increase after that to take advantage of high prey numbers, then decline and bounce around as it has for the past half century.

TR: 13-30 wolves.

TV: The minimum number of wolves released for re-establishment on Isle Royale is unknown and unpredictable. You could get lucky with a single male and female or you may need to release more or supplement until a pair forms and a pack starts.

RW: See discussion above.

AW: Initially 4 unrelated individuals with single males and single females released at both end of the island if they successful bred and established territories may be a reasonable minimum. A more optimum situation would be to release and third pair in the middle of the island. If any wolves die before breeding or establishing a territory, it should be replaced by a new individual of the same gender, until 2 or 3 fully functioning packs have occurred. A definition of a functioning pack might be a pack holding a specific territory, reproducing and having at least one surviving pup by the end of the nest winter.

2.2.2. If multiple source individuals or breeding pairs are desired, how should genetic relatedness/inbreeding concerns be minimized?

BP: Wolves can disperse considerable distances (Gese and Mech 1991; Treves et al. 2009) so without full genetic profiling of all individuals in all candidate packs (which would take considerable time and require a soft release which is unnecessary and ill-advised for many reasons) it will be nearly impossible to be certain that neither member of the breeding pair of one pack isn't closely related to any breeding pair member of other released packs. However, given median dispersal distances of < 50 km (Gese and Mech 1991; Patterson et al. unpublished data) ensuring that candidate packs for release originated from distances > 50 km apart should help ensure non-relatedness of breeding pair members among reintroduced packs.

RP: Obtaining wolves from a variety of local sources would tend to minimize inbreeding issues. Wolves released on the island would be able to avoid inbreeding to the same extent as dispersing wolves would on the mainland. It can be assumed, I think, that wolves possess mechanisms to reduce inbreeding when two wolves meet without earlier association. A breeding pair from a single locale would be fine, otherwise, try for some geographic separation (or provide for some unaffiliated wolves to be released if several members of a pack were also released, so that pack members could "disperse" and select unrelated mates. For logistical reasons, it might be advisable to hedge bets and attempt capture in several locations (for example, adjacent Ontario could provide a goodly number, plus additional wolves from NE Minnesota, and perhaps even a few (if an experiment can be tolerated) from Michigan's Upper Peninsula (this would be politically expedient and it would have to be realized that wolves from Michigan would be deer-dependent but might learn quickly if teamed up with wolves experienced with moose – that would be the "experimental" component).

DP: Much of this depends on whether you would be attempting a reintroduction that would be as similar as possible to immigration that would have happened had ice bridges been more common. I believe that most packs would have come from areas close to the ice bridges. If these ice bridges were typically wide, you could get the wolves from as far apart as possible along the mainland shore side of the ice bridge. I would consider minimizing genetic inbreeding by bringing in a dispersal-aged wolf (about 1.5-2.5 years-old) or two every wolf generation or so. Dispersing individual wolves can travel great distances (up to about 800 km in our studies), so getting these wolves from close to the ice bridge would not be necessary.

TR: DNA fingerprint or haplotype wolves and select individuals based on genetic dissimilarity. Alternatively, source wolves from different geographic regions.

TV: See answers to 2.1.3. c. and d.

RW: See discussion above. Genetic testing should be done to rule out close kinship between breeders and a history of inbreeding.

AW: Attempts should be made to capture no more than one yearling or adult wolf from source packs, and attempt capture of other individuals at least distance of one pack territory from packs where a wolf has been removed. Individuals for release should have coefficient of inbreeding of < 0.10, and levels of heterozygosity approaching or exceeding 0.60.

2.3. Logistics and timing of release on IRNP

2.3.1. What level of health-related concerns during translocation and holding should be addressed?

BP: The greatest stress to the wolves will come from being restrained and kept in captivity. Wolves should be hobbled, blind-folded and administered a long acting tranquilizer such as Clopixol-Acuphase, then released as a pack on the island as soon as possible.

RP: Ivermectin or equivalent drug should be part of an anti-parasite treatment. Consideration should be given to vaccination for rabies (perhaps that would be required by state or federal laws), distemper, and canine parvovirus. NPS veterinarians could (and should) weigh in on this question. The animals should be free of mange and internal helminths. They will pick up *Echinoccocus granulosus* soon enough once they are on Isle Royale, as all moose >3 years old are infected. Standard veterinary health check should be done.

DP: Same as 2.1.2

TR: This is outside my area of expertise. I am not even sure how to answer this.

TV: Evaluation during translocation and holding should insure that wolves are optimally healthy (see answers to 2.1.5.). This means especially that they are free from injuries or disease or other conditions that may preclude breeding or hunting.

JV: A wildlife vet would more readily answer this question than I.

RW: This is beyond my expertise and I defer to others on the panel.

AW: Hypothermia and hyperthermia after being tranquilized are always concerns for translocating wolves. Conditions of temperature and weather extremes should be avoided. Adequate ventilation on carrying crates, use of water and other cooling agents need to be present when transporting on warm days. Covering to hold in heat will be critical if transporting on cold days. Excessive noise and visual stimulation needs to be avoided near wolves. Holding pens should be in area with adequate shade and some kind of shelter. Wolves should be supplied with native prey items (road kills or carcasses provided by trappers). Regular amounts of clean water should be supplied. Human contact should be very limited, and the holding site should be away from any areas used by dogs. Areas to hold wolves should generally be at least 20-25 m2 if held for several weeks.

2.3.2. Discuss how distance and timing of transporting animals to IRNP may or may not affect viability of the translocation?

BP: If the wolves are captured on the mainland in adjacent MN or NW Ontario it should be possible to transport them to the island via aircraft (i.e. twin otter) and release them as an intact pack within 24 hours of their initial capture.

RP: The shorter the distance and time associated with transport, the better. Animal health will suffer with prolonged captivity, especially if restrained. I will defer to NPS veterinarians on how to make 1-2 days in a craft tolerable. The Yellowstone introduction dealt with this variable and it would be worth checking with Doug Smith on this detail.

DP: The time between capture of wolves on the mainland and release on IRNP should be minimized to the extent possible. If packs are moved, as I recommend, the 4-5 wolves from a pack could probably be captured by helicopter in one day, then transported to the Park.

TR: This is outside my area of expertise. I am not even sure how to answer this.

TV: Increasing distance from capture/holding site and Isle Royale release sites increases costs associated with logistics and stress to the individuals associated with transportation. If the jurisdictional issues of coordinating and moving wolves across an international border were trivial, than the logical model would be to capture and hold wolves in north central Ontario near Lake Superior's north shore (see answer to 2.1.1) – otherwise the areas near Duluth or Houghton might be reasonable options.

JV: If wolves are translocated from somewhere in the Great Lakes region then it should be readily possible to transport them in a timely manner. The details of such transport would depend on other issues that should be decided first. The first decision would be where precisely should the wolves come from. Logistical constrains – aside from transportation – would likely play a larger role in deciding where wolves come from. If the NPS desires more detail from me on this point, I would be happy to provide it.

RW: This is beyond my expertise and I defer to others on the panel.

AW: A logical location for holding pens would be on or near Grand Portage National Monument, or perhaps somewhere on the Grand Portage Indian Reservation through cooperative agreement with the tribe. A location near Grand Portage would be within an hour or two of most likely wolf trapping areas, and near the boat landing for transporting to Isle Royale.

2.3.3. Discuss the pros and cons of a soft release versus a hard release approach, and should timing of either approach depend on whether winter or summer season releases are conducted?

BP: I recommend a hard release because this will minimize chances of habituation and minimize stress to the wolves while being logistically easier for the NPS. I further recommend capture and release during early winter. Hunting and travel conditions on the island should be good at this time of year and any pups left "orphaned" from the source pack captures should be reasonably self-sufficient by this time. During winter, and after many years of little to no predation pressure, there should be a sufficient number of old, vulnerable moose available to be predated by the wolves. Summer is generally a more difficult time energetically for wolves (wolf body condition tends to be at a seasonal nadir in summer and kill rates of moose are lower than during winter) so wolves released in summer into unfamiliar territory might face more difficulty meeting nutritional needs. Also, follow-up monitoring of released wolves will be easier when snow will facilitate observation of their behavior and social dynamics from the air.

RP: I favor hard release, regardless of season. Logistical considerations dictate that soft-release, particularly in winter, would be rather difficult. Soft release would mean construction of holding facilities and a feeding operation, which would be justifiable only if there was concern the wolves could escape from the island.

DP: Soft release was shown in the Yellowstone and Central Idaho areas to be very successful. Winter would be the preferred time for the capture and release operation for reasons mentioned earlier and because the lack of visitor presence will make the operation easier.

TR: Fritts et al. (2001) reported success with both hard and soft releases in winter. I am unaware of any data from summer season releases, but have concerns about capture and release of animals during this period as pups are still developing.

TV: Soft releases are recommended to offset the homing tendency of translocated wolves (Bradley et al. 2005). Soft releases are more difficult and expensive because managers need to construct and maintain holding pens, procure and provide food and water at the release site, and monitor wolves prior to release (Weise et al. 2014). These costs are increased for Isle Royale because of its remoteness. An added complication given that Isle Royale is a national park, is the need to keep park visitors from disturbing wolves at a soft-release holding site.

JV: The purpose of soft release is to increase the chance that released animals do not disperse too far from the release site.

If wolves are released shortly after ice out or during mid-summer, then the released wolves would likely have established territories and formed pair bonds. These wolves we would expect to stay on Isle Royale in the unlikely event of an ice bridge. If wolves are released with that kind of timing, then a hard release would be appropriate.

One concern with releasing wolves in, e.g., May is that some of the wolves captured in the source population might be lactating females. These females should be released without being considered for translocation to Isle Royale.

RW: This is beyond my expertise and I defer to others on the panel.

AW: The pros of a soft release are useful because they allow animals to localize, and habituate to a specific area. Soft release allows observation of the animal to determine they are healthy and displaying normal behavior. Soft release provides better assurance that animals stay in or near desired recovery areas, and allows manager to somewhat dictate where activity centers will occur.

The con of soft release includes costly creation of structures in the environment, requiring some destruction of native vegetation, and soil disturbance that may allow invasion of exotic plants. The soil disturbance in a soft release pen may also increase risk of spread of exotic plant. Soft release sites require careful monitoring and regular human visits to water and proved food, and thus becomes labor intensive, as well as increase risk of animals becoming habituated to people. Soft release sites are also somewhat at risk to vandalism. Once created, soft release sites become unsightly human infrastructures on the landscape or will require work to remove and restore the site to natural conditions.

The pros of hard release include: ease of operation; no structure to be created or maintained; no major disturbances are made to the release site; does not require costly monitoring or maintenance; and reduces risk of wolves becoming habituated. Hard released wolves behave more like natural dispersing wolves thus the selection of home range and territory is more of a natural process than human induced process.

The cons of hard release include higher risk that wolves don't habituate to the desired area. In winter time, released wolves might completely leave the island. During other periods released wolves may roam too broadly for establishing the desired system of 2 or 3 distinct packs. Risk of leaving the island are probably minimum if hard releases are done in non-winter periods.

2.3.4. Discuss the role of location of the release site in terms of individual animals or mated pairs. The island is 45 miles long and 9 miles wide and contains 132,000 acres.

BP: I would release the 2 packs simultaneously on either end (E- W) of the island as intact packs. This will maximize the chance that each pack is able to feed and establish a comfortable territory before meeting and potentially engaging in antagonistic encounters with other released pack(s).

RP: If wolves to be released come from the same pack or otherwise show affiliation for each other, they should be released together. Otherwise, wolves could be released singly or "together" (approximately same time and place) at one or more locations. If boats were used to transport wolves to Isle Royale, places with remote docks would seem best, e.g., Todd Harbor, McCargo Cove, Duncan Bay, Hidden Lake, Moskey Basin, Chippewa Harbor, Malone Bay, Siskiwit Bay. If wolves arrived by float plane Windigo and Tobin Harbor could be made to work with temporary closures to unnecessary human traffic. If wolves were transported by air in winter, Windigo would provide the most reliable ice (intensively monitored for thickness and condition) for ski-equipped aircraft.

DP: Pens for wolves that will be soft-released should be relatively close to facilities where their caretakers can stay (but not so close as to lead to human-wolf problems). I would put the pens at the southwest (probably near Windigo?) and toward the northeast end (I'm less familiar with facilities for people there). This would increase the probability that these packs would stay close to where they were released and hopefully minimize the chances of early inter-pack strife. If releases of individual wolves occur later (simulating dispersal), I would consider hard releases where wolf pack territories adjoin.

TR: Released pairs or packs should be spaced at the maximum possible distance. For example, if there are three packs of 5 animals being released, the release sites should be at island coordinates (east to west) 0, 22.5, and 45 miles.

TV: The release site should be easily accessible by managers to facilitate transport of captive wolves and maintenance and monitoring while wolves are being held. Maintenance of the holding facility will be a challenge so sites where exposure and forest conditions seem favorable for wind throw should be avoided. At the same time it should be remote enough from the shore line and from hiking trails and canoe routes that it is invisible to park visitors. Secondarily, it may make sense to have the release site near areas with relatively higher levels of moose activity.

JV: Isle Royale is relatively small with respect to the distances that wolves travel.

And, we should not expect that a wolf released from, e.g., the west portion of Isle Royale to establish a territory on the west portion of Isle Royale. Similarly, we should not expect that two wolves (male and female) released together will eventually form a pair bond. The released wolves are likely to sort those social issues out in a way that is not so easy to precisely predict.

That being said, it would probably be good to release all of the wolves at pretty much the same time and from, say, three locations on Isle Royale (roughly western region, middle region, and eastern region). Those regions represent, roughly, the historical distribution of pack territories on Isle Royale.

If the wolves were released in a more staggered fashion (a few at a time), the concern would be that the first wolves would establish a territory that occupies the entire island, making it a bit more difficult for subsequent packs to form.

RW: This is beyond my expertise and I defer to others on the panel.

AW: The size and shape of Isle Royale is well suited for formations of three wolf pack territories. Adjacent Minnesota has in recent years had average pack territories at ~140 km2 (Erb and DonCarlos 2009), and including 37% interstitial area would cover an area of 575km^2 for three territories, similar to the size of Isle Royale. The long narrow configuration of Isle Royale facilitate in the establishment of pack areas on western edge, eastern edge, and middle of the island, without too much disruption among groups. While Isle Royale has had as many as six packs in the early 1980s, three packs has been the more common condition during much of the history of wolf occupancy of the island.

2.3.5. Discuss the pros and cons of providing dead prey during the initial release phase.

BP: Should be unnecessary (e.g. Yellowstone release, and previous natural recolonizations of IR) and may be counterproductive in terms of promoting self-sufficient moose-eating wolves. During winter, and after many years of little to no predation pressure, there should be a sufficient number of old, vulnerable moose available to be predated by the wolves.

RP: Note that there would be a high density of moose calves and beaver, so I would recommend NOT providing food or any other reason for wolves to hang around at the release site. Note that some wolves may not pass this test, and that is OK.

DP: With any soft release, dead prey would have to be provided. I see no alternative with a soft release. If a hard release is chosen and the moose population is very high, many moose would be available for the wolves and some would be quite susceptible to predation. In this situation, provisioning with dead prey would not be necessary. If the moose population is lower and in great shape, some provisioning with dead moose may be necessary.

TR: This is outside my area of expertise. Carrion would provide some insurance against a failed establishment. Ideally, the dead prey would be moose. I cannot think of any cons.

TV: The advantage associated with providing dead prey during the initial release is that this is a continuation of soft-release techniques and would likely help offset wolf homing tendency (Bradley et al. 2005). The cons include the costs and logistical difficulty of procuring and transporting (moose!) it. There is also a risk that wolves will begin to associate humans with food, but this risk can probably be managed or mitigated (see 2.3.6. below).

JV: The “con” associated with providing dead prey during the initial release phase is an increased risk of wolves associating food with people. It is, I believe, quite difficult to quantify the risk of this outcome. The “pro” associated with providing dead prey is that it <u>might</u> increase the survival rate of translocated wolves during the first few weeks of presence on Isle Royale. Because wild wolves are capable predators, the survival rate of released wolves might be plenty high enough without food provisions.

At the same time, other considerations suggest that a boost to survival rate might be valuable. This would be suggested possibly by considering the life history of dispersing wolves and recognizing that not all wolves are equal in their capacity to lead a kill of large prey.

An additional consideration would be to release wolves on Isle Royale shortly after moose calves are born. Moose calves are relatively easy prey to capture.

The best available and relevant science on the topic of seasonal variation in wolf foraging can be found by consulting Metz et al. 2011, 2012, the references cited therein, and papers that have cited Metz et al. 2011, 2012. If more time had been granted to respond to this Questionnaire, I would have been willing and able to review that literature.

RW: This is beyond my expertise and I defer to others on the panel.

AW: Providing dead carcasses near release sites would likely encourage wolves to habituate near specific areas, and help supply food after the stress of capture, captivity and transport. Such food sources would also help hold wolves over while they are learning to hunt in this new environment. Carcasses will be necessary in any kind of soft release, but may also be useful in a hard release. Carcass would help in distributing wolves across the island and encourage the establishment of 3 founder territories.

Providing carcasses may cause wolves to become more habituated. Carcasses obtained by shooting could risk lead poisoning to wolves and scavengers unless non-lead bullets are used. The stress associated with release may cause wolves to avoid the area where they are released and perhaps may not find carcasses, and thus make no use of the carcass.

2.3.6. What measures are there available to decrease the probability that wolves become habituated to humans?

BP: Hard release, minimize degree to which wolves see and hear humans, completely decouple human presence from any food wolves receive while in captivity.

RP: Don’t provide dead prey or supplemental food.

DP: Release wolves in winter. Minimize interactions with humans while wolves are in captivity, similar to what was done when wolves were reintroduced into the western U.S.

TR: This is outside my area of expertise. Wolf translocation workers could wear costumes, as some do in rearing endangered birds. But given the wolf’s sense of smell, this seems difficult to achieve.

TV: Strategies for offsetting habituation include minimizing contact and human-scent contamination (of food and facilities) and short holding times.

JV: USFWS staff from the Mexican wolf program and red wolf program should be consulted. They have the most experience with this issue.

RW: This is beyond my expertise and I defer to others on the panel.

AW: Measures for detecting habituations to humans would include, wolves coming close to campsites and campers, not showing fears or avoidance when within 100 m of people, attempting to steal food from campers, growling or acting aggressive to people nearby. Regular travel in areas of regular human activity during daylight hours.

2.4. Monitoring of released wolves

2.4.1. If released wolves are to be monitored, what is the purpose of this monitoring and how might this purpose influence monitoring approaches?

BP: The purpose of any wolf reintroduction would be to restore ecological integrity and natural ecosystem functioning, thus any monitoring should be focused on:

1) The success and results of the wolf reintroduction in terms of wolf population demography and health, and
2) The role of the reintroduced wolf population in restoring natural ecosystem functioning.

RP: The purpose of monitoring should be to gauge the success of the effort and to reinstate a research program similar to that conducted for the past 58 years. That is, which wolves have survived, and what are the resulting social relationships and reproductive success of territorial packs? There has also been a history of telemetry monitoring of wolves at Isle Royale as part of the annual research effort, and the primary purpose of telemetry monitoring in this respect is to efficiently find wolves during winter for observation, determination of predation rate, determination of wolf population size, collection of feces for DNA ID, and other information that can be gained by direct observation and detailed aerial photography.

DP: Yes, any released wolves should be monitored. This monitoring would have at least 2 purposes: 1) to see whether the release was successful (determine survival, estimate reproduction, are ascertain whether further releases necessary); and 2) to follow the genetics of the population. For both of these monitoring purposes, radio telemetry of released wolves would be optimal. Mortality switches can determine whether wolves are living or dead, and causes of death could be determined (which could help with future releases). Radio locations would assist in finding dens and rendezvous sites.

TR: The primary goal of monitoring would be demographic—are the wolves increasing or decreasing in numbers? A secondary purpose would be to monitor the genetic condition of the wolf population. Fixed wing counts in winter could provide census data, while game camera traps could provide data on genetic deformities.

TV: I think that he re-introduced wolves should be monitored. The primary purpose for monitoring would be to determine whether the wolves form one or several packs leading to a population that may persist (with the caveats outlined in my answer to 1.1.2). A secondary purpose for monitoring would be to contribute to the important and long-term database on the Isle Royale wolf-moose system (Nelson et al. 2011).

Given the setting in a National Park, wolf monitoring should find a way to be as non-invasive as possible (such as camera trapping and aerial counts rather than capture and collaring) while still maintaining compatibility with long-term research and monitoring.

JV: The requisite monitoring depends, again, on the purpose of releasing wolves on Isle Royale. If the purpose is merely to know if the longevity of the wolf population has been increased (as stated in Alternative B), then it would be important to monitor for the presence of wolves and that's all the monitoring that would be required.

If the purpose of releasing wolves is to restore predation as an unimpaired ecological process, then the requisite monitoring would include the metrics outlined in section 1 of this document.

Reproduction has been monitored in recent years by fecal DNA. This is a particularly non-intrusive means of monitoring reproduction. That method would also seem to match the management goals associated with Alternative C. More invasive methods would yield more information, but are unnecessary for science or management. None of the other alternatives would seem to suggest the need to monitor reproduction.

The most effective, non-intrusive means of monitoring immigration to Isle Royale would be the methods that have been employed in recent decades.

RW: Monitoring is key, as we need to learn from the reintroduction to enhance future management actions and improve reintroduction prospects on the island and elsewhere. Yellowstone provides a guide here, as monitoring efforts decreased as the population increased and stabilized.

AW: Monitoring would be critical to determine the fate of translocated wolves, whether territories have been established and where, hunting success, and whether the wolves successfully reproduce. Monitoring can help inform on areas of major use, den sites, and rendezvous sites so these areas can be protected and avoid disturbing these areas. Telemetry can help inform on movement patterns and interactions among packs.

2.4.2. Define critical data for long term wolf population management and how it should be collected. Explain the various options you considered and why you defined critical data the way you did.

BP: Using my answer to 2.4.1 as guidance, long term monitoring of the wolf population should assess:

1. Number and size of packs
2. Complete genetic pedigree of all island wolves going forward
3. Look for evidence of inbreeding depression
4. Look for evidence of natural immigration from mainland
5. Assess prey use of and kill rates by all packs and individuals

RP: There should be no need for population management other than ensuring adequate genetic viability. The parameters that would be important for evaluating genetic viability and prey availability in relation to wolf density and population trend would be: number of wolves in winter, reproductive success the previous summer, survival patterns, full pedigree data, number of moose, number of active beaver colonies, skeletal integrity (presence of congenital abnormalities). These parameters can be determined from a winter study with island-based survey aircraft for counting wolves, determine kill rate, counting wolves and moose, recovering dead wolves (indicated by telemetry), collecting and analyzing fecal DNA.

DP: I spent a good part of my career conducting research in National Parks and know that the least intrusive methods that will get needed results are preferred. Critical data for long term wolf population management would include the wolf population (estimated during winter flights; at first this will be quite easy with radio collared individuals). Dens can generally be found where the alpha female stops moving. The number of pups can sometimes be determined through flights over den sites. Another method that could be used would be to go to rendezvous sites shortly after the wolves have left and collect individual scats for genetic work. Individuals, their sex, and genetic heterozygosity can be determined using genetic techniques. These data could be used to estimate when additional wolves could/should be introduced.

TR: Critical data would include: number of individuals in year t, birth rate in year t, death rate in year t, population growth rate R, variability in population growth rate var(R). The number of individuals can be obtained from winter fixed wing surveys. Birth rates are difficult to obtain but could be inferred from changes in pack sizes from one year to the next. Death rates would probably need to be estimated, unless carcasses can be identified in the field. Growth rates and VarR can be calculated once there are population size estimates for two years of data, and can be recalculated with each additional year. With birth and death rates and a population growth rate, a simple population model can be build that (1) incorporates demographic and environmental stochasticity, and (2) can be used to estimate a probability of extinction over the 20-year management horizon.

TV: Critical data include long-term population trends for wolves and moose and effects on key plant communities and park-visitor attitudes about these things. To my knowledge the drivers for discussing re-introduction are primarily about the ecological functioning of the wolf-moose-plant system and a sense among people who care about Isle Royale that wolves "are supposed to be there." In my mind, these are the monitoring goals that minimally serve the park's mission and maintain the scientific value.

Variation in population trend can be the signal for more intensive research. For example, observation in a moose decline over time could be the motivation for more intensive/invasive research (collaring, collection of biological samples) to determine mechanisms.

RW: Critical data include activity patterns of individuals are how they are associated with other wolves and prey, agnostic and affiliative behaviors within and between packs, pack membership, hunting dynamics and success, injury and causes of mortality, including age, disease and nutritional factors. As in Yellowstone, such data allow a better understanding of population levels factors leading to demographic regulation and success. These data can enhance management and the likelihood of population persistence.

AW: All wolves released onto the island should be radio-collared probably with satellite collars. Genetic samples should be collected from all wolves released onto the island to determine inbreeding coefficients, heterozygosity, detect any hybridization with other canids, connect the wolf by assignment test to its original population, and allow individual identification when scats are collected. Collections should be made of scats during specific surveys and opportunistically. Intense collections of scats should specially be done around rendezvous sites to determine new wolves produced in the population. During winter collection of scats should especially focus around kill sites. Aerial flight during winter study will continue to be important to get winter counts on packs and compare the counts to genetic samples collected. Data should continue to be collected on wolf kills of moose and other prey. Data on survival, mortality factors, reproductive performance, and impact on prey populations are all critical measures for assessing the success of wolf reintroduction and measure the impact of wolves on the Isle Royale ecosystem.

2.4.3. What are the least intrusive methods of monitoring the offspring of reintroduced wolves and what data can be provided by those methods? If telemetry or methods that involve handling animals is added, what are the additional information data sets and hypothesis that could be explored?

BP: The number and genetic identity (i.e. genetic fingerprint) of all pups can be assessed by collecting non-invasive DNA samples (primarily hair, e.g. Ausband et al. 2010) from dens and rendezvous sites (after the wolves naturally leave these sites). Once the genetic fingerprints of all new wolves entering the population have been established, continued non-invasive genetic monitor would reveal how long these pups survive, movement among packs, or if any of these pups eventually become breeders. This should be enough data, however, if precise data on pup movements (i.e. among packs or as transients post dispersal) or fate is desired then implant transmitters (e.g. **Mills et al. 2006; JWM)** would be required, but this is highly invasive and labor intensive. Implanted pups could then be recaptured via helicopter netgunning during their first winter of life to further monitor their fates and lifetime reproductive success.

RP: The least intrusive methods for monitoring any wolf, offspring or not, is analysis of individual identification from fecal DNA collected in winter from wolf-kill sites. This information places the individual into a population-wide pedigree, which will provide total lifespan, reproductive success, year of birth and death, and pack affiliation. Adding telemetry-based monitoring will provide information on cause of death and greatly increase the possibilities of learning details of social relationships by increasing observation time in winter.

DP: Genetic methods (see above) are quite non-invasive. Telemetry enables ease of finding rendezvous sites so that researchers can get in and out of areas used by wolves quickly. Without telemetry, finding rendezvous sites would be a time- and personnel-intensive activity. Keeping one or two wolves radio collared within each pack through time is a bit intrusive when the collaring occurs but would enable the collection of genetic data in a much less intrusive manner.

TR: The least intrusive methods for monitoring offspring might include (1) collecting scats and genotyping them using multilocus microsatellite markers, or (2) identifying regularly-used areas, and setting up game cameras with SIM cards to transmit images or video back to a central location. Genetic data can be used to track individuals through time using Jolly-Seber methods, provided scats were collected continuously, and assess levels of inbreeding within the population. Video data could be used to screen for deformations and identify individuals to track survivorship.

TV: The least intrusive monitoring techniques include aerial surveys, camera trapping, and snow tracking. Except for small populations where animals are individually identifiable, these techniques provide, minimum counts for estimating population trend (aerial surveys, snow tracking) or site-level estimation of detection probabilities and occupancy for individuals of easily recognized classes (e.g. pup or non-pup). The analytical techniques for this sort of data is becoming very sophisticated (MacKenzie et al. 2006) leading to rigorous inferences on space use dynamics, population trend, and even point-estimates of population size (Royle and Nichols 2003, Mackenzie et al. 2006).

Telemetry enables more fine-scale estimation of behavior and demography. For example, one can infer population trend with observations of unmarked animals but to evaluate how reproduction or survival contribute to population trend you need techniques that enable repeated encounters of at least some uniquely identifiable individuals (telemetry, mark-recapture, mark-resight). Similarly, one could infer spatio-temporal patterns in habitat use with enough cameras, enough sampling, and a sufficiently dense camera trapping grid but it's more efficient to use telemetry. In general, telemetry enables research on mechanisms (e.g. causes of mortality, age-specific fecundity) that are drivers of population trend.

RW: Telemetry provides highest resolution for movement and activity patterns and sources of mortality. As the founder wolves will be handled anyway, the advantage of not placing telemetry collars on individuals is not clear, and would seems like a lost opportunity for science and informed conservation management.

AW: Genetic sampling with scat collection is probably one of the least intrusive monitoring methods. Collections at rendezvous sites are especially useful for determine the identity of most pack member (Stansbury et al. 2014). There is a slight risk that such activity may cause abandonment of rendezvous sites, but the risk is relatively low. Winter aerial monitoring of pack as has been done on Isle Royale since 1958 (Mech 1966) also is a nonintrusive means for monitoring wolf packs. Placement of trail cameras along trails, at kill sites, and rendezvous sites would be another nonintrusive means for monitoring wolves. Such trail cameras might also be set near rendezvous sites, but such placement would risk abandonment of den sites. While radio-collaring is more intrusive, for wolves captured on the mainland, processed, held in captivity and transported to the island, the collar placement is probably a relatively low stressor compared to the other treatments. The fate and behavior of these founders will be especially important in assessing the success of the reintroduction program. Telemetry would help determine more precisely movement patterns, and territory areas as well as degree of extra territorial movements. Timing and causes of mortality would more likely be detected with telemetry. Exact locations of den and rendezvous sites can be determined with use of telemetry. Hypothesis on timing and location of den sites, predation patterns, timing and locations of mortalities, and patterns of space use can be tested with telemetry.

2.4.4. How should reproductive success of released wolves be assessed?

BP: Movements of GPS collared wolves in each pack will quickly and definitively reveal any denning behavior in spring (e.g. Mills et al. 2006; Benson et al. 2013). See above for option using non-invasive genetic monitoring at den and RV sites to enumerate number and genetic fingerprint of all pups. Otherwise, howling surveys in late summer could confirm presence of pups with each pack or visual observation in winter and documentation of increases in pack size.

RP: This can be done most comprehensively, and least obtrusively, from fecal DNA (winter scat collections at kills) that would enable long-term tracking of every member of the population.

DP: As mentioned earlier, flights over den sites in June/July could result in an estimate of pup production fairly early; collection of wolf scats at abandoned rendezvous sites would give a later summer estimate of pup survival; flights during early winter would provide an even later estimate of pup survival. Reproductive success of individual released wolves can be determined genetically.

TR: Reproductive success might be monitored via winter tracking and identification of females in estrous, as indicted by blood in elevated urine markings. If the den or rendezvous site can be located, identification and number of offspring can be determined by genotyping scats. I do not know how to monitor reproductive success of each pack in a cost-effective manner beyond this.

TV: Reproductive success of re-introduced wolves can be assessed through aerial composition counts, camera trapping, or telemetry research. Post-hoc and historic reproductive success can be assessed by using genetics to reconstruct family trees.

RW: Genetic sampling and analysis of founders, followed by non-invasive monitoring using feces.

AW: Reproduction can be assessed several ways including detection of collared females at den sites; genetic sampling of all wolves at rendezvous sites; placement of trail cameras near rendezvous sites; reports of wolf howls by members of the public, NPS staff or opportunistically by researchers; winter wolf pack counts; and examination of placental scars of any adult females dying.

2.4.5. How, and how often, should natural wolf immigration to IRNP be monitored?

BP: If non-invasive genetic monitoring were conducted on an ongoing basis (i.e. samples collected from all known den & rendezvous sites in summer, and from snowtracking all known wolves in winter), and a complete pedigree of the island population constructed, any immigrants to the island should be detected within the year.

RP: This should be done annually, in winter, using fecal DNA (see 2.4.4).

DP: Immigration is only marginally important biologically unless the immigrants reproduce. A genetic sample from each of the wolves released onto the island should be taken. If wolves are monitored genetically (as suggested above), new genes coming into the population will be an indicator of immigration. Monitoring the occurrence and frequency of ice bridges should also occur.

TR: Wolf immigration to IRNP would be difficult to monitor real-time, but might be detected after the fact as documented by Hedrick et al. (2014). If scats were collected for genotype analysis, new wolves should be fairly easy to detect with the aid of a population geneticist.

TV: Monitoring natural wolf immigration would be difficult unless researchers were able to identify individuals reliable enough to infer that new previously-undetected individuals were immigrants. Contributions of immigrants to the island gene pool, which is arguably the most important effect of immigration can be asses periodically through genetics – and this can be done non-invasively by collecting hair or scat.

RW: Again, exhaustive fecal sampling during summer would provide an annual record of birth, possibly death and migration.

AW: Monitoring of natural immigration should be an ongoing process. Collection of scats opportunistically throughout the year as well as during other scat genetic sampling would help determine the presence of previously unidentified wolf that shows no close relationship to other wolves on the island. Winter aerial surveys would help in location single wolves not accounted for by genetic sampling and radio tracking.

<u>Additional input provided</u>

JV: The questions associated with Item 2 (MG: see the SME's questionnaire) merit more detail than provided here. Limited time prevented me from providing any more detail (see footnote 1, page 1; see also section 5 of this document [MG: this parenthetical refers to the SME's questionnaire]).

3. **Alternative B: The NPS would bring wolves to Isle Royale as a one-time event over a defined period of time (i.e. over a 36 month period) to increase the longevity of the wolf population on the island.**

3.1. During the re-introduction time period, can you identify any issues that should be monitored if it affects the characteristics of the startup population; i.e. wolf on wolf predation is high- affecting an age distribution?

RP: The most obvious parameter to monitor would be survival in introduced wolves. If I understand this alternative correctly, the goal is to increase genetic variability right at the start. It is likely that the initial few wolves introduced will pair off and attempt reproduction. Once reproduction has been achieved, it seems unlikely that new wolves would provide options for new mates for wolves already reproducing.

DP: I don't think there is anything beyond what you would be doing in any release. Monitoring the survival of reintroduced wolves through mortality switches on radio transmitters would address the question you raised.

TR: This is outside my area of expertise. I do think the main variables to monitor are: population size of wolves, and successful breeding of wolves. Wolf on wolf predation may be a reflection of a density-dependent process and might not provide informative data for management intervention. However, successful breeding in packs will be informative for management purposes.

TV: I don't understand the question. If the question is about critical phenomena that should be monitored to understand the success or failure of an ongoing reintroduction, that would be simple counts (if the animals were not monitored using telemetry) or the individual fates (in animals were monitored using telemetry).

RW: Again, the number of animal moved to the island is critical, moving whole packs may enhance stability or lead to higher levels of inter-pack strife. Introduced at a modest density approaching carry capacity as suggested by prey abundance may enhance resilience of the system.

AW: Issues that warrant special attention during start up would include the success of pack /territory establishment and the extent territories spread across the island. High rates of pack conflict that restrict other packs from establishing, may warrant release of additional wolves to the island to replace killed wolves. But once three packs have established, additional reintroductions would probably not be necessary.

<u>Additional input provided</u>

JV: Item 5 MG: see the SME's questionnaire] pertains to Alternative B, which states "*The NPS would bring wolves to Isle Royale as a one-time event over a defined period of time (i.e. over a 36 month period) to increase the longevity of the wolf population on the island*." The questions asked of Subject Matter Experts focus on how to best implement Alternative B. As indicated in section 1.0 of this document (MG: see the SME's questionnaire), knowing how to best implement a plan in the service of some goal can depend greatly on knowing the broader purpose of that goal (Vucetich et al. 2016 and references therein).

The Federal Register indicates that the management goal of Alternative B is to "bring wolves to Isle Royale… over a 36 month period" and that the purpose of this goal is "to increase the longevity of the wolf population." However, item 3.3 of the Questionnaire suggests rather directly that the purpose of Alternative B is considerably broader, i.e., to achieve "*the best chance of long term viability without further addition via human intervention. Note additional natural immigration events are assumed to be limited*."

Furthermore, the purpose implied by item 3.3 is not possible to achieve through Alternative B. That is, long-term viability is almost certainly impossible to realize by bringing wolves to Isle Royale on a one-time basis, given that additional natural immigration events are assumed to be limited.

As of March 2016, there were believed to be two wolves on Isle Royale. If Alternative B were selected, if would likely not be implemented until sometime after 2017. Insomuch as the purpose of Alternative B is merely to increase the longevity of the wolf population, then wolves could be brought (on a one-time basis) by almost any means and the result would be to increase the longevity of the wolf population on the island. I fully realize that this response risks sounding disrespectful. No disrespect is intended. Rather the critical and inescapable concern is that the best means for how to achieve a goal depends importantly on knowing the purpose. The purpose of Alternative B maybe, unless I have missed something, inadequately articulated.

3.2. Discuss timing factors for the release of animals.

BP: As per above, I recommend capture and release during early winter. Hunting and travel conditions on the island should be good at this time of year and any pups left “orphaned” from the source pack captures should be reasonably self-sufficient by this time. During winter, and after many years of little to no predation pressure, there should be a sufficient number of old, vulnerable moose available to be predated by the wolves. Summer is generally a more difficult time energetically for wolves (wolf body condition tends to be at a seasonal nadir in summer and kill rates of moose are lower than during winter) so wolves released in summer into unfamiliar territory might face more difficulty meeting nutritional needs. Also, follow-up monitoring of released wolves will be easier when snow will facilitate observation of their behavior and social dynamics from the air.

DP: To the extent possible, wolves should be released at the same time using soft release techniques. As I wrote above, I believe winter is the best time for release. Late winter is when prey is most vulnerable to predation and so newly released wolves should do well from a food standpoint.

TR: This is outside my area of expertise. However, I think that late fall or early winter would be outside of the breeding season for wolves, and it corresponds to the onset of snow cover that gives wolves an advantage during predation.

TV: As with my answer to question 2.1.8., I will defer to subject matter experts with wolf-trapping experience on the issue of timing of the release.

AW: The timing of a reintroduction should be conducted within a year or two of the last of original wolves having died off.

As stated previously wolf releases in late summer or fall would likely be the most optimum situation for releasing wolves on the islands, and minimizing disruption of mainland packs from where wolves are removed. A fall release would allow males and females to pair bond well before winter breeding season and allow the pair to become well acquainted within their territory before winter, and reduces chance they would leave the island if an ice bridge forms to the shore.

3.2.1. Should the release of wolves at different IRNP sites be simultaneous or staggered? When should animals be released?

BP: I would release the 2 packs simultaneously on either end (E- W) of the island as intact packs. This will maximize the chance that each pack is able to feed and establish a comfortable territory before meeting and potentially engaging in antagonistic encounters with other released pack(s).

RP: Simultaneous would present no problems. Logistics may well dictate a staggered release, provided wolves are moved quickly to the island after initial capture. I favor release as soon as possible after transport to Isle Royale. The transportation itself can be difficult and delayed by weather, so it will be important to have back-up plans in that event.

TR: This is outside my area of expertise. If this were a mainland release, a staggered release would mimic the natural process of dispersal and pack establishment associated with increasing populations. However, because the island sets a hard limit on wolf population size, a simultaneous release might be less risky. I trust my colleagues to provide a better answer of this question. I think a late fall or early winter release would be best for the reasons I outlined above.

TV: This question is unanswerable without knowing first whether a soft-release is planned. If a soft-release is used, I would recommend a concurrent release of all individuals to maximize the probability of pair-formation. If hard-releases were planned, I would trap/transport/release wolves as soon as practical (effectively a staggered release) to minimize time spent in captivity and the logistical costs of holding and maintaining wild-captured wolves.

RW: Simultaneous might be better, prior to territory establishment.

AW: Although the release of wolves onto the island does not need to be completely simultaneous, ideally a close time period for release would improve probability individuals of three pairs finding each other and starting to establish similar sized territories. Presence of a member of the opposite sex in an area of suitable habitat with adequate prey, generally are the factors that cause a pair to localize activity and begin defending a territory. Without a potential mate present and no barrier of other pack territories to restrict movement, lone wolves would be encouraged to roam widely. If one pair established well ahead of one or two other pairs, the original pair may try to defend and hold larger portions of the island, making it more difficult for second and third pack to develop.

3.3. Define what should be the genetic and health characteristics of wolves chosen for reintroduction so that the packs that form have the best chance of long term viability without further addition via human intervention. Note additional natural immigration events are assumed to be limited.

BP: For reasons discussed above, all or most wolves (including both breeders) from 2 or more packs, separated by > 50 km from each other, from adjacent populations in the western GL states or NW Ontario should be sufficient. If desired you could ensure that the breeders are below a certain age threshold (to maximize lifetime reproductive output on IRNP) and at or above the typical weights for adult wolves in the region (e.g. Mech 2006) to help ensure good predatory performance on moose.

RP: The wolves should be given a basic health evaluation by a DVM, plus appropriate immunization. Genetic variability to the extent possible should be maximized, but this will be subject to the often challenging logistics of wolf capture on the mainland. Over a 36-month period, of course, different capture areas could be used, but I would recommend spreading out the source area to two or three generation locations to obtain the initial animals, as these wolves will pair off and self-organize. I would tend to resist the temptation to manage all aspects of genetic diversity and selection of individuals, as the wolves themselves will probably do what they can to avoid inbreeding provided they have sufficient choice.

DP: Given the size of IRNP, I do not believe that any mix of wolf genetics introduced will result in long-term viability without human intervention given limited or no natural immigration events.

TR: Lynch et al. (1995) modeled the effect of small populations accumulating mildly-deleterious mutations, and found that populations with effective sizes (Ne) of less than 100 individuals are highly vulnerable to extinction via mutational meltdown in less than 100 generations. This risk of extinction increases as Ne gets smaller. Without some gene flow, there can be no long-term viability (> 100 generations) of wolves on Isle Royale. There may, however, be viability over the life of the wolf recovery plan of 20 generations. It would be best to select animals for reintroduction on the basis of a high degree of polymorphic loci and a large number of alleles per locus for codominantly-inherited loci. This could slow the rate at which the inbreeding coefficient rises for the IRNP wolf population.

RW: As above, individuals that have pair bonded and introduced as a functioning pack, without evidence of high kinship or past inbreeding would be best. Wolves should otherwise be in good condition and hunt moose in similar environments found in the island.

AW: Genetically healthy individuals chosen for reintroduction should have coefficient of inbreeding of < 0.10, and levels of heterozygosity approaching or exceeding 0.60. Wolves selected should not be showing signs of sarcoptic manage or be afflicted by any major canid disease. Larger individual should be selected to assure they are effective predators on moose including males > 35 kg and females > 30 kg. Release of three unrelated males, and three unrelated females in pairs on east end, middle and west end of island should produce a fairly diverse population. If initial pair members die before functioning packs occur, they should be replaced by same sex individuals until three functioning packs establish.

3.4. If wolves leave IRNP during the translocation period, what effort should be made to translocate additional wolves?

BP: Once committed to translocation, translocation should repeated as needed until ≥2 packs are established and successfully reproducing.

RP: Wolves should be added until a viable population is established.

DP: As I mentioned above, I recommend introducing packs. If an individual leaves, I would not see a reason to introduce another wolf. However, if a pack leaves shortly after release, another pack should be introduced onto the end of the island least used by the wolves that still inhabit the island.

TR: It depends on conditions when the wolves leave the island. If the wolf population is high, dispersal from IRNP to the mainland may be a means by which the wolf population is undergoing density dependent regulation. If the moose population reaches a record low, wolves may be leaving to establish a territory with a larger prey base. In both of these cases, no translocation should take place. If the wolf population is low and the moose population is high, translocation may be appropriate and should be pursued.

TV: If translocated wolves leave the island, I think that managers should continue to translocate additional wolves until a pair forms and evidence of successful mating is seen.

RW: I would not recommend bringing them back, but would consider replacing them with new wolves. Such dispersing wolves are likely to continue to be problematic.

AW: If wolves die or leave the island before three functioning packs exist, they should be replaced by members of the same gender.

4. **Alternative C: The NPS would bring wolves to Isle Royale as often as needed in order to maintain a population of wolves on the island for at least the next 20 years, which is the anticipated life of the plan. The wolf population range and number of breeding pairs to be maintained on the island would be determined based on best available science and professional judgment.**

Please note thresholds for translocating additional wolves to IRNP should be based on the wolves function as the apex predator and possible effects to IRNPs current ecosystem, including effects to both the moose populations and forest/vegetation communities.

4.1. What threshold(s) or ecological criteria should be considered for augmenting the IRNP wolf population and why are they important? Consider: wolf and prey density, wolf demographics, habitat, and/or social parameters, (growth rate, juvenile mortality, number of successful breeders, number of packs, etc.), on the ability to perform an expected ecological role as apex predators (predation rate), moose population growth rate, herbivory metrics, etc.

BP: As stated above, "thresholds for translocating additional wolves to IRNP should be based on the wolves' function as the apex predator and possible effects to IRNPs current ecosystem" however, once ≥2 packs have been established the NPS will have little (no) control over how well the wolves perform their desired ecological service (ie. reducing herbivory and landscape manipulation by moose and beaver). Also, it may take some time for herbivory to be reduced to desirable levels so trend towards reduced herbivory should be sufficient in the short term, rather than strict adherence to hitting an unrealistic target of low herbivory within a few years. Once the wolf population on the island is saturated (i.e. no vacant space), even if herbivory is still higher than desired it is unlikely that further wolf reintroductions will increase the population or resulting predation rates because of the expected strife and social stress that introduction of additional wolves would cause. Barring unforeseen catastrophes or stochastic events, he wolves will govern their based on available space and prey resources (Pimlott et al. 1969, Kuzyk and Hatter 2014).

RP: I think it is difficult and generally pointless to predict what wolf population size "ought to be" at any single point in time. I suggest two approaches for determining the viability of the wolf population, one based on genetics and one based on prey-to-wolf ratio. If the first case, viability could be judged by maintaining the population below a level of inbreeding that is likely to reduce reproductive success (this level can be determined both generally from other wolf populations (e.g. Sweden) and also specifically from Isle Royale given the detailed pedigree information available from 1999-present. An inbreeding coefficient (*F*) calculated from detailed pedigree could be used to trigger intervention if F exceeded a predefined threshold level. In the second case, monitoring numbers of wolves in relation to moose population size will indicate when inbreeding is likely to be reducing reproductive success (and therefore viability). For example, a threshold ratio of moose to wolves of >100 could trigger additional intervention, based on the record of the past 58 years when moose:wolf exceeded 100 only when it was known that genetic inbreeding was having an important negative effect – early 1990s and 2012-2016).

Similarly, a threshold ratio >75 could be a red flag that specifies immediate consideration of additional intervention (if other reasons for the skewed ratio can be excluded).

DP: Many of us tend to think of these systems as staying the same through time…but they don't, especially when the system we're interested in is as small as IRNP when it comes to species requiring a lot of space with limited immigration possibilities. I mentioned earlier that wolves on IRNP without any anthropogenic influence probably would have been high at times, low at times, and absent at times. Trying to hold them at a level where they are always controlling the moose and hence influencing the vegetation is unnatural. The criteria you specify in this section are not as important (I don't think) as maintaining the *processes* within this system.

The decision that needs to be made is whether you want a continuous population of wolves on the island *or* whether you want to provide for the process of immigration but beyond that let the wolves, moose, and vegetation do what they are going to do. If you want a continuous population of wolves, you might consider bringing in an immigrant wolf once per wolf generation (and for that to work, the immigrant must also become a breeder). Otherwise, provide an artificial immigration event when wolves disappear and see what happens.

TR: Wolf-prey density. This does not seem to provide a good threshold for management purposes, because of the coupled dynamics of each. Wolf demographics, habitat, social parameters. Number of wolves and number of packs appear most relevant. If the wolf population dropped below 10 animals or below 2 packs, augmentation would be beneficial. Ability of wolves to perform ecological role as apex predators. Wolves are one among many modulators of moose population growth rates and vegetation responses. Responses of ecosystem components to wolves are often not immediate (there are time delays), the effect sizes are not always large, and the effects of wolves can be increased or decreased by other environmental factors (snow depth and winter severity, drought, etc.). Therefore, I do not think the use of some type of apex predator indicator or threshold would be practical for management purposes. It lacks the sensitivity needed for managers. The best threshold or indicator should be the number of wolves present on IRNP.

TV: Many of the ecological criteria listed in this question such as "wolf and prey density wolf and prey density, wolf demographics, habitat, and/or social parameters, (growth rate, juvenile mortality, number of successful breeders, number of packs, etc.)" presuppose an equilibrium paradigm or at least a benchmark relative to long-term averages that may be considered normal or desirable. Given the extreme variation displayed (Vucetich and Peterson 2009) I am not convinced that point estimates of many of these values are meaningful except in very broad terms.

Again without knowing the Park Service's goal for wolf restoration, I would suggest that there are 2 over-riding concerns: 1) ecological function and 2) persistence of wolves because of their iconic and scientific association with IRNP. Hence, I think the most meaningful threshold for decision-making would be multi-year trend in the growth of the wolf population. Population growth rate is an integrator of all the intrinsic and extrinsic influences on demography and life-history (Sibley and Hone 2003) and IRNP and its collaborators have protocols for estimating population size. If population growth is demonstrated, one can infer that some meaningful ecological function (i.e. moose predation) is occurring. The "population" of wolves on the island will always be very small (Vucitich and Peterson 2009), consequently year growth rates will be highly variable and probably negative from time to time (Stenglein and Van Deelen 2016). For this reason, growth rate should be measured as a multi-year trend using, for example, a 5 to 10 year window for decision-making.

The one caveat to this would be to also monitor browsing by moose. Overbrowsing can be unsightly for visitors and can have long-term effects on the productivity of the island for moose. Questions like this one assume that the wolf-moose-fir system is regulated top-down (McLaren et al. 1994) given the variation in the system and the possibility of additional productivity through climate change, that assumption may not always be valid (Vucetich and Peterson 2004).

JV: The ecological function of a wolf population includes its capacity for predation without being impaired by inbreeding. That ecological function, predation, is most appropriately quantified as predation rate, which is calculated as the per capita kill rate times the ratio of wolves to moose. As such, predation rate integrates many aspects associated with narrower senses of viability (e.g., vital rates, such as survival and reproduction, given the availability of food).

The central importance of predation rate is further acknowledged by the NPS's cooperative agreement for researching wolves in Isle Royale National Park, which states that one of the basic objectives of researching wolves on Isle Royale is, "Determining the rate of predation of wolves on moose as a key indicator of the health and viability of the wolf population."

Predation rate. – The predation rate of Isle Royale wolves has been estimated for several decades – to our knowledge, longer than in any other free-ranging vertebrate. The methodological details for these estimates and analysis of these estimates are reported in Vucetich et al. (2011). Some of these empirical relationships and temporal patterns are worth reviewing here:

- Predation rate has been estimated directly (i.e., as the per capita kill rate times the ratio of wolves to moose) each year for the period, 1971-2015. Over that period, predation rate is the most important predictor of population growth rate in the moose population and explains about 56% of the variance in growth rate (Fig. 1).

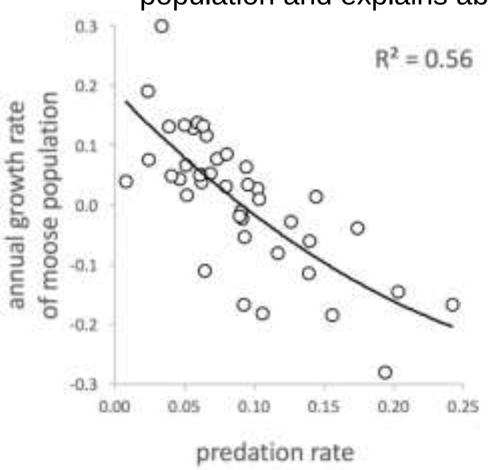

Fig. 1. The relationship between predation rate and growth rate of the moose population in Isle Royale National Park, 1971-present.

- Estimates of predation rate also indicate that much of the variation in predation rate is predicted by the ratio of wolves to moose (Fig. 2). Because estimates for the ratio of wolves to moose do not exist for the period, 1959-1970, there is value in generating a hindcast of what predation rates were likely to have been for that earlier period. The hindcast will be useful *only* for providing a very rough sense of what predation rate is likely to have been during that earlier period.

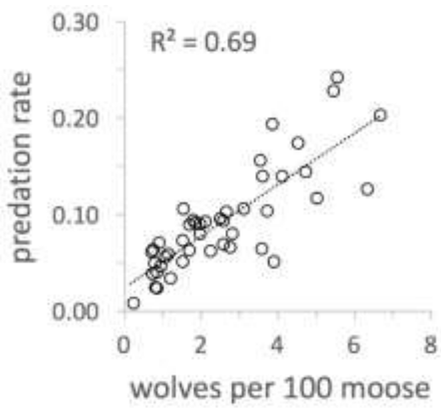

Fig. 2. The relationship between the ratio of wolves to moose and predation rate in Isle Royale National Park, 1971-present.

- Figure 3 depicts temporal variation in estimates of predation rate. The values are both direct estimates for the period 1971-2015 and hind casts for the period 1959-1970, based on the ratio of wolves to moose. Over the past 56 years, there have been two periods during which predation rate was particularly low and likely impaired by inbreeding. One period is recent, from approximately 2012 to the present.

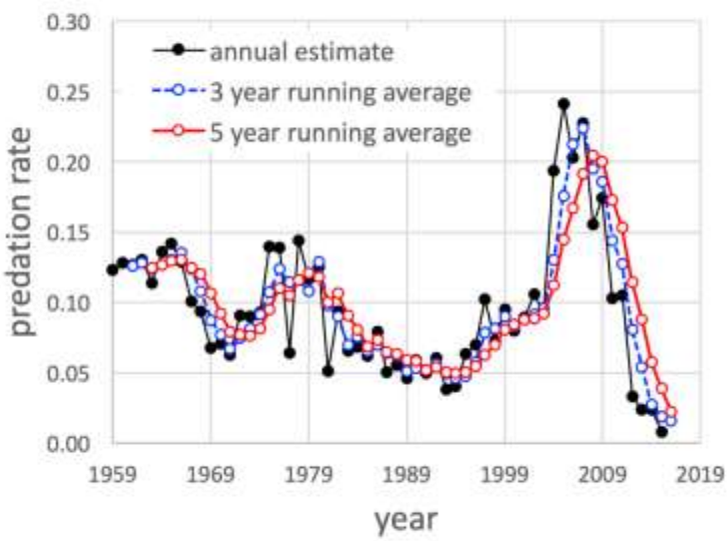

Fig. 3. Temporal variation in predation rate for the wolves and moose of Isle Royale National Park, 1959-2015.

The other period is the early 1990s. That period of low predation rate followed the introduction of canine parvovirus and the subsequent collapse of the wolf population. The wolf population recovered from that crash slowly, despite an abundance of prey. Inbreeding was proposed as an explanation for that slow recovery (Peterson et al. 1998). Moreover, that recovery is likely the result of a genetic rescue that occurred in 1997 (Adams et al. 2011).
Inbreeding.

Several patterns of inbreeding in the Isle Royale wolf population are also worth noting:

- The demography of Isle Royale wolves suggests that its effective population size is approximately 3.8 (Peterson et al. 1998). That statistic provides a basis for expecting the inbreeding coefficient (*F*) to increase (in the absence of immigration, inbreeding avoidance, and selection against homozygotes) from zero to 0.24 in two generations, where the length of a wolf generation is approximately 4 years. While this estimate represents useful context, it should not be taken as a particularly precise estimate projection.
- Another basis for anticipating the rise in *F* are estimates of *F* derived from a pedigree that exists for the population since the late 1990s. Those temporal trends are depicted in Fig. 4.

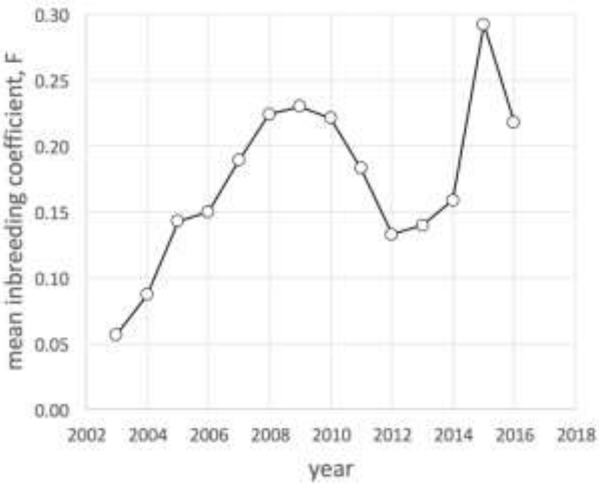

Fig. 4. Temporal variation in the mean, expected inbreeding coefficient for the wolf population on Isle Royale. Data taken from Hedrick et al. (in review).

- A combination of evidence (demographic, genetic, and behavioral) suggests that rates of immigration from 1950 to 1995 had been on the order of two immigrants every third generation (p. 1116 and Fig. 5 of Hedrick et al. 2014). While this estimate represents useful context, it should not be taken as a particularly precise estimate of past rates of gene flow.

Recommended threshold for future augmentations. – On the basis of the considerations outlined above, augmentation should occur when two conditions are met:

(i) the X-year moving average of predation drops below Y, *and*
(ii) the mean inbreeding coefficient of the population (F) exceeds Z; *or* when other plausible evidence suggests that inbreeding has impaired predation rate to the point of falling below the threshold described in (i).

The threshold is presented with variables (X, Y, Z) to first highlight its structure. Key elements of the threshold are: using predation rate to quantify functional viability (*sensu*, Soulé et al. 2005), recognizing that temporal fluctuations in predation are normal, but also recognizing that predation rate can be impaired by inbreeding. Those elements reflect the purpose of Alternative C, as expressed within the wording of Alternative C.

More specifically, consider values for X, Y, and Z that would correspond to the three-year moving average of predation rate dropping below 5% and *F* exceeding 0.15. An important perspective for judging the appropriateness of this threshold is that these conditions were met in 2014 for the first time to our knowledge (Except, Fig. 6 in Hedrick et al. 2014 indicates that the threshold could have been passed in the 1990s after the wolf population collapsed following the introduction of canine parvovirus). Moreover, the current condition of the Isle Royale population was anticipated several years prior to 2014, indicating that future crossings of a threshold are likely to involve enough advanced warning to allow for planning of a subsequent augmentation. For emphasis, this recommended threshold would be a threshold for when augmentation should be implemented, not a threshold for beginning to discuss or plan augmentation.

An inescapable trade-off - Judging appropriate values for X, Y, and Z represents an inescapable trade-off between two important principles. On one hand, setting a “high” threshold for augmentation (i.e., low predation rate and high *F*) is attractive insomuch as a high threshold will prevent active management that may not be absolutely necessary. On the other hand, setting a “low” threshold for augmentation (i.e., high predation rate and low *F*) is attractive for an equally important reason. That is, a low threshold represents the restoration of unimpaired predation rate after a longer delay or time lag.

Management actions that are time lagged tend to amplify variance in population dynamics beyond what would normally be observed (Fryxell et al. 2010). Amplified variance may be undesirable for its own sake and would plausibly be associated with damage for Isle

Royale forests through amplified variance in moose herbivory. Similarly, delayed restoration is significant because restoring predation after its absence does not necessarily restore an ecosystem (e.g., Schmitz 2004). For example, the absence of wolf predation in Yellowstone allowed elk to outcompete beavers, greatly reducing the abundance of willow and beaver. The resulting alterations to hydrology appear to be not readily reversible, even after restoration of wolves (Marshall et al. 2013).

A note on "plausibility." – The recommended threshold states "…when other plausible evidence suggests that inbreeding has impaired predation rate …" The word "plausible" deserves emphasis. Inbreeding depression is notoriously difficult to detect even when it is strong and even when considerable effort is expended to detect it. The wolves of Isle Royale are an important example of this circumstance.

It is also important to highlight the phrase "other plausible evidence." The recent and current problems with the wolf population were not indicated by any single observation or on the basis of observations that were anticipated. For example, while inbreeding depression had been occurring for many years and while researchers were searching for evidence of inbreeding, the first signs of inbreeding depression were not detected until 2009 and were inferred on the basis of bone deformities (Räikkönen et al. 2009). And, definitive evidence that severe inbreeding had been impacting population dynamics was inferred only because of an unanticipated and chance event (i.e., the arrival of an immigrant wolf at a particular time in the population's history, Adams et al. 2011). Those events led the broader community of conservation geneticists to redefine the criteria by which genetic rescue is judged (i.e., Hedrick et al. 2011).

RW: Unclear which of these would be the best metric for augmentation. If the goal is to control moose numbers, a population size near the carrying capacity might be best, alternatively, a value near ½ the carrying capacity might sustain the highest mortality without decline.

AW: If a diverse wolf population is established on IRNP, I am not sure I can conceive of a situation where non-genetic criteria would be used for determining the need for wolf population augmentation before genetic criteria. Factors that might be considered could include extremely low kill rate on moose with wolves seeming to be ineffective predators, drastic decline in balsam fir from moose browsing, or near lack of any pup production or recruitment. In general, I think genetic metrics would be more useful for determining need for wolf population augmentation, and ideally such criteria will result in genetic rescues before major ecological damage or demographic problems develop

4.2. If using wolf demography and social structure alone to inform augmentation, what would be the pros and cons to this type of approach?

BP: Once ≥2 packs have been established the NPS will have little control over how well the wolves perform their desired ecological service (ie. reducing herbivory and landscape manipulation by moose and beaver). Once the wolf population on the island is saturated (i.e. no vacant space), even if herbivory is still higher than desired it is unlikely that further wolf reintroductions will increase the population or resulting predation rates because of the expected strife and social stress that introduction of additional wolves would cause. Ultimately the wolves will establish their own density with regard to available space and food resources as wolves always do, particularly in protected areas (e.g. Pimlott et al. 1969, Kuzyk and Hatter 2014; WSB). As such, this is not a measure of success that leads to many practical management options. However, if monitoring reveals low pack size and persistence reproductive failure then genetic assessment would be warranted to determine if inbreeding may be responsible. If so further introductions may be warranted.

RP: Negative aspects to this include ignoring the ecological role that the wolves are serving in relation to moose as prey. Positive aspect would be simplicity, provided there is adequate monitoring.

DP: See 4.1.

TR: Pros—it is easy to measure and use. It can be used to provide gene flow and offset any adverse consequences of inbreeding. Cons—it would not include any information about the genetic status of the population. It also does not consider any potential catastrophic and unforeseen collapse in the moose population for reasons other than predation.

TV: Yearly point estimates of demography (age- and sex-specific mortality and fecundity) likely are not very meaningful given the small population size expected for wolves re-introduced to Isle Royale. Demography also has the disadvantage of being difficult and expensive to estimate because it usually requires a dedicated research effort using marked animals.

ocial structure is vital – at least in its broadest terms. Managers should monitor for pack formation and evidence of reproduction within a pack. This seems to be the fundamental socio-biological signal that re-introduced wolves are likely to persist (Fuller et al. 2003).

RW: Unclear what the goal is here, is restoration about controlling moose, restoring ecosystem function or restoring the natural dynamics of wolf packs? Alternative C is my least favorite option, as it requires heavy management and utilizes dubious assumptions.

AW: The pros for using genetic data for setting metrics to determine need for augmentation includes that such data is easier to determine and likely to encourage augmentation before ecological damage or demographic problems occur.

A con of genetic testing for setting criteria for augmentation, genetic metrics require specialized testing and is not based on features that can be viewed in the landscape or can be easily observed in the demographics of populations.

4.3. If genetic factors are considered in determining the need to augment the population of wolves inhabiting IRNP, what are genetic factors or phenotypic characteristics that could be considered in determining whether additional wolf translocations to IRNP are necessary?

BP: The NPS can use history to inform what levels of wolf abundance (number and size of packs) and predation rates are possible and expected. If wolf numbers, reproductive success, or kill rates are below what would be expected, then genetic monitoring of wolves in all packs should reveal whether inbreeding is at levels sufficient to make it the likely cause of impaired population performance. If so further introductions may be warranted.

RP: Commenting on specific genetic characteristics is beyond my expertise. One obvious phenotypic character to monitor over time would be skeletal abnormalities, but these data would accumulate too slowly to provide a trigger for augmentation (and of course the animals would be dead when evaluated).

DP: Genetic specialists should handle this one.

TR: The particular phenotypic characters that might indicate a genetic problem cannot be anticipated. We cannot anticipate and simply look for the appearance of “black eyes” or “crooked tails” or some other phenotypic trait that indicate inbreeding depression. There are simply too many possibilities. However, if wolves do begin to show signs of inbreeding depression in the form of failed or reduced reproduction, augmentation would be recommended.

TV: The great danger to the persistence of wolves on Isle Royale in terms of genetics is inbreeding. Inbreed can be quantified for individuals on the basis of heterozygosity (Mills 2013) and the effects of inbreeding can be observed physically (Räikkönenet al. 2009, 2013). Observing phenotypic signatures of inbreeding requires, minimally, the collection and measurement of wolf skeletons. This is expensive and it's not clear how phenotypic signatures associate with population growth.

RW: Genetic evidence of inbreeding, close kinship among breeders, congenital defects or decreased survivorship among pups and juveniles and decreased reproduction could all be considered if not attributed to disease, prey density or other factors.

AW: Phenotypic characteristics such as development of lumbosacral transitional vertebrate (Räikkönen et al. 2009) or other skeletal malformations spreading through large segments of the wolf population should serve as a metric for considering genetic augmentation to the wolf population.

4.3.1. If inbreeding is to be accounted for, how should inbreeding be estimated and what threshold inbreeding coefficients, measures of heterogeneity, or levels of genetic diversity would be considered problematic and trigger translocation of additional wolves?

BP: See Adams et al. 2011, Hedrick et al. 2014, Hedrick and Lacy 2015. Not my area of expertise.

RP: Inbreeding can be determined from analysis of the full pedigree for the population over time, which should be a priority research/monitoring goal. A quick perusal of inbreeding coefficient (*F*) since 2000 suggests that a *F*>0.15 is associated with poor reproduction and declining viability.

TR: Inbreeding should be measured using the inbreeding coefficient F—the probability that two genes at a locus are identical by descent. Data can be collected from scats or hair traps (from which DNA can be isolated), and F can be estimated based on deviations from Hardy-Weinberg frequencies. Unfortunately, there is no level of inbreeding as measured by F that corresponds clearly to population declines. The wolf population size persisted throughout the 1980s and 1990s despite a high F, prior to the arrival of individual no. 93. We can anticipate that regardless of the measure of heterozygosity or genetic diversity, these values will decline over successive generations. It would be prudent to consider augmentation at least once during the life of the plan, regardless of measured data.

TV: Inbreeding is best estimated through analysis of genetic material that can be obtained from captured wolves (tissue) or obtained non-invasively (hair catchers, scat collection). Mean heterozygosity associated with negative population growth in a small Scandinavian wolf was about 0.50 and positive growth was associated with immigration and breeding of a new individual leading to a mean heterzygosity of 0.62 (Vilá et al. 2002). This value, 0.50, might be a reasonable benchmark for dangerous levels of inbreeding.

RW: Some preliminary data from Swedish wolves (Liberg et al. 2005) indicates a decrease in juvenile survivorship by approximately 15% with an increase in the inbreeding coefficient of 0.1. This perhaps could be a value triggering action.

AW: Augmentation to the wolf population should be considered if heterozygosity starts to fall below 0.6, and coefficient of inbreeding starts to increase above 0.10, as well as other measures of declining genetic diversity.

4.3.2. Should phenotypic signs of inbreeding depression be the primary trigger for augmentation? If the inbreeding coefficient is considered problematically high, but wolves continue to reproduce without clear phenotypic or functional role indications of inbreeding depression, should translocations nonetheless occur? Why or why not?

BP: No, by the time congenital defects are apparent population performance will likely have suffered considerably. This is not a sufficiently sensitive indicator. (MG: Regarding the inbreeding coefficient:) No, this entire program is motivated and premised on restoring ecological function, thus remedial measures are not warranted as long as ecological function is within normal expected ranges (and reasonably expected to continue to be in that state).

RP: Phenotypic variables are not likely to be useful as appropriate data on living wolves will generally not be available. A high *F* (>0.15) should, by my recommendation, prompt introduction of new individuals. As a basic alternative, I encourage consideration of a low-level program of translocation, to simulate immigration events of the past – this might mean translocating/monitoring one-two individuals every three-five years. If translocated animals survive to reproduce, this would go a long way toward alleviating inbreeding. Hedrick et al. (2014) suggested that approximately two successful (breeding) immigrants for every three generations may have described the population for much of its history.

TR: There are reasons to maintain genetic diversity within populations that extend beyond the avoidance of inbreeding depression. Genetic diversity provides adaptive capacity for the population. Even if the inbreeding coefficient is problematically high and wolves are reproducing without evidence of inbreeding depression, translocations most certainly should occur.

TV: I don't think phenotypic signs of inbreeding should be the primary trigger because I don't think we know the relationship between phenotypic signatures and reproductive dysfunction. High inbreeding coefficients should be considered signals of impending dysfunction and should be triggers for intervention under a precautionary principle. The wolves are going to become inbred. It's a normative judgment but I think interventions to prevent the deleterious effects of inbreed are a better strategy than waiting until those effects show up.

RW: This is an important indicator and should be part of the argument that needs to include genetic data. I would not wait until breeding is affected, it is a clear prediction from empirical data and theory that if inbreeding is increasing, it will eventually affect fitness, so I would be inclined to take preventative action (see above comment).

AW: Augmentation should be done based on genetic criteria to avoid manifestation of phenotypic signs or the large scale development of skeletal malformations. Quick response to declining heterozygosity or increasing inbreeding depression by releasing new wolves on IRNP will reduce the probability of genetic problems developing.

An alternative to setting criteria for augmentation is routinely release a new wolf onto Isle Royale every generation (4-5 years). Wolves could be released if no known migrants occurred during the interval. Additional wolves beyond the single release every 4-5 years would be considered if genetic problems start to occur.

Other input Received

JV: According to basic principles of natural resource management (Vucetich et al. 2016 and references therein), an appropriate response to Alternative C would depend, inescapably, on the underlying purpose of Alternative C. The purpose of Alternative C is unstated. Nevertheless, the Subject Matter Expert Questionnaire, Version 4 implies that the purpose is “based on the wolves (*sic*) function as the apex predator.”

5. **Alternative D: The NPS would not take immediate action and would continue current management, allowing natural processes to continue. One or more resource indicators and thresholds would be developed, which could include moose or vegetation-based parameters. Once a threshold is met, wolves would be translocated to Isle Royale as a one-time event (per alternative B) or through multiple introductions (per alternative C).**

5.1. Assessing wolf-mediated resource thresholds

5.1.1. What aspects of prey and habitat health are a concern, and why. How may they be mediated or affected by wolves through top-down control? (i.e. winter ticks)

BP: Hyper-abundant moose can all but eliminate balsam fir regeneration on IR leading to pronounced changes in forest structure. Wolves may reduce herbivory and facilitate natural rates of forest regeneration by reducing the number of moose and beaver (lethal effects) and by effecting behavioral responses in moose and beaver (fear effects, e.g. Montgomery et al. 2013, Peterson et al. 2014).

RP: Degradation of the forest community with disappearance or functional absence of species may result from moose hyperabundance. Elimination of a functional balsam fir component at the west half of Isle Royale, for example, will over decades progressively reduce this primary winter forage species for moose, not to mention other species for which balsam fir is a critical habitat component. Other forage species may be likewise affected, particularly in little-studied and difficult-to-monitor aquatic habitats. For example, in 2016 I’ve observed shoreline habitats where beavers have excavated roots during foraging – such areas can readily cover tens of square meters where topsoil is dug out, much like feral hogs might do.

How may they be mediated or affected by wolves through top-down control? (i.e. winter ticks) Of primary concern here is reduction and potential absence of wolf predation, which is beyond a doubt the major limiting factor for moose at Isle Royale (Peterson et al. 2014).

DP: Dramatic moose population increases and population crashes occurred on Isle Royale from the time moose first arrived through the present. Moose population highs were apparently not as high following wolf immigration as prior to the presence of wolves, and the time between moose highs and lows was longer in the presence of wolves. Wolf persecution on the mainland in the early decades of the 20th century may have kept wolf numbers sufficiently low that dispersal to Isle Royale was unlikely then.

At high population levels, moose had a dramatic influence on the vegetation. Species that moose rarely ate, such as spruce, did quite well while species that moose ate at almost every opportunity, like yew, declined.

My point is that wolves were nonexistent and very abundant at times during the historic period. Moose were not present at the beginning of that period and extremely abundant at other times. Humans had very little effect on this until now, when the probability of immigration by wolves has declined. Starving moose or vegetation that moves toward or away from species eaten by moose would not be parameters that I think should be considered. The parameter that is out of whack is immigration by the top predator, the wolf. And that process, with Park Service assistance in moving animals, can be addressed.

TR: With respect to prey, the moose population, by virtue of being isolated on an island, is vulnerable to inbreeding depression. A warming climate, winter ticks, etc. will be of concern if the moose population undergoes a sustained population decline. Wolves are unlikely to halt such declines. Habitat health as measured by sustained seedling recruitment of vulnerable species like balsam fir and aspen is likely affected by top-down control. However, direct effects are likely weak. Interactions between wolf predation and other factors, such as climate, are probably as important if not more important than top-down control alone.

TV: The wolf-moose-fir system on Isle Royale is highly variable and demonstrates both top-down, bottom-up, and stochastic abiotic influences that vary over time (and probably, space; McLaren and Peterson 1994, Vucetich and Peterson 2004, Wilmers et al. 2006). The concepts of prey health and habitat health in this question are subjective because every individual and every ecosystem has some level of dysfunction. Monitoring needs to acknowledge and embrace this problem. Decisions about the moose should focus on visitor expectations and perceptions as well as on ecosystem function. The park, to the extent that it manages moose, should strive to avoid overabundance where browsing pressure impairs productivity in key plants and visitors see lots of sickly and dying moose due to density dependent mechanism like malnutrition and disease. If you assume top-down control of the moose, than enhancing the wolf population is reasonable management intervention although effects of management may not be immediate. If winter-tick infestations are density-dependent or have a synergistic relationship with predation, then more wolves would reduce the incidence or severity of infestation.

RW: This again is a little beyond my expertise, but I don't think data exists that will allow specific vegetation limits, and considering lag periods for habitat and vegetation, it may be unwise to use this as indicator. Essentially it may be too late for constructive action by the time these effects are manifest.

AW: Wolf predation has improved growth of balsam fir on Isle Royale in the past and likely to continue to be a factor, but may be modified based on climate change (McLaren and Peterson 1994, Post et al. 1999). Fluctuations in the moose population mediated by wolf predation may be a factor in winter tick abundance and ecology, but abiotic factors at times may be more important than top-down factors (Vucetich and Peterson 2004). But this recent reduction in effectiveness of wolf predation may be partial due to reduced effectiveness of predation after disease outbreak and genetic problems being faced by wolves (Wilmers et al. 2006). Climate change will likely impact mainland ecotonal-temperate-boreal forest, and it will be important to examine the extent such changes occur on Isle Royale (Frelich et al. 2012). Trophic cascade effects are also being detected in recolonized wolf range south of Lake Superior (Bouchard et al. 2013, Callan et al. 2013, and Flagel et al. 2016).

As wolves again re-establish as effective predators it will be critical to evaluate changes in moose populations and impact on balsam fir and other browse species. It will be important to also evaluate impacts on winter ticks and other parasites as wolves exert greater influence on moose abundance and again become major selection factors on moose.

5.1.2. What are historic baselines available for Isle Royale and the surrounding mainland ecosystem that would inform identifying thresholds?

BP: McLaren and Peterson 1994, Janke et al. 1978.

RP: The most relevant data from other ecosystems comes from the Scandinavian peninsula and the maritime Canadian provinces. Across all of Sweden, there is a management goal to maintain (through hunting) moose density at two/sq.km or less (LB Keith, personal comm, ca. 1983), to reduce deleterious environmental impact of moose herbivory. Parks Canada has determined that current hyperabundance of moose in Cape Breton and Gros Morne national parks (approx. five/sq.km) needs to be reduced to approx. two/sq.km for the same reason, with special concern for the future of balsam fir in the forest community (Parks Canada 2014, Knight et al. 2015). It should be pointed out that Parks Canada expended $13 million over a five-year period in the 2000s to assess the effects of hyperabundant moose (several Parks Canada ecologists, personal comm. with ROP in 2014). Since wolf predation (now absent) in the areas surrounding these parks is socially unacceptable, Parks Canada is attempting to reduce moose through shooting (this began in 2015). At Isle Royale, there are impacts of moose on vegetation at densities of two/sq.km. (Snyder and Janke 1976, McLaren and Peterson 1994), but these impacts became quite dramatic (reducing moose body size and vigor) in the 1990s when moose density approached five/sq.km. before the population crashed from starvation (see annual reports by Peterson in the 1990s).

DP: Not applicable.

TR: Historic baselines are useful for understanding current dynamics in a broader context. However, historic baselines should not automatically be considered targets when identifying thresholds. Useful baselines might include: mean and variance of annual recruitment rates of both tree seedlings and moose, and proportion of stems browsed and its relationship to seedling recruitment rates.

TV: The most relevant baseline ecological information likely will be research and monitoring done in Ontario where wolves prey primarily on moose. I cannot speak to the monitoring data available in Ontario. Mainland wolf systems in Wisconsin, Michigan and, to a lesser extent, Minnesota are primarily deer-based and are less comparable.

AW: Ohmann and Ream (1971) and Heinselman (1993) work in the Boundary Waters Wilderness to west in Minnesota, and work on Isle Royale by Linn (1957) and (Hansen et al. 1973) provide important baseline studies of vegetation that can be used to assess changing patterns and identify potential thresholds. Area that serve as baselines include virgin forest areas in the boundary waters area listed by Ohmann and Ream (1971), exclosures on Isle Royale (Krefting 1974), and smaller islands around Isle Royale the receive little or no browsing by moose.

5.1.3. What prey and plant species should be monitored?

BP: Moose, beaver, balsam fir.

DP: If there are endangered plant species on the island that are preferred foods of moose, those species should be monitored.

TR: Moose population size and number of calves per 100 animals should be monitored. As for plant species, (a) annual recruitment of tree seedlings, mainly balsam fir, white spruce, sugar maple, and trembling aspen into size classes (<10 cm tall; 10-29 cm tall; 30-99 cm tall, and >100 cm tall). This should include a few plots in which individual seedlings are followed through time to get estimates of growth rates and size class recruitment, and several plots with spot counts of seedlings in each size class. Recruitment can be measured annually or estimated based on surveying 20% of plots each year. IRNP should also monitor percent moose browsing on each of size classes, and how this is changing through time.

TV: The primary prey species to monitor are moose and beavers. The primary plant species to monitor is balsam fir although I think that our understanding of important moose-wolf effects on plant community dynamics as a component of the Island's ecosystem (e.g. Bump et al. 2009a,b) are still relatively poorly understood and should be further researched.

AW: The main prey to be monitored would be moose and beaver, although changes in abundance of snowshoe hare and small mammal communities are also of interest. Plants that should be monitored include species important as moose browse such as balsam fir (*Abies balsamea*), aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), mountain maple (*Acer spicatum*), yew (*Taxus canadensis*), mountain ash (*Sorbus decora*), as well as trees/shrubs that are generally poor moose browse that may become more abundant including white spruce (*Picea glauca*) and alder (*Alnus crispa*) (Pastor et al. 1993). Changes in wetland species/ upland species that change with fluctuations in beaver populations would also be important monitor.

5.1.4. What prey or vegetation demographic or community measures should be monitored?

BP: Species diversity, relative abundances.

RP: Slow-growing coniferous tree species such as northern white cedar and balsam fir are important forage species, and balsam fir is highly favored over cedar (Parikh et al. 2016). Aquatic ecosystem status is likely to be severely degraded because moose prefer aquatic plants, and the focus here should be on interior beaver ponds, because of more dramatic effects of moose and beaver herbivory (Bergman and Bump 2015).

DP: I feel a need here to say that monitoring should occur to learn more about systems with and without wolves in the absence of humans being a predator.

TR: Annual recruitment of tree seedlings, mainly balsam fir, white spruce, sugar maple, and trembling aspen into size classes (< 10 cm tall; 10-29 cm tall; 30-99 cm tall, and > 100 cm tall) would provide a good measure of community dynamics appropriate for the time horizon of the plan. IRNP could also measure the frequency of flowering and average height of wild sarsaparilla (*Aralia nudicaulis*) as an indicator species of browsing intensity. This would likely be positively correlated with recruitment of browse-sensitive woody species and negatively correlated with recruitment of white spruce.

TV: Moose should be monitored with aerial surveys and effects should be monitored with browse surveys (e.g. McLaren and Peterson 1994). Beavers should be monitored with aerial counts of active lodges during the fall (Hay 1958). I will defer to other SMEs on techniques for monitoring plant communities.

AW: Features to monitor in moose, and beaver would include general demographics, distribution, abundance and changing impacts they are having on vegetation and the landscape. Changes in abundance, distribution, growth forms, and reproduction in balsam fir and deciduous trees and shrubs should be monitored, as well as the plants and communities that replace them. Beaver impacts to study should include examination of the areas of beaver-created wetlands, successional patterns in beaver created habitat, and alteration in plant growth caused by beaver feeding especially on aspen, willow and birch.

5.1.5. What threshold(s) of prey population size or prey vital rates would result in the translocation of wolves to IRNP? What has been the range of variability for population sizes for species of concern?

BP: I would be more concerned with the impacts of overabundant prey on lower trophic levels than with absolute prey abundances or vital rates per se.

RP: The key parameter of interest in the past is predation rate – if this is <5% then it is clear that wolf predation is not functioning, which I suggest is a reasonable trigger to prompt translocation of wolves to IRNP. The long-term average moose annual mortality is on the order of 13% (Peterson 1977), with wolf predation providing most of this mortality. In the period 2012-2016 wolf predation rate was on the order of 1-2% or less (Vucetich and Peterson 2014 and subsequent annual reports) and there was little moose mortality from other causes (Peterson and Vucetich, unpubl data from >250km of off-trail coverage annually). For context, moose in Minnesota have been declining seriously for the past decade, when annual mortality exceeded 20% (G. DelGiudice, MN DNR, pers. Commun. to R Peterson, 2015).

DP: None.

TR: For moose, a threshold population size above 500 would be sufficient to support wolves. Moose recruitment rates above 0.125 would be sufficient to support wolves. I am not sure what species of concern refers to in this question, but typically for species without outbreak dynamics (like forest tent caterpillars), a coefficient of variation of less than 100% is a normal range of variability.

TV: The focus for decision-making should be the moose population. Moose numbers varied from <500 to roughly 2500 during 1957-2007 (Vucetich and Peterson 2009). The high point followed a period in the late ‘80s and early ‘90s when wolf populations were in the 10-13 range. This suggests to me that 2000-2500 moose is probably an indication of current or pending overabundance – although this should be verified with vegetation surveys. Typical moose densities elsewhere are <1.0 moose/km2 (Karns 1997 in Vucetich and Peterson 2009). This density would be roughly 544 moose.

JV: My thoughts on thresholds for translocation are detailed above in section 1 (MG: see the SME’s questionnaire) of this document.

AW: It is probably not possible to come up with any one threshold of prey population size or vital rates that can be used as a threshold. A combination of factors may be necessary in combination with genetic markers to determine threshold for releasing new wolves on the island. Some factors to consider would be moose population rising above 1500 or greater, above levels observed until after wolf die-off from canine parvovirus between 1980 and 1982 and genetic problems affecting wolves since then. Along with a moose population eruption and wolves not showing normal predatory numerical or functional responses to prey increase might indicated genetic or disease problems impacting wolves. When diseases or parasites such as winter ticks in moose, and tularemia in beaver become more important factors than wolf predation as mortality factors, release of additional wolves may be necessary.

5.1.6. For plants, what thresholds of population size, vital rates, or aspects of vegetation structure or composition would result in wolves translocated to IRNP?

BP: Insufficient forest succession to maintain current and historical plant/ forest communities. See McLaren and Peterson 1994, Peterson et al. 2014.

RP: For the most part these data do not exist, but for balsam fir at the west half of Isle Royale it is clear that regeneration is on a knife-edge at present (Peterson et al. 2014), and with increased moose herbivory regeneration could easily fail, leading to long-term decline and likely elimination of this species in the understory.

DP: See 5.1.3. Otherwise, none.

TR: Thresholds can be based on recruitment of tree seedlings, mainly balsam fir, sugar maple, and trembling aspen into size classes (< 10 cm tall; 10-29 cm tall; 30-99 cm tall, and > 100 cm tall). A reasonable threshold to trigger reintroduction would be mortality of > 75% in the 10-29 cm size class before reaching the next size class, and mortality of > 75% in the 30-99 cm size class before reaching the next size class. The limitation of this measure and other measures based on seedling recruitment is that it is a slow indicator relative to management needs. A faster but less precise way to identify a browsing threshold on vegetation involves examining browsing rates on the indicator plant, wild sarsaparilla (*Aralia nudicaulis*) in the spring. Browsing rates in excess of 70% on flowering plants would be above a historical norm when wolf populations were high in the 1970s (Edwards 1985).

TV: I must defer to other SMEs on this question.

AW: Factors to consider for assessing balsam fir would include lack of reproduction elimination of sapling tree stages, major reduction in balsam coverage, replacement of balsam by spruce, growth and expansion of white spruce savanna, would be some factors to consider. For aspen and birch being impacted by both moose and beaver, factors to consider would include levels of regeneration, lack sapling trees, and drastic decline including flooding of aspen stands by beaver flowages.

5.1.7. If natural colonization of IRNP by wolves occurs, but prey or vegetation-based thresholds are nonetheless triggered, should translocations of additional wolves occur?

BP: Once the wolf population on the island is saturated (i.e. no vacant space), even if herbivory is still higher than desired it is unlikely that further wolf reintroductions will increase the population or resulting predation rates because of the expected strife and social stress that introduction of additional wolves would cause. Ultimately the wolves will establish their own density with regard to available space and food resources as wolves always do, particularly in protected areas (e.g. Pimlott et al. 1969, Kuzyk and Hatter 2014). As such, this is not a measure of success that leads to many practical management options. However, if monitoring reveals low pack size and persistence reproductive failure then genetic assessment would be warranted to determine if inbreeding may be responsible. If so, further introductions may be warranted.

RP: Yes.

DP: No.

TR: Yes, translocations of additional wolves should occur. A small group of wolves colonizing the island will be susceptible to the genetic and demographic perils of small populations, so additional translocations would buffer against these risks.

TV: Probably, yes. Natural colonization are likely to be done by very small numbers of wolves. This would introduce a longer lag between recolonization and desired population- or ecosystem-level effects.

AW: If natural colonization does occur, vegetation triggers should not be used to reintroduce more wolves until the effect of new colonization can be adequately evaluated. The time frame for evaluation of immigrant impacts would probably be within wolf generation time, or a period of about 4 to 5 years.

5.1.8. What are the pros and cons of basing the translocation of wolves on a primary indicator or multiple indicators?

BP: Multiple indicators are always preferred and likely to garner the most robust support from stakeholders and interest groups on all sides of the issue.

RP: Primary indicators (one or two) are simpler, of course, and provide an unambiguous answer, which is preferable given all the ecological uncertainties that are inevitable. But one or two indicators may not provide sensitivity and they are necessarily the product of partial understanding (error is possible). Multiple indicators potentially provide a more comprehensive evaluation but they require a level of research and monitoring that has historically been difficult to maintain.

DP: Again, the natural process that is not occurring at a normal rate is immigration.

TR: The pros are that it creates a semi-objective, agreed-upon standard to trigger management action. The cons are that primary indicators and multiple indicators are imprecise. They best guesses based on a collective understanding of past events. We expect the future to resemble the past. However, the future always brings novel surprises that force us to re-examine our understanding of the past. When we decide insulate an attic to an R-50 standard, we can create the same effect regardless of which house we are insulating. Not so with indicators and thresholds—they are sloppy and imprecise, and should be applied only in combination with good judgment.

TV: The pro of basing translocation of wolves on ecological indicators is that it puts the focus on ecological function and the desired outcomes for ecological feature in the park. The cons for this strategy are: 1) an underlying assumption of top-down control that may not be valid, 2) a difficulty in identifying sensitive thresholds, 3) logistical and financial costs associated with obtaining rigorous estimates of threshold quantities, and 4) the strategy ignores the human component (visitor expectations, iconic nature of wolves on Isle Royale, research value).

AW: Pros of use of primary indicators are that such indicators are more obviously detected and visible on the landscape or observable with populations, unlike genetic testing that can be evaluated only by specialized test.

Cons on using primary indicators would include that major ecological damage may occur before actions are taken.

5.1.9. How does the potential for climate change influence the suggested thresholds?

BP: Vegetation based indicators should account for projected changes in plant growth/ forest succession attributable to changing climate independent of the influence of predators. (i.e. the expected "baseline" change should be accounted for). See Frelich et al. (2012).

RP: Elsewhere I have commented on the difficulty of evaluating specific effects of anticipated climate change in a small location such as IRNP. I do not care to guess how climate change may change specific thresholds. However, the general pattern of climate change and its effect on winter climate (frequency of ice bridges) is relatively clear, and I would venture to predict that ice bridges are likely to continue to decline.

DP: This is the reason immigration is less likely now and the reason introductions should be considered.

TR: Over the 20-year planning period of the document, climate change will mostly likely manifest largely as discrete events, rather than a slow creeping trend. This can be in the form of fires, drought, kinetic events (derechos and other windstorms), heat waves, or extreme precipitation events. While transient in nature, these will create landscape legacies. Climate change can cause indicators to malfunction, giving false positive or false negative threshold readings. For this reason, I encourage IRNP to include monitoring fire danger levels, the North Atlantic Oscillation, and fire events. These could provide explanations for "wrong" threshold readings. Every year is an unusual year—a wetter than normal spring, a warmer than normal summer, a fall heat wave—but unusual years are not the same as extreme events. Extreme events will be apparent at the time.

TV: Climate change is likely to change the boreal character of the island to a more temperate deciduous one although fine scale community effects are difficult to predict (Frelich et al. 2012). This will increase primary productivity and plant biomass which in turn could reduce the degree to which wolf-ungulate systems experience top-down controls (Crete 1999). Isle Royale is at the southern edge of moose range on the North American continent. Sufficient global warming and habitat change may impair moose ability to cope with summer heat stress (Street et al. 2015) which could make them more vulnerable to a coursing predator like wolves or make Isle Royale unsuitable over the long term.

JV: Moose in northeastern Minnesota have been declining precipitously over the past decade. Some believe the decline is driven by climate warming.

Moose on Isle Royale and northern Minnesota experience essentially the same inter-annual variability in climate. In spite of the similar climates, the Isle Royale moose population has increased dramatically. These very different outcomes, under similar climates suggests that the impact of climate on moose populations (and the ecosystem function of moose populations, i.e., herbivory) may be considerably more complicated than is often appreciated.

The current state of knowledge is inadequate to anticipate – with adequate reliability or precision – when or by how much climate will impair moose population dynamics and their ecosystem function on Isle Royale (This sentiment is also expressed, more generally, in Weladji et al. (2002). That sentiment still applies today). This claim is especially true given the timeframe we were asked to focus on (i.e., the next 20 years). As with many management issues, this one is shrouded in non-trivial uncertainty.

The loss of predation from this moose-dominated ecosystem is currently a critical loss to the ecosystem's health. The impact of climate change *on moose demography and herbivory* is a potential threat to the ecosystem's health (over the next 20 years). As such, it would be best to preserve predation in an unimpaired manner until such time that one can more precisely anticipate the timing of these impacts. In the meantime, there would be value in developing indicators of moose demography and herbivory that would alert us and contingencies about how to respond.

The claims made here can be substantiated in more detail. I am also able to provide more detailed thoughts with respect to indicators and contingencies. Time prevented me from offering any more detail, here in this document, than I did (See footnote 1 on page 1 [MG: see the SME's questionnaire]). If more detail is useful, let me know and I can review the relevant science in a more formal manner.

AW: Climate change may alter value of thresholds. Warming conditions may stress species such as balsam fir, aspen and birch, beyond stress of browsing by moose or cutting by beaver. The advantage and replacement of fir by white spruce would likely change with major warming that would also reduce the spruce. It is possible if the climate becomes too warm, moose would eventually die off on the island regardless of any mitigations. With warming climate it becomes more critical just to maintain a healthy wolf-moose predator-prey community as long as possible, and will probably require more reliance on genetic thresholds.

Additional input provided

JV: An appropriate response to Item 5 requires understanding the meaning of Alternative D. In this regard, the NPS does not explain the meaning of the phrase "allow natural processes to continue." If that phrase means "allow non-anthropogenic process to continue" then Alternative D may well contain an internal inconsistency. That is, the wolf population on Isle Royale and the function of that population (predation) have been and continue to be impaired by anthropogenic climate change. Therefore "not tak[ing] immediate action" would be inconsistent with the premise of Alternative D, i.e., "allowing natural processes to continue." This odd internal inconsistency reflects the great challenge of managing for "naturalness" in the face of anthropogenic climate change. An essential element of meeting that challenge is to specify and justify more precisely the purpose of management.

6. **Alternative A (No action alternative) The NPS would not intervene and would continue current management. Wolves may come and go through natural migration, although the current population of wolves may die out.**

Please address these questions with respect to changes to the broader ecosystem as a result of not taking action.

6.1. The life of this EIS is intended to cover about a 20 year period, what changes to habitat and the ecosystem might occur as a result of our decision under this alternative (IRNP without a top predator)?

BP: As per Frelich et al. 2012, and Peterson et al. 2014, on western IRNP balsam fir may soon become functionally absent if moose herbivory is not reduced.

RP: It is likely that aquatic systems, especially interior beaver impoundments, will be degraded by moose foraging and trampling of shoreline areas. This has already begun in ponds dominated by the native floating aquatic plant, watershield (*Brassenia schreberi*). Further, it is possible that the "last chance" cohort of regenerating balsam fir on the western half of Isle Royale will be browsed sufficiently to reverse height growth that began when stems were released from moose herbivory in the 2000s (because of low moose density caused by high wolf predation pressure, Peterson et al. 2014 and Peterson and Vucetich 2016). As in

Yellowstone following the 1988 fires (Turner et al 1997), should there be any fires that burn on Isle Royale during this period, high moose herbivory would likely eliminate regeneration of deciduous shrub and tree species that are important for foraging moose, thus accelerating the conversion of the forest community to a simplified and species-depauperate ecosystem.

DP: Under this alternative, the moose population would probably increase, crash, and start to increase again during the 20 year period under this Alternative. Changes to the vegetation along the lines of what has happened in past build-ups of moose will occur; some of those species would be released following a moose population crash. The species that I am familiar with on Isle Royale (personal observation) that moose prefer include mountain ash and willow, which are quite resilient, and yew, which is not.

TR: We might expect a decrease in the recruitment of tree seedlings and an increase in the abundance of moose browse resistant or tolerant vegetation, including white spruce, grasses and sedges. Over time, this could increase fire risk. We might also expect a breakdown of forest gap dynamics, with savanna or other tree-free vegetation types establishing in forest canopy gaps.

TV: The most direct ecological effects of wolf extirpation and a decision not to re-introduce wolves on Isle Royale likely would be an increase in the number of moose and possibly an eruption (steady increase in numbers followed by a density dependent decline; Caughley 1970). With increasing moose numbers, one would expect indirect impacts on the foods that moose eat – with the most severe impacts on understory woody browse plants (balsam fir, Canada yew, eastern hemlock, (possibly) northern white cedar) that grow slowly.

RW: Work by Rolf Peterson and colleagues have shown that 20 years is sufficient for vegetation to changes in the presence of wolves.

AW: Without a wolf population impacting moose and beaver populations, both populations are likely to rise to high levels, and possibly crash after exhausting forage or possibly crash because of disease or major parasite. Impact of moose and beaver are likely to leave island ecosystems highly impoverished. Future growth of both populations are likely to be greatly reduced. Although neither species is not likely to totally disappear during this time frame, over a longer period, drastic fluctuations in the moose population, along with warming climate may cause moose to eventually to become extinct on the island. Maintaining moose in healthy predator-prey relationship with wolves would likely dampen drastic population fluctuations and improve likely persistence for moose on IRNP.

With expanding moose population balsam fir is likely to decline and likely to be drastically reduce. Probably little if any reproduction will occur, with seeding and saplings mostly disappearing. As older trees die off or get blown down there will be few young trees to replace them. Other trees such as aspen, birch, mountain ash, and various deciduous shrubs will likely have reduced regeneration, low vigor and show gradual decline. White spruce in savanna like settings with exotic bluegrass (*Poa* spp.) understories will likely expand, but warming climate may also reduce eventually reduce spruce abundance.

With expanding beaver population will result in a maximum extent of wetlands across the island. Such beaver wetland expansion may benefit some species, but would be detrimental to portions of the forest ecosystem. Tree species such as aspen and birch are likely to decline near beaver ponds, and with lack of wolf predation, beaver will likely travel further from ponds to cut down trees.

6.2. What other factors associated with climate change might alter the environment regardless of wolf being present?

BP: Not my area of expertise.

RP: Lee Frelich (U MN forest ecologist), points out that Isle Royale sits on the line (from global climate models) where precipitation might either increase or decrease over the next 50 years. According to Thomas Dietz (MI State Univ climate policy analyst), the current climate change models are not of sufficiently small scale to properly account for the effect of the Great Lakes on regional climate; therefore, one must be circumspect in interpreting results of these computer-based simulations of future climate for local areas at the scale of Isle Royale, located in the middle of the largest of the Great Lakes. Further, Wallace Broecker (oceanographer at Lamont-Doherty Observatory, Columbia Univ.) believes that future climate changes are likely to be surprising, possible arising from shifts in ocean currents which are poorly understood (Broecker 1991). Therefore, it seems inadvisable to plan today's management actions on the basis of uncertain expectations for tomorrow.

DP: I suspect that climate change models specifically for IRNP (or nearby areas) have been developed but I am not familiar with them.

TR: Fire frequency could increase. Drought could become more frequent, making vegetation more susceptible to insect outbreaks and/or pathogen outbreaks.

TV: With climate change, the vegetation of Isle Royale will begin losing its boreal character in favor of a temperate deciduous forest vegetation. Individual plant populations will respond differently subject to their tolerance for the changes driven by a changing climate (Frelich et al. 2012).

RW: Climate warming, infectious disease and other stressors may affect tree growth, but it would seem most of the conceivable stressors are negative, hence herbivory would likely enhance the effect of stressors, and would be even more important to control.

AW: Increased temperatures, lower rainfall, and lower snow amounts are likely to cause decline or disappearance of certain plants. Conifers such as balsam fir and white spruce would especially be at risk. Other species such as paper birch and aspen may also decline. Warming weather may also change abundance of insects and other arthropods, potentially leading to greater abundance of winter ticks that could become detrimental to moose.

6.3. What monitoring should be conducted, with what goals, and how should these monitoring protocols for wolves and the broader animal and plant community be undertaken?

BP: Monitoring of changes in forest structure with emphasis on shifts in forest communities and lack of succession of any members of the current guild of plants/ trees making up the forest communities.

RP: Given the management alternative described in 6, there would be no wolves present (otherwise another of the alternatives provided would take over). It would then seem that NPS would have to demonstrate that the management direction the agency selected did not violate the spirit or letter of the Organic Act of 1916, the Wilderness Act of 1964, the "Thomas Act" of 1999 (mandating an appropriate science program for NPS), as well as Director's Order 41 and other internal policies. Specific suggestions are provided in my answer in Section 1.1.1. An explicit decision that reduces the future presence of an apex predator would significantly increase the necessity of monitoring ecosystem components such as in Section 1.1.1.

DP: Nothing different than what has been discussed above.

TR: The goal should be to monitor forest recruitment dynamics, and ascribe potential drivers of those dynamics. Key monitoring activities should include: (a) annual recruitment of tree seedlings, mainly balsam fir, sugar maple, and trembling aspen into size classes (< 10 cm tall; 10-29 cm tall; 30-99 cm tall, and > 100 cm tall). This should include a few plots in which individual seedlings are followed through time to get estimates of growth rates and size class recruitment, and several plots with spot counts of seedlings in each size class. Recruitment can be measured annually or estimated based on surveying 20% of plots each year. (b) Percent moose browsing on each of size classes, and how this is changing through time. (c) The North Atlantic Oscillation and snow depth, and how these relate to growth rates and annual recruitment of seedlings. (d) Number of days per year with fire danger levels rated as high, very high, and extreme. (e) Number and extent of fires per year.

TV: As outlined above, priority should be given to monitoring moose growth rate and browsing impacts. Monitoring of understory browse species (balsam fir, Canada yew, eastern hemlock) should receive priority as well. That said, it is likely that impacts of moose herbivory on other plant and animal communities because of browse-mediated changes in litter accumulation, shading, and soil chemistry (McInnes et al. 1992, Pastor et al. 1993, Bump et al. 2009a,b). This should be a research priority so that effective targeted monitoring programs can be designed.

RW: As above, monitoring abundance, recruitment and the rate and extent of herbivory island wide.

AW: Monitoring should continue to focus on any remaining wolves and annual search for migrant wolves should be done while conducting moose counts in winter counts each winter. Beaver counts should be done on a minimum of every other year. Vegetation surveys should be done to determine abundance, vigor, and reproduction of balsam fir, aspen, and paper birch. Monitoring for wolves should also be done opportunistically to search for wolf scats. If a known wolf population occurs on the island, specific surveys should be done to obtain scats on all individuals for genotyping to determine number of individuals and genetic conditions of the wolf population.

The goal of monitoring should be determining number of wolves, moose, and beaver while assessing demographic characteristics and determining the impact they have on each other. Vegetation surveys should have a goal of determining abundance and condition of major browse species, and examining impact of moose, beaver and wolves have on vegetation of Isle Royale.

6.4. Describe ecological processes important to monitor to assess changes in the system.

BP: Monitoring of changes in forest structure with emphasis on shifts in forest communities and lack of succession of any members of the current guild of plants/ trees making up the forest communities. There is enough evidence for bottom up regulation (in concert with top down control) that healthy status and functioning of lower trophic levels over the long term may be considered indicative of potential health of ecological function at upper levels.

RP: In general, the important processes are predation, competition, nutrient and energy flows, and interspecific trophic and other interactions that determine species distribution and abundance.

DP: Depending on what climate scientists have predicted under 6.2., I would monitor those processes that they feel will change or have the potential to change.

TR: I think seedling recruitment is the most important process to monitor, as it will be sensitive to changes in moose numbers, climate, pathogens, and disturbances.

TV: This is a difficult question to answer because large scale ecological processes (e.g. nutrient cycling) are difficult to monitor and are indirectly related to the presence of the wolf. Smaller-scale ecological processes like the regeneration of select browse-sensitive plants is probably the priority here although it probably requires some directed research first (see answer to 6.3).

RW: Disease, herbivory and changes in climate.

AW: Important processes to monitor would include predation of wolves on beaver and moose; herbivory of moose on balsam fir and other browse species; beaver herbivory on aspen and birch and other trees; and nutrient cycling under different moose abundance and with changing vegetation and climate.

6.5. Describe what components of the IRNP ecosystem are specifically important to preserve (we ask since there are other ways to protect and manage park resources other than using wolf).

BP: All trophic levels should be healthy and ecological processes and functioning within and among trophic levels should be occurring at natural and expected levels (i.e. maintenance of ecological integrity).

RP: Firstly, I suggest that the health and vigor of the moose population would emerge as a significant ecological and public value that would have to be assured, even if the species is not listed as Threatened after species review by the U.S. Fish and Wildlife Service (a process begun in 2016). Of course, if the moose is listed as a Threatened Species, then the NPS has additional legal requirements to review its actions for any negative effects on moose. Health and vigor of the moose population would be indicated by many of the measures outlined in Section 1.1.1. It is abundantly clear that management of a moose population by human knowledge, skills, and tools does not and cannot substitute for the subtle nuance that wolf predation brings to the task of predation (chapter three in Darwin 1859, Peterson 1977). Secondly, the integrity of small interior watersheds and ponds (indicated by plant and animal species present, water flow, water volume, erosion potential, and water chemistry would also be specifically important because the prey animals likely to be hyperabundant in the absence of an apex carnivore – moose and beaver - are partially or completely aquatic; recall the decades-long expression of concern about effects of moose on aquatic plants, voiced first by Murie (1934), then fish biologist Walter Koelz, ecologist Stanley Cain, and ecologist Durward Allen (summarized by Allen 1979, pages 194-195).

DP: Maintaining ecological processes within some “natural” range of variability should be the goal of national parks. The natural range of variability on Isle Royale may be different than on the mainland due to its relatively isolated location. That is why I am less concerned with a short term absence of the apex predator – which would happen with or without climate change - than I am with the ability of a population to be rescued through immigration or recolonize after it disappears.

TR: Maintaining forest dynamics is important if IRNP is to remain a forested park. This might be accomplished through the establishment of exclosures, monitoring of existing exclosures, or the use of moose culling activities.

TV: I think the components of the IRNP that are most important to preserve are those most impacted by the absence of wolves. These are likely to be some sort of regulation of the moose population below and absolute food-regulated carrying capacity and the resilience and capacity for regeneration in the plant populations that moose depend on. Management plans should indicate a maximum density goal for moose and a desired condition for browse-sensitive plants in terms of their spatial extent on the island, their capacity for regeneration, and for long-lived species, a diversity of age- or developmental stage classes.

JV: Isle Royale is characterized by a top predator that is un-persecuted by humans, an un-hunted ungulate population, and a forest that is no longer logged. That circumstance – a food chain of large mammals that is entirely unexploited by humans is remarkably rare. Even in Yellowstone wolves have been killed often enough by humans to affect their behavior and often enough to be a non-trivial force on their population dynamics. The rarity of such a natural wonder and basic ecological phenomenon is the component of the ISRO ecosystem that is specifically the most important to preserve. The most important component to preserve, in this particular case, is predation itself.

RW: Community structure and diversity, the full web of interactions among trophic levels as means to enhance stability, persistence and resilience.

AW: Maintaining a healthy ecotonal temperate-boreal forest that remains unaltered by human activity is an important component of IRNP ecosystem, and maintaining the natural ecological processes within that system will be important to protect. Maintaining the key large herbivore of this system, the moose at healthy population levels, will be an important component to conserve.

6.6. Are there aspects of the ecosystem that will be better served by allowing ecological processes to continue unimpeded by any intervention?

BP: No, I think the non-intervention decision would be based more on the philosophy of non-intervention, and done so despite the negative and demonstrated impairment to normal, intact ecological function (ecological integrity).

RP: None of which I am aware.

DP: Our knowledge of the role of apex predators could be enhanced by having time periods with and without wolves.

TR: Beaver populations and beaver-associated habitats might increase, at least to the extent beavers serve as an alternate prey source for wolves. This could lead to declines or slower increases in aspen populations.

TV: I cannot think of any

RW: It may be argued that humans are intervening even without any management actions. Humans have affected the climate and weather, the likelihood of ice-bridge formation, the abundance of plant and animal disease and its transmission through visitation, the genetic composition of source individuals (coyotes are only recent migrants to the Great Lakes area). So doing nothing is not natural, it's merely letting other anthropogenic events affect the system. We should use science and logic to accomplish goals of stability and resilience in maintaining biodiversity and try to restore the ecosystem given human-induced effects beyond our control.

AW: Anytime interventions are used in natural areas there are potentials for unintended consequences. The components of those consequences can't always be predicted but conservation planning that use major intervention should be prepared to deal with consequences from such management actions. Species such as white spruce are likely to expand and increase abundance across the island. Unpalatable plants would likely increase without interventions. Without wolf predation, beaver will likely be able to exert maximum wetland coverage across the IRNP, and would benefit species adapted to these type of wetlands.

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