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Harvest and group effects on pup survival in a cooperative breeder

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Recruitment in cooperative breeders can be negatively affected by changes in group size and composition. The majority of cooperative breeding studies have not evaluated human harvest; therefore, the effects of recurring annual harvest and group characteristics on survival of young are poorly understood. We evaluated how harvest and groups affect pup survival using genetic sampling and pedigrees for grey wolves in North America. We hypothesized that harvest reduces pup survival because of (i) reduced group size, (ii) increased breeder turnover and/or (iii) reduced number of female helpers. Alternatively, harvest may increase pup survival possibly due to increased *per capita* food availability or it could be compensatory with other forms of mortality. Harvest appeared to be additive because it reduced both pup survival and group size. In addition to harvest, turnover of breeding males and the presence of older, non-breeding males also reduced pup survival. Large groups and breeder stability increased pup survival when there was harvest, however. Inferences about the effect of harvest on recruitment require knowledge of harvest rate of young as well as the indirect effects associated with changes in group size and composition, as we show. The number of young harvested is a poor measure of the effect of harvest on recruitment in cooperative breeders.

1. Background

Group living has evolved across a wide range of taxa and species. Many species that live in groups display cooperative breeding behaviour. Cooperative breeding generally refers to the shared care of related, or even unrelated, young by helpers (i.e. non-breeding individuals) within a group [1]. In mammals, both manipulative and observational studies have shown that the presence of helpers can be critical to breeder fitness and group persistence [1–5].

The number of helpers in a group can positively influence recruitment (i.e. number of young reared to maturity [1,6,7]), but group composition (i.e. number of different age and sex classes) may also have an important influence on recruitment and population growth in cooperative breeders. For example, selective removal of male African lions (*Panthera leo*, Linnaeus) resulted in lower recruitment through increased infanticide [8]. Breeder turnover (i.e. death or expulsion of a breeder) can lead to reduced recruitment and survival of the group [9]. Further, changes to group composition that lead to reduced genetic relatedness within groups can lead to reductions in helping behaviour and ultimately recruitment [1,10,11]. Group composition may also be important because not all age and sex classes help equally within a group. Individuals in groups of grey wolves (*Canis lupus*, Linnaeus), for example, vary widely in the amount of pup-guarding behaviour they display [12,13]. Given the importance of pup-guarding to recruitment in African wild dogs [4] (*Lycaon pictus*, Temminck 1820), groups that have diverse sex and age classes may also have experienced adult helpers that contribute more to rearing young than young helpers [14] and ultimately increase fitness of breeders. Many social canids are territorial and individuals living in large groups are often more successful during intraspecific confrontations than those in small groups [15,16]. Whereas group size can increase because of inclusive fitness (i.e. helpers have increased fitness by rearing related young), an advantage of increased group size would also support predictions from group augmentation theory [17], where individuals, particularly those that are philopatric, assist in rearing young because increased group size is beneficial to future fitness. Additionally, when there is sex-biased dispersal, group augmentation theory would predict that the philopatric sex (i.e. females relatively more philopatric in grey wolves) may help more because they can inherit a breeding position in their natal pack [18]. Group demographics in social canids may also be explained by food availability and dynamics related to density dependence. For example, increased food availability after high-mortality events (i.e. control) can lead to increases in recruitment at the population level in some canids [19] (e.g. coyotes, *Canis latrans*, Say 1823). We used grey wolves as a focal species to study the relationships between harvest (i.e. regulated human-induced mortality), group size and composition, and pup survival in cooperative breeders because of their complex social structures [20], territorial defence that relies in part on large group size [16] and exposure to persistent (i.e. annual) harvest.

Wolf groups not exposed to high rates of mortality typically comprise a breeding pair and two to three generations of offspring that remain in their natal group and help care for subsequent offspring [20]. In the Rocky Mountains of the USA, wolves generally do not disperse from their natal group until 3 years of age even though they are reproductively mature at 22 months [21]. If selection has favoured breeding wolves to retain offspring within their group then pup survival may be negatively affected by events that reduce group size. The effects of harvest on recruitment can compound. For example, the number of adults in a group has been shown to positively influence pup survival in a protected population of wolves, whereas harvest, particularly trapping, has been shown to disproportionately target pups, although recent data from Idaho, USA, indicate that this may not apply to the populations we studied [5,22,23]. Groups of grey wolves in Idaho, USA, had significantly lower pup recruitment after public harvest was initiated, but the number of pups harvested could not entirely account for the decline in recruitment (18–38% due to direct harvest mortality), suggesting both direct and indirect effects of harvest [22]. Indirect effects of harvest (i.e. reduced group size, breeder turnover) may explain the observed changes in recruitment that go beyond the number of pups harvested. Generally, studies of cooperative breeding have not evaluated human harvest; therefore, the effects of recurrent, annual harvest on group characteristics and recruitment in cooperative breeders are poorly understood.

Our study used, in part, a natural experiment to provide insights about the indirect effects of harvest and groups on pup survival in a cooperatively breeding social carnivore. Ausband *et al.* [22] suggested there were indirect effects of harvest on wolf pup recruitment, and we use data from their study areas to attempt to identify those indirect effects. Furthermore, we evaluated whether harvest appeared to be an additive source of mortality by comparing pup survival rates in harvested and unharvested wolf populations, and by exploiting a natural experiment (i.e. manipulation via human harvest) measuring pup survival and group size before and after harvest. Initially, one population we sampled was unharvested and we assumed pup survival would be similar to a second unharvested population. After harvest began, however, we predicted pup survival would be similar to a third harvested population of wolves. Wolves can compensate for harvest mortality in multiple ways: increased immigration into the group, changes to other vital rates and increased litter sizes. Declines in both recruitment and group size, however, would suggest that compensation mechanisms are not keeping pace with harvest. We used non-invasive genetic sampling and 18 microsatellite loci to identify individuals, construct group pedigrees and estimate the probability of survival for grey wolves under three different management regimes ranging from heavily harvested to fully protected. We hypothesized that harvest reduces pup survival because of (i) reduced group size, (ii) increased breeder turnover and/or (iii) reduced number of female helpers. Alternatively, harvest may increase pup survival possibly due to increased *per capita* food availability or it could be compensatory with other forms of mortality.

2. Study areas

We conducted our study in Idaho, southwest Alberta, Canada, and Yellowstone National Park, Wyoming. The three study areas represented a wide range of human-caused mortality from heavily harvested and agency-controlled (i.e. wolves killed for livestock depredation; southwest Alberta and central Idaho) to fully protected (Yellowstone National Park). Public harvest in Idaho and Alberta generally began in autumn and continued through the following winter (approx. September–March [23]; electronic supplementary material, S1). Harvest in Idaho is largely opportunistic, pups are not more vulnerable to harvest than adults and adult males are harvested more than females during rifle season [23].

From 2008 to 2014, we genetically censused 8–10 wolf groups annually in Game Management Units (GMUs) 28 (Salmon Zone), 33, 34 and 35 (Sawtooth Zone) in central Idaho. Public harvest of wolves began in Idaho in 2009, temporarily ceased in 2010 and began again in 2011 [23]. Annual population harvest rates in our Idaho study areas average 24% [22]. Control actions to address livestock depredations are rare in our study groups in Idaho and accounted for less than six animals killed by agency personnel over the course of our study.

During summers 2012–2014, we also sampled wolves in five to six groups in Yellowstone National Park. Wolves exist at relatively high densities and there is no human hunting inside Yellowstone National Park. Although some wolves are harvested when travelling outside Yellowstone National Park, the number is typically small (less than five individuals annually).

Lastly, during summers 2012–2014, we also sampled wolves in two groups in southwest Alberta. Southwest Alberta is a highly diverse landscape where mountainous forests meet the dry short-grass prairie region. Wolf densities are thought to be maintained at low levels in southwest Alberta. There was a wolf bounty, livestock depredation control actions and harvest; thus, we posited that overall mortality was higher in southwest Alberta than in the Idaho study areas. Population estimates and harvest rates were not available for Alberta; thus, we assessed the influence of harvest as present or absent (i.e. binary).

3. Methods

(a) Field methods

We collected scats for genetic analysis at rendezvous sites (i.e. locations where pack members congregate for several weeks) used by wolf packs. When available, we used radiotelemetry locations of wolves to locate rendezvous sites. In areas that did not contain radiocollared wolves, we surveyed at historical rendezvous sites and sites predicted by a habitat model [24] to be highly probable (greater than or equal to 70% suitability). We sampled all rendezvous sites once each at dawn or dusk [25]. After howling to determine the presence of wolves, two technicians would separate and survey the site for 30–45 min looking for wolf signs. At occupied or recently occupied sites, we located the activity centre and collected pup and adult scat samples for 3–4 h, radiating out from the activity centre on trails to ensure we collected scats from all available adults in the pack [24,26]. We considered scats less than 2.5 cm diameter to be pup scats (at three months of age) [24,26] and those greater than 2.5 cm to be adult wolf scats [27]. Previous studies estimating pup counts using genotypes based on this distinction in size were found to be accurate [26,28]. Our approach generated 125–200 samples per pack per year, thus potentially providing genotypes for each animal in the pack [29]. We attempted to locate and resample each group every year. If a pack could not be thoroughly sampled (fewer than 65 scats collected), they were excluded from analyses in an effort not to bias pup survival low. We analysed 40 adult and 25 pup scats from each pack based in part on rarefaction results regarding sampling effort [29].

(b) Laboratory methods

DNA analyses were performed at the University of Idaho's Laboratory for Ecological, Evolutionary and Conservation Genetics (Moscow, ID, USA). We extracted DNA using Qiagen stool kits (Qiagen, Valencia, CA, USA) in a room dedicated to low-quantity DNA samples and using negative controls to monitor for contamination. We used nine nuclear microsatellite loci and sex identification primers to identify individuals and gender [28]. We generated an additional nine microsatellite loci on the best sample for each unique individual (i.e. total = 18 loci) and for samples that differed at only one locus out of initial nine loci to verify matches or mismatches [28,29]. We used an Applied Biosystems 3130xl capillary machine (Applied Biosystems, Foster City, CA, USA) to separate PCR products by size and verified peaks individually by eye with GeneMapper v. 3.7 (Applied Biosystems). We used Genalex v. 6.5 [30] to match genotypes from scat samples and we required greater than eight loci to confirm detections of the same individual. We initially amplified all samples twice and required successful amplification of alleles at five or more loci for the sample to continue for an additional one to three replications. We discarded samples that amplified at less than five loci. For each locus, we required at least two independent PCR amplifications for consensus of a heterozygote and at least three independent PCR amplifications for consensus of a homozygote. We included a negative control in all PCRs to test for contamination. We cross-checked all genotypes in Structure v. 2.3.3 [31] with reference samples of known wolves ($n = 66$), domestic dogs ($n = 17$) and coyotes ($n = 40$) at $K = 3$ groups under the general admixture model, with a burn-in of 100 000, and 500 000 additional Markov chain Monte Carlo repetitions and 10 iterations to estimate individual ancestry and remove samples highly probable as dogs or coyotes from the dataset. We used Reliotype [32] to test the accuracy of unique genotypes detected in only one sample (i.e. single captures) by ensuring that the genotype attained a 95% accuracy threshold. In 2008 and 2009, we analysed all collected samples. After 2010, we analysed 40 adult and 25 pup scats from each pack. We analysed additional samples to obtain 10 more consensus genotypes if a pack had more than two individuals detected only once and additional collected samples were available.

(c) Analysis methods

For each year and study area, we included all sampled adult males and females as potential parents and all sampled pups as potential offspring and then determined breeders and their offspring by constructing pedigrees using maximum likelihood in Colony v. 2.0.5.5 [33]. In addition to adults we sampled at rendezvous sites, we also included genotypes of any radiocollared animals present in the study areas. We calculated allele frequencies for each study area and year in Coancestry v. 1.0.1.5 [34] and then imported those into Colony for use in pedigree analyses. We allowed for polygamy in both males and females and assumed an allelic dropout rate of 0.01. In cases where parentage was undetermined from Colony, we further examined offspring genotypes against the likely parents of the remaining offspring in the group and allowed for a two-allele mismatch owing to allelic dropout between parent and offspring to verify parentage across the 18 loci.

We sampled the same groups of wolves across consecutive years, and from the resulting pedigrees we estimated the number of individuals in each age and sex class (breeding females, breeding males, 1-year-old non-breeding females, greater than or equal to 2-year-old non-breeding females, 1-year-old non-breeding males, greater than or equal to 2-year-old non-breeding males, unknown age females, unknown age males, female pups, male pups) and recruitment (pup alive at 15 months). We assumed similar rates of other sources of mortality (i.e. road kills) over the years of our study and that changes to group composition were the result of harvest. We obtained such detailed group compositions before and after harvest in Idaho as well as in Alberta (harvest) and Yellowstone National Park (no harvest). We documented breeder turnover from pedigree analyses. Causes of breeder turnover were not always known and could include loss of a breeding position through death, expulsion from the group or having a breeding position behaviourally usurped. We estimated the number of adults present in the group when pups were 3 and 15 months old. Because we sampled only reproductively active groups, breeder replacement is inherent in our data. Ages of pups (i.e. 3 months, 15 months) were approximate assuming a birthdate of 15 April and an average sampling date of 15 July (i.e. age = 3 months; range: 20 June–27 August) with an actual average of 362 days until subsequent sampling when pups would have been approximately 15 months of age [22]. We defined adults as any non-pup individual (i.e. helpers; non-breeding females and males, and breeders). Non-breeding adults were further split into groups of 1 year old (actual age between 1 and 2 years), greater than or equal to 2 years old, and 'likely older' individuals, which included animals initially detected as adults and subsequently detected for more than 1 year (i.e. 3+ years). Only helpers greater than or equal to 2 years old were counted at $t = 15$ months because 1 year olds at $t = 15$ months are the response variable: pups still alive. We treated each sampled pup as a case, considered whether they were alive or dead at 15 months a binary response, and defined pup survival as the probability of being alive at 15 months of age (electronic supplementary material).

(d) Differences among study areas

We first wanted to test for differences in litter and group sizes among the study areas as well as differences in the probability of pup survival before and after harvest was initiated. We used count data and a generalized linear model (model form: litter size at three months~(as a function of) study area) with a Poisson distribution to first assess whether litter sizes at three months were different among the three study areas. We used a *t*-test to assess differences in group size (no. of adults) when pups were three months of age before and after harvest in Idaho.

We used logistic regression with pup fates ($0 = \text{dead}$, $1 = \text{alive}$) as the response variable, and before and after harvest as independent variables to look for potential differences in pup survival in Idaho. To test our assumption that pup survival was similar among harvested and protected areas, we used probabilities of pup survival estimated from logistic regression to test whether pup survival before harvest in Idaho was similar to pup survival in the protected Yellowstone National Park and whether pup survival after harvest in Idaho was comparable with levels in the harvested population in southwest Alberta.

(e) Pup survival with and without harvest

We used mixed effects logistic regression to assess the simultaneous influence of harvest (i.e. binary; presence/absence in the calendar year pups were born) and group composition on pup survival. We assumed that other sources of mortality (e.g. road kills) were similar among years during our study and that changes observed in group compositions were largely due to the introduced annual harvest. In addition to covariates of harvest and number of individuals in each sex and age class, we also included a study area covariate:

$$\begin{aligned} y = \beta_0 &+ \beta(\text{harvest}) + \beta(\text{no. of 1-year-old non-breeding females when pups 3 months}) \\ &+ \beta(\text{no. of 1-year-old non-breeding males when pups 3 months}) \\ &+ \beta(\text{no. of likely} \geq \text{2-year-old non-breeding females when pups 3 months}) \\ &+ \beta(\text{no. of likely} \geq \text{2-year-old non-breeding males when pups 3 months})\ ) \\ &+ \beta(\text{no. of breeders when pups 3 months}) \\ &+ \beta(\text{no. of} \geq \text{2-year-old non-breeding females when pups 15 months}) \\ &+ \beta(\text{no. of} \geq \text{2-year-old non-breeding males when pups 15 months}) \\ &+ \beta(\text{no. of breeders when pups 15 months}) + \beta(\text{study area}) + \text{random effect(pack)}. \end{aligned} \tag{2.1}$$

We included a random effect for pack to account for potential non-independence of pup survival among packs. In 2008, the ages of five non-breeding adults were unknown and they were included in the non-breeders greater than or equal to 2 years old category for analyses. Data are provided in the electronic supplementary material.

To test our hypotheses about the indirect effects of harvest on pup survival, we used multiple logistic regression to assess whether group size (number of adults at $t = 3$ months and $t = 15$ months) or breeding male and female turnover had a larger influence on pup survival only in years when wolves were harvested. Prior to modelling, we tested for collinearity between number of adults at 3 and 15 months and breeding male and female turnover using a correlation of fixed effects matrix from glmer in R (v. 3.2.2).

(f) Effects of group composition on pup survival in years with harvest

Lastly, using just years with wolf harvest in Idaho, we used multiple logistic regression to assess the influence of each adult sex and age class (equation (2.1)) on pup survival. The majority of such survival data were from Idaho; thus, we did not include two wolf groups from Alberta in an attempt to construct a more parsimonious model. We used the Akaike information criterion and model weights [35] (w_i) to evaluate the relative support for competing models given the data. We used R (v. 3.2.2) for statistical analyses.

4. Results

(a) Differences among study areas

The probability of identity for siblings (i.e. chance that two individuals would have the same genotype) ranged from 3.54×10^{-4} to 1.18×10^{-3}. We detected 279 adults and 193 pups through genotyping in 10 groups in Idaho during 2008–2014. We detected 31 adults and 35 pups in 2 groups in Alberta, and 85 adults and 47 pups in 4 groups in Yellowstone National Park during 2012–2014. Litter sizes at three months of age were 5.0 (s.e. = 0.47), 5.8 (s.e. = 0.95) and 4.1 (s.e. = 0.40) for Idaho, Alberta and Yellowstone National Park, respectively (figure 1). The assumptions of a Poisson distribution using count data of litter size were generally met ($\bar{x} = 4.6$, s.d. = 2.8) and litter size was not significantly different (range of $p = 0.22$–0.50) among the three areas. The mean group size declined after harvest began in Idaho from 10.45 (s.d. = 3.1) to 6.97 (s.d. = 3.3) adults per group ($t = 6.39$, $p < 0.0001$). The number of adults at 3 and 15 months were not strongly correlated ($r = -0.19$), neither were breeding male and breeding female turnover ($r = -0.28$); thus, all variables were retained in models. We found no evidence that the probability of a pup surviving differed between Yellowstone National Park and Idaho before harvest (0.62 versus 0.67; $p = 0.66$). We found evidence pup survival rates differed after harvest began in Idaho, however (0.67 before versus 0.37 after; $p = 0.003$). The probability a pup survived in Alberta (0.13) was lower than in Idaho (0.37) even after harvest began ($p = 0.04$). Overall, we found evidence that the number of pups recruited (survived to 15 months) per group declined in Idaho in years when wolves were harvested (3.69 versus 1.65 pups per group; $p < 0.0001$).

(b) Pup survival with and without harvest

Across all study areas and years, harvest was associated with greater than six times decrease in the probability of pups surviving to 15 months of age (odds ratio (OR) = 0.14; 0.06–0.33, 95% CI; figure 2). The addition of each breeder in a group (which may or may not have been the original parents) was associated with a doubling of the probability of pups reaching 15 months of age (OR = 2.19; 1.32–3.63, 95% CI; figure 2). The number of non-breeding males greater than or equal to 2 years old when pups reached 15 months of age was associated with a negative effect on the probability of survival (figure 2). The presence of older (greater than 2 years)

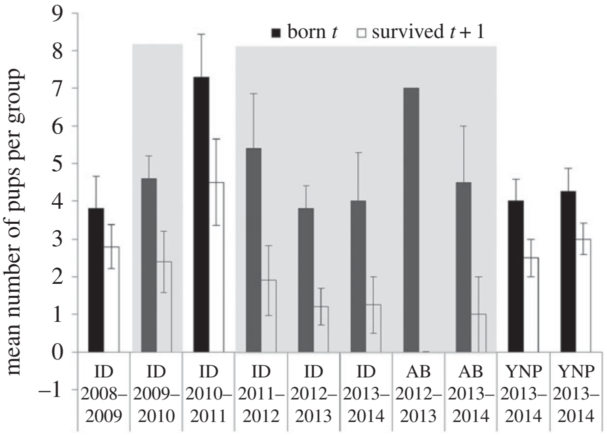

Figure 1. Mean number of pups born and survived to 15 months old in Alberta (AB, 2012–2014), Idaho (ID, 2008–2014) and Yellowstone National Park (YNP, 2012–2014). Error bars represent the s.e., grey shading indicates years with harvest.

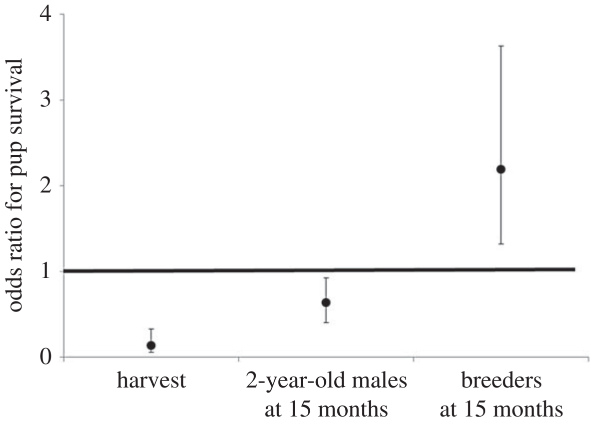

Figure 2. Odds ratios for influential variables from model of wolf group sex and age classes predicting the probability of pup survival (i.e. 3–15 months) in Alberta (2012–2014), Idaho (2008–2014) and Yellowstone National Park (2012–2014). Variables less than 1.0 had a negative effect on pup survival, whereas those greater than 1.0 had a positive effect.

non-breeding females was not correlated with increased pup survival. Area did not have a significant influence on pup survival (Idaho $\beta = 0.69$, s.e. $= 0.76$; Yellowstone $\beta = 0.35$, s.e. $= 0.93$; Alberta = reference category) and the inclusion of pack as a random effect was not influential ($p = 0.34$).

(c) Effects of group composition on pup survival in years with harvest

Competing models of group size and breeder turnover explaining the indirect effects of harvest on wolf pup survival in Idaho were indistinguishable (table 1). During years with harvest, the average effect of one additional adult when pups were three months of age was associated with a 1.14 times (figure 3) increase in the probability of pups reaching 15 months of age, although the 95% CI did overlap 1.0 (0.97–1.36). Turnover of breeding males, however, was associated with more than three times (0.09–0.87; OR 95% CI; figure 3) decrease in the probability of survival during years with harvest.

When assessing the effects on pup survival from each sex and age class during years with harvest, we found that each additional greater than or equal to 2-year-old non-breeding male present when pups reached 15 months of age was associated with a nearly three times (0.13–0.84; OR 95% CI; figure 4) decrease in the probability of pup survival. An increase of 1 unit in the number of breeders present at 15 months, however, was associated with a nearly four times increase (1.45–10.9; OR 95% CI; figure 4) in the probability of survival during years with harvest.

5. Discussion

Increased group size when pups were young (three months) was associated with increased pup survival. When pups are relatively young (i.e. less than six months), non-breeding individuals can help through provisioning and guarding behaviours [12,36], both of which may be particularly important early in pup development. The positive effect of group size on recruitment has been found for groups of wolves in unharvested populations [5] as well other canids with similar life-history strategies (e.g. African wild dogs [3]; black-backed jackals, *Canis mesomelas*, Schreber [37]). Harvest generally occurred when pups were 6–15 months of age. Group size at the beginning of pups' first year of life (i.e. three months) may have influenced pup survival via a dilution effect (i.e. more individuals in a group dilute the potential for harvest mortality of any one individual).

The number of breeders present when pups reached 15 months of age was a strong predictor of pup survival across all study areas and years. This may be because in some cases, non-breeding helpers changed status during the year and became breeders as the pups neared 15 months of age. Mortality can create breeding vacancies where helpers may contribute more to rearing young if they can acquire a breeding position in the group during the pups' first year of life. Such individuals would be expected to help more and thus potentially increase recruitment as predicted from group augmentation theory [17]. Breeder turnover has been found to reduce survival of young in cooperative breeders [8,9,11], and we found that turnover of breeding males in particular had negative effects on pup survival in years with harvest. Male vacancies were often filled by males adopted from outside the group (71.4%, $n = 14$), and these males were unrelated to other group members and may have helped less as a result. Additionally, adoption of unrelated males may lead to earlier dispersal ages and lower recruitment may be an artefact of our sampling. Ausband *et al.* [22], however, found little evidence of dispersal during the pups' first year (4% or less than 1 sampled wolf per year) in study areas in Idaho. Newly adopted males may be at a disadvantage compared with former resident males because they did not have adequate time to establish stable social hierarchies and develop knowledge of the group's territory and hunting patterns. By contrast, vacancies caused by losses of breeding females were generally filled by non-breeding females within the group (78.9%, $n = 19$).

While increasing group size generally had a positive effect on pup survival, not all classes of individuals had positive effects on survival of young. The presence of older (greater than 2 years) non-breeding males over the pups' first year of life was associated with reduced pup survival. Individuals such as older non-breeding males may avoid helping (i.e. cheat) to increase the benefits of group living for themselves; such behaviour has been widely documented [38,39]. Female wolves in our study areas were philopatric and older non-breeding male helpers may not have

Table 1. Log-likelihood (−2LL), number of parameters (*K*), Akaike information criterion (AIC) value, change in (Δ) AIC value and Akaike weight (w_i) of multiple logistic regression models predicting the probability of wolf pup survival (i.e. alive at 15 months) in years when there was public harvest, Idaho (2009, 2011–2014). BF, breeding female; BM, breeding male. Breeder turnover = loss of a breeding position through death, expulsion or behaviourally usurped.

model	−2LL	*K*	AIC	ΔAIC	w_i
breeder turnover (BF turnover + BM turnover)	137.4	4	145.4	0.0	0.40
global (group size$_{(t=3\ \text{months})}$ + group size$_{(t=15\ \text{months})}$ + BF turnover + BM turnover)	133.6	6	145.5	0.1	0.38
group size (group size$_{(t=3\ \text{months})}$ + group size$_{(t=15\ \text{months})}$)	138.6	4	146.6	1.2	0.22

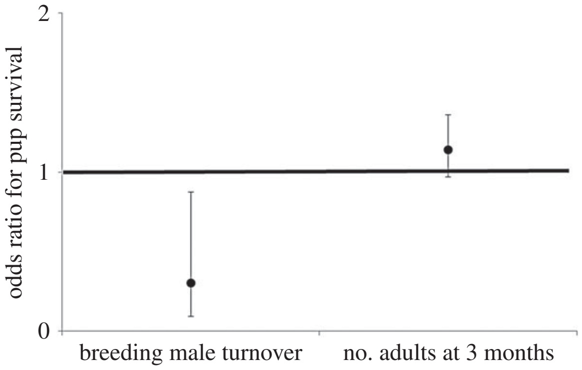

Figure 3. Odds ratios for influential variables from equally supported competing models predicting the probability of wolf pup survival (i.e. 3–15 months) in years of harvest in Idaho (2009, 2011–2014). Variables less than 1.0 had a negative effect on pup survival, whereas those greater than 1.0 had a positive effect.

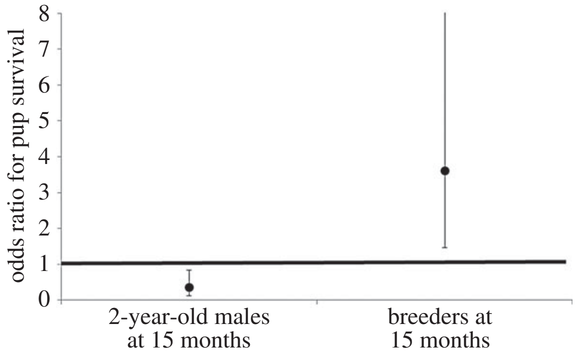

Figure 4. Odds ratios for influential variables from model of wolf group sex and age classes predicting the probability of wolf pup survival (i.e. 3–15 months) in Idaho during years with harvest (2009, 2011–2014). Variables less than 1.0 had a negative effect on pup survival, whereas those greater than 1.0 had a positive effect.

participated as much as female helpers in provisioning or guarding young, at least during portions of the pup-rearing season [7,22]. Older (greater than 2 years) non-breeding male helpers may increase their fitness by dispersing rather than waiting to inherit a breeding position in their natal group as females commonly do [18]. Given the lower tendency, we observed for inheriting a breeding position in their natal group, one might expect males to be selfish (i.e. to improve their individual fitness by growing large and helping less). Although they may help increase prey acquisition rates, older non-breeding adult males may also consume more at kills due to their large body size [40,41]. Conversely, adult males may be expelled from the group because of the negative effects that sexually mature males have on the fitness of breeders as we measured.

Contrary to our hypothesis, we did not find evidence that the presence of older (greater than 2 years) non-breeding females was associated with an increase in pup survival (odds ratio overlapped 1). Nevertheless, because the negative effect of older non-breeding males was so strong, our model predicts that during years with harvest, pups in groups with two breeders and only two adult male helpers had a 0.12 probability of surviving to 15 months, whereas pups in groups with two breeders and only two adult female helpers had a 0.33 probability of surviving as long (using Alberta as reference category). If relatively large, older males are critical during intergroup conflicts and territory maintenance [16], our model results suggest provisioning and helping, and not direct intergroup competition, may be the mechanism driving recruitment. Our findings also suggest that selection might favour groups that expel adult males and recruit adult females. We found evidence that males dispersed earlier than females, perhaps due to expulsion. Of the helpers that stayed with their natal pack for at least 3 years, 29% were males and 71% were females. This sex-biased philopatry allowed female helpers to obtain a breeding position in 10 cases, whereas male helpers bred in their natal pack only four times. While there is some evidence that such breeding inheritance can lead to inbreeding [29], breeding males regularly turnover and inbreeding does not appear widespread, at least in our Idaho study area where such data are available. We found no evidence that pup survival increased after harvest, as found in a heavily controlled population of coyotes [19]. Perhaps wolves in our study areas were not food stressed or harvest was sufficiently high to dilute any potential positive effects of increased food availability at lower wolf densities after harvest. Our analyses focused on reproductive groups because they could be adequately sampled with confidence when individuals congregated at rendezvous sites, thus years when groups did not have pups were not included in our analyses. We therefore probably underestimate the effect of harvest on recruitment at the population level because in some years, small groups failed to reproduce or were no longer extant after harvest began and they are not represented in our sample. Lastly, because we did not sample neonatal pups (less than 3 months of age), it is possible that we missed early season mortality of this age class. The litter sizes we measured in our study, however, are similar to those reported for wolf populations elsewhere [20] and we suspect survival of neonates was similar.

Illegal harvest can have strong effects on population growth [42]. We assumed our findings related to mortality were due to harvest, although it is plausible that similar results could be seen if mortality were due to illegal harvest. We evaluated post hoc the known fates of 278 radiocollared wolves monitored statewide in Idaho during the years of our study; combining known illegal kills with all unknown mortalities of collared wolves (liberally assumed to be 'illegal') showed only 10.0 radiocollared wolves per year were killed illegally statewide (8.2% of the population annually). If the radiocollared wolves are a representative sample, it is unlikely that illegally killed wolves would produce the effects we measured in our study areas.

It appears that the introduction of harvest in Idaho represented a novel source of mortality that was at least partly additive (i.e. not compensated for by immigration or increases in other vital rates) because both pup survival and group sizes declined significantly. Pup survival in Idaho before harvest was similar to levels measured in unharvested Yellowstone National Park but was significantly lower after harvest was initiated. Alberta had much lower pup survival rates than either Idaho (after harvest) or Yellowstone National Park. Sample sizes were limited for Alberta (two groups over 3 years), but turnover within packs in Alberta was high among all age classes. Only 22% of the 41 wolves sampled that were available for recapture in Alberta were detected again the following year and only 1 wolf was detected during all 3 years of our study despite reasonably high detection probabilities using our sampling methods [26,28]. These animals may have dispersed out of the study area and not died, but the resulting change to group composition between years is the same. It is difficult to discern what factors beyond harvest influence pup survival in Alberta, although prey availability and the density of other sympatric carnivores may also be influential. Given the very low levels of pup survival we measured in southwest Alberta, it appears this population of wolves is likely to be dependent on immigration for population persistence.

Recruitment, although important, is just one component for measuring fitness in cooperatively breeding carnivores. Behaviours such as foraging and territory maintenance contribute to both survival and recruitment, and thus affect fitness indirectly. For example, individuals in group-living carnivores that rely on capturing large prey can fulfil different roles during foraging [40,41]. Maintaining diverse sex and age classes in a group may enhance foraging success and lead to better condition and larger body size in breeders, thus positively affecting fitness. Group size can also influence territory maintenance and defence [15,16], leading to increased fitness for breeders. Harvest can influence group size and composition, which in turn affect recruitment, foraging success, and territory maintenance and defence. Determining how persistent mortality due to harvest also influences group-living benefits such as territory defence and foraging success can enhance understanding of the evolution and maintenance of group living in managed populations of cooperative breeders.

If the patterns we observed are representative, there are implications for wolf conservation. Because harvest appears additive, recruitment can be expected to play a major role in how wolf populations respond to harvest. Harvesting pups can reduce recruitment, but there are also indirect effects of harvest (i.e. reduced group size, breeder turnover) that further reduce survival of young [22]. Inferences about the effect of harvest on recruitment require knowledge of harvest rate of young that incorporates the indirect effects of harvest due to changes in group size and composition. The number of young harvested is alone a poor measure of the effect of harvest on recruitment.

If the goal is to maintain a sustainable harvest over time, managers can craft seasons and quotas to ensure that some groups in the population grow in size and have non-breeding adults available to assist in pup-rearing in the spring when pups are young. Further, designing harvest seasons to not overlap wolf breeding season for extended periods would minimize breeder loss, which we showed has a strong negative effect on pup survival.

Ethics. Grey wolves were captured and radiocollared by management agencies as part of monitoring and research efforts, and by University of Montana personnel (Animal Use Protocol 008-09MMMCWRU).

Data accessibility. All data used in this manuscript are available in table format in the electronic supplementary material.

Authors' contributions. D.E.A. designed and implemented the study and authored the bulk of the manuscript; M.S.M. assisted with hypothesis development and helped write the manuscript; C.R.S. conducted fieldwork and DNA laboratory analyses, and assisted with manuscript writing; J.L.S. conducted fieldwork and DNA laboratory analyses, and assisted with manuscript writing; L.P.W. conducted fieldwork, supervised DNA laboratory analyses and assisted with manuscript preparation.

Competing interests. We declare we have no competing interests.

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