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GROUPS AND MORTALITY: THEIR EFFECTS ON COOPERATIVE BEHAVIOR AND

POPULATION GROWTH IN A SOCIAL CARNIVORE

By

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Groups and mortality: their effects on cooperative behavior and population growth in a social carnivore

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  Cooperative breeding refers to the cooperative care of related, or even unrelated, young. Helpers can increase the survival or reproduction of the breeders in the group which increases helper fitness indirectly. We have a poor understanding of how mortality, particularly human harvest, affects cooperative breeders. Given their complex social structures, territorial defense that relies on group size, and persistent harvest regimes, gray wolves (*Canis lupus*) are an ideal species for studying the ecological relationships between mortality, group size and composition, and population growth in a cooperative breeder.
  Chapter 1: How does group size affect vital rates of individuals and population growth? Furthermore, how do density and immigration of individuals into groups influence the effect of group size on population growth? I used historic data from Idaho and Yellowstone National Park as well as the scientific literature to populate a metapopulation model and explore the simultaneous influences of group size, density, and immigration on population growth.
  Chapter 2: What is the effect of harvest on recruitment in a cooperative breeder? Are there both direct (i.e., mortality from harvest) and indirect effects (i.e., reduced survival because of breeder turnover, reduced group size) of harvest on recruitment? I used a natural experiment and genetic sampling to assess the influence of harvest on pup recruitment. I compared genotypes of sampled pups to harvested pups to determine whether harvest had both direct and indirect effects on recruitment.
  Chapter 3: How does mortality, in the form of persistent public harvest, affect group size, composition, and ultimately recruitment in a cooperative breeder? I genetically sampled wolves across a broad range of human-caused mortality in western North America. I used the resulting data to assess the influence of harvest on group size, group composition, breeder turnover, and ultimately recruitment.
  Chapter 4: How do individual, group, and environmental factors influence helping behavior in a cooperative breeder? I used location data from satellite-collared wolves in western North America to explore the influences of sex, individual status within a group, group size, and predation risk on pup-guarding behavior.

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To my wife Liz, thank you for encouraging me to pursue this degree. Thank you for putting up with me being away – even the times when I was at home but wasn't really there. Your perception of my abilities far outstrips reality but there are times when I rely on your belief in me. I'm pretty glad we had bird class together.

To Sam: I know my little degree is a minor achievement as far as the great, big, wide world is concerned. I wanted, in part, to show you that just because something is hard doesn't mean you avoid it or give up once you start. Working hard doesn't always ensure success but it sure does help most of the time. I'll share a secret though; find your passion, follow it wholeheartedly and it won't ever really feel like hard work anyhow. Thanks for helping me in the field all those times. I cherish every day you were there.

And finally, thanks wolves. For all of it.

Contents

Groups and mortality: their effects on cooperative behavior and population growth in a social carnivore

Introduction

Group living has evolved across a wide range of taxa and species. Not all group living species display cooperative breeding behavior, however. Cooperative breeding refers to the cooperative care of related, or even unrelated, young (i.e., helping; Solomon and French 1997). Helpers can increase the survival or reproduction of the breeders which increases helper fitness indirectly (Emlen et al. 1991). In mammals, both manipulative and observational studies have shown that the presence of helpers can be critical to fitness of the breeders in the group and persistence of the group as well (Solomon and French 1997; Courchamp et al. 2000; Courchamp and Macdonald 2001; Courchamp et al. 2002).

Most studies of cooperative breeding in mammals have focused on Rodentia and Primates (Solomon and French 1997) and studies exploring the effects of human harvest on cooperative breeders are few. Harvest can have both direct and indirect effects on groups of cooperative breeders. Direct effects are when animals are harvested whereas indirect effects result from changes to group size or composition through harvest. For example, harvest can lead to reductions in group size which in turn can lead to indirect effects such as lower recruitment or an inability to successfully defend a territory (Courchamp and Macdonald 2001; Courchamp et al. 2002; Stahler et al. 2012; Cassidy et al. 2015).

Studies across a broad range of species affirm that number of helpers is positively related to group fecundity (Tardif et al. 1984; Solomon and French 1997; Clutton-Brock 2006), however, group composition (i.e., the number of sex and age classes within a group) can also influence group success and ultimately population growth. Changes to group composition can be

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subtle and may not change group size drastically but their effect can still be quite large. For example, modeling of African lion (*Panthera leo*) populations showed that the selective harvest of large males led to increased infanticide and reduced population viability (Whitman et al. 2004). Additionally, female elephants (*Loxodonta africana*) in groups disrupted by poaching had lower reproductive success despite many of the surviving females being prime reproductive age (Gobush et al. 2008). Lastly, breeder mortality in groups of wolves (*Canis lupus*) led to reduced recruitment and higher group dissolution rates, although these were both mitigated somewhat by increased group size (Brainerd et al. 2008). Group composition may be influential in part because not all age and sex classes contribute (i.e. help) equally within a group. For example, nonbreeding (i.e., helper) gray wolves will guard offspring within packs foregoing what is presumably valuable foraging time for themselves. Wolves within a group vary widely in the amount of pup-guarding behavior they display, however (Thurston 2002; Ruprecht et al. 2012). Given the importance of pup-guarding to pup survival and fecundity in African wild dogs (*Lycaon pictus*; Courchamp et al. 2002), groups of wolves that contain multiple sex and age classes may be at an advantage because of this diversity.

Given their complex social structures, territorial defense that relies on group size, and persistent harvest regimes, gray wolves are an ideal species for studying the ecological relationships between mortality, group size and composition, and population growth in a cooperative breeder. Wolves have evolved to live in groups and in the absence of harvest generally attain a pack structure containing 2-3 generations of offspring. If selection has favored breeding wolves that retain mature offspring and foster diverse group structures then population growth may be driven more directly by characteristics of groups rather than characteristics associated with individuals. This may be particularly true in saturated populations with high

levels of intraspecific competition. Thus, modeling the vital rates of groups should provide more useful insights into factors that drive population growth in this cooperatively breeding species. Furthermore, if group size and composition influence recruitment and survival (Solomon and French 1997; Brainerd et al. 2008) then management actions that affect such group characteristics may also affect individual behavior, group persistence, and ultimately population growth.

Despite the influence that human-caused mortality has on group size and composition little work has been conducted on how population management affects groups of cooperatively breeding species. Furthermore, even though cooperative breeding species live and breed in groups I know of no study that has explored how vital rates of groups rather than individuals ultimately affect population growth. I have been collecting highly detailed data on groups of gray wolves in Idaho since before public harvest began (2008) and have continued to sample every year after harvest providing an ideal natural experiment for assessing the effects of human-caused mortality on groups. Further, additional sampling in Alberta and Yellowstone National Park, WY (2012-2014) helped ensure I encompassed a range of human-caused mortality from heavily exploited to wholly protected. I used these data, along with detailed historic data collected by wolf managers in the northern Rocky Mountains of the U.S., to explore facets of cooperative breeding in a large carnivore. Specifically, I tested hypotheses about 1) the relationships between vital rates of groups, density, immigration, and population growth, 2) how harvest affects group size, composition and ultimately recruitment, and 3) how characteristics of groups affect helping behavior.

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Chapter 3: How does mortality, in the form of persistent public harvest, affect group size, composition, and ultimately recruitment in a cooperative breeder? I genetically sampled 670 individual wolves across a broad range of human-caused mortality in Alberta, Idaho, and Yellowstone National Park. I used the resulting data to assess the influence of harvest on group size, group composition, breeder turnover, and ultimately recruitment.

Chapter 4: How do individual, group, and environmental factors influence helping behavior in a cooperative breeder? I used location data from 92 satellite-collared wolves in Alberta, Idaho, Montana, and Yellowstone National Park to explore the influences of sex, individual status within a group, group size, and predation risk on pup-guarding behavior.

Title: The influence of group size on population growth in a cooperatively breeding carnivore depends on density and immigration

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Abstract

In cooperative breeders, large group size is often positively related to reproductive success as well as to territorial defense and persistence. We have a poor understanding, however, of how group size affects individual vital rates and population growth particularly as density and immigration vary. Conceivably, in suitable habitat and at low densities, individuals in small groups may be able to secure the resources they need just as well as individuals in large groups. Selection, however, has favored the evolution of relatively large family group sizes in many cooperatively breeding mammals. Thus, we can expect larger groups to have an advantage over smaller groups particularly as density and competition between groups increase. We hypothesized that 1) at low densities populations composed of small and large groups have similar growth rates, 2) at low densities populations of large groups grow slightly faster than populations of small groups when both have low levels of immigration, 3) at high densities populations composed of small groups have lower growth rates compared to populations consisting of mostly large groups presumably because of competition, 4) a lack of immigration exacerbates this effect at high densities.

We tested our hypotheses by simulating metapopulation growth while allowing vital rates of individuals to vary as a function of group size. We estimated vital rates from gray wolves (*Canis lupus*) in Idaho and Yellowstone National Park, USA during 1996-2012. Group size had a positive effect on most individual vital rates. Group size also had positive effects on colonization rates of new groups and metapopulation growth in the absence of immigration. The benefits of living in a large group increased with density but generally declined as immigration increased. Abundance of individual wolves (not wolf groups) declined at high densities in all metapopulations however, metapopulations of large groups declined the least and were still able to increase the total number of groups by 20% over 5 years. We show that group size positively affects individual vital rates, group persistence, and metapopulation growth. The influence of group size on fitness and metapopulation growth weakens as immigration increases and density declines. Studies examining the importance of group size on fitness can benefit by simultaneously considering the influences of density and immigration because of their marked effects on metapopulation growth in cooperative breeders.

Key words: *Canis lupus*; cooperative breeding; gray wolves; groups; metapopulation; population growth

Introduction

Cooperative breeding generally refers to the cooperative care of related or unrelated young (Solomon and French 1997). In mammals, both manipulative and observational studies have shown that the presence of nonbreeding helpers in a group enhances reproductive success, fitness of breeders, and persistence of the group (Solomon and French 1997; Courchamp et al. 2000a; Courchamp and Macdonald 2001; Courchamp et al. 2002; Clutton-Brock 2006).

The benefits of living in a large group may be particularly marked for territorial carnivores. Large group size can increase hunting success (Fanshawe and Fitzgibbon 1993; Creel and Creel 1995; Carbone et al. 2005; MacNulty et al. 2014) although there can be intermediate group sizes that lead to maximum per capita benefits for group members (Creel and Creel 1995). Larger group size can also increase the ability to successfully defend a territory and offspring from predation (Creel and Creel 1995; Packer et al. 1990; Courchamp et al. 1999; Courchamp et al. 2002; Whitman et al. 2004; Cassidy 2013). Yet the benefits of larger group size may vary with conspecific density when resources are patchy and limited. For example, as density increases individuals in larger groups may be able to secure and defend high quality territories (i.e., those with abundant limiting resources) and provision and guard offspring more successfully than those in smaller groups (Courchamp et al. 1999; Ruprecht et al. 2012; Cassidy 2013).

In obligate cooperatively breeding species there can be a threshold for the minimum number of helpers required for group existence and population growth. Failure to maintain a threshold group size is one reason for the high frequency of group extinction observed in such species (Courchamp et al. 1999). When populations of other individuals are nearby, however, immigration can buffer the effects of mortality loss within groups (Courchamp et al. 1999). Field studies of cooperative breeders have shown that immigration does contribute to group persistence and population stability in harvested species (Adams et al. 2008; Rutledge at al. 2010).

In some populations, immigration mitigates the effects of mortality over relatively short timescales but such mortality may affect group social structure, learning, helping behavior and evolution over longer time periods (Haber 1996; Rutledge et al. 2010). Because of their

hierarchical structure and dependence on others in the group, mortality can affect group-living species in complex ways. For example, individuals in groups of African elephants (*Loxodonta africana*) that experienced higher rates of poaching, and particularly had lost older females, had lower reproductive rates despite the continued survival of reproductively prime females (Gobush et al. 2008). Additionally, the extinction rate for groups of cooperatively breeding gray wolves (*Canis lupus*) was 33-38% after breeder loss, but survival of the remaining pups was greater in groups that had more nonbreeding helpers (Brainerd at al. 2008; Borg et al. 2014). The effects of mortality in group-living species can be more than simply subtracting 1 animal from the group's size; effects can depend on the status of the animal lost but also which individuals remain in the group. To gain a better understanding of the mechanisms affecting population dynamics of cooperative breeders we need to know how vital rates of individuals are affected by both density and group dynamics (Bateman et al. 2011).

We wanted to know how group size affects individual vital rates and metapopulation growth in cooperatively breeding species. Furthermore, how do metapopulation density and immigration of individuals into groups influence the effect of group size on metapopulation growth? At low metapopulation densities and in suitable habitat, individuals in small groups may be able to secure the resources they need just as well as individuals in large groups. If true, such metapopulations should have stable or positive growth rates and immigration should increase the rate of growth regardless of the initial group size distribution in the metapopulation until the population reaches carrying capacity (K). Selection, however, has favored many cooperatively breeding species to live in relatively large, multi-generational family groups (Solomon and French 1997). Thus, at high metapopulation densities the benefits of living in a larger group should become more pronounced as competition for limited resources between groups increases.

Subsequently, metapopulations composed of small groups should have lower growth rates when habitat is saturated. Immigration of individuals into groups should bolster small groups and eventually lead to stable or positive growth rates for metapopulations composed of mostly small groups.

Gray wolves are cooperative breeders who live in groups (i.e., demes) thus their populations can be viewed as metapopulations. Wolf populations can also be strongly influenced by immigration (Adams et al. 2008). Reintroductions to vacant habitat in the northern Rocky Mountains of the U.S. provide an ideal framework for assessing the relative influence of density on metapopulation growth. We simulated metapopulation growth in gray wolves using varying immigration rates, different initial group size distributions and individual vital rates that varied as a function of group size. We hypothesized that at low densities metapopulations composed of small and large groups would have similar growth rates. Low levels of immigration would make metapopulations of larger groups perform slightly better than those with small groups. At high densities, however, metapopulations composed of small groups would have lower growth rates compared to metapopulations consisting of mostly large groups. A lack of immigration would exacerbate this effect. We compared growth rates from simulations to those predicted by our hypotheses. We further assessed growth rates from our simulations by comparing them to growth rates observed during wolf recovery.

Methods

We used Program Vortex (Version 10; Lacy and Pollak 2014) to model wolf metapopulation growth (i.e., $r = \ln(\lambda)$) over a 5-year time interval. We used 1,000 iterations for each model. We considered each group (i.e., wolf pack) to be a subpopulation within a larger metapopulation under 2 scenarios where n = 10 and n = 25 groups at $t_{(0)}$. We chose to model 10 groups because it

is the minimum recovery criteria for wolves in each of 3 states (Idaho, Montana, Wyoming) in the northern Rockies. Furthermore, the 3 states are to maintain at least 30 groups total for successful delisting, therefore we also modeled 25 groups while permitting 20% growth in the number of groups (i.e., n = 30). We varied group sizes at $t_{(0)}$ and conducted simulations using 3 different group size distributions (Table 1); all small groups (n≤4 wolves), approx. 50% small groups, and no small groups (n≥8) wolves. We permitted colonization of 20% more groups via dispersal by seeding 2 and 5 additional groups to have 0 individuals at $t_{(0)}$ in each of the 2 scenarios (n = 10 and n = 25, respectively). Each group reached carrying capacity (K) at 30 individuals and K was implemented as a probabilistic truncation on survival across all age classes when group size was >30. The largest wolf group recorded in YNP was 37 animals although their association was brief; the largest group recorded in Idaho since 1995 was 26 wolves. To reflect the demographic potential for growth of a population, Vortex calculates growth rates before truncation for K. To further assess the influence of K (i.e., density dependence), we performed focal simulations (n = 1,000) for metapopulations of 10 small and 10 large groups at high densities with no immigration where we varied the mortality rate and variance for pups, litter size, and proportion of females that had litters when group size reached >15 and >20 individuals.

Initial age distribution for each group was set to reflect the family structure commonly observed in wolf packs. For example, an adult breeding pair and their offspring from previous years where group size determines how many generations of offspring are present (Table 2). To assess the influence of initial age distribution on our simulations we performed focal simulations (n = 1,000 simulations) where we added 1 year and subtracted 1 year from the age of all

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individuals in simulations of 10 small and 10 large groups without immigration and at high densities.

Dispersal rates at low (1995-2002) and high wolf densities (2003-2008 only) were derived from wolves in the northern Rocky Mountains (NRM; Jimenez et al. In Revision). We allowed individuals between the ages of 1-7 to disperse and join other groups in the metapopulation when their group size >3. Lastly, we allowed immigration of wolves from outside the metapopulation. This outside source of immigrating wolves was an infinitely-sized population of individuals that were wholly separate from the metapopulation being modeled. These individuals were unaffected by group sizes and their contingent vital rates until they immigrated into the metapopulation. Immigrants joined groups when they entered the metapopulation. Immigration varied from none to low (1 adult individual into each group every 5 years while alternating sexes between groups) to high (2 adult individuals, 1M and 1F, into each group every year). We considered a group extinct when only 1 individual remained.

We modeled reproduction as long-term polygyny where pairs remained mated until one died. We set the age of first reproduction at 3 for males and females (Fuller et al. 2003), assumed equal sex ratios in the offspring, and a maximum age of 10 and 11 for breeding in female and males, respectively (Kreeger 2003). Each female could have 1 litter per year with a maximum of 8 pups. We allowed >1 breeding female in a group when the number of adult females >4. Maximum age for individuals was 14 years (Ausband et al. 2009).

Pup mortality rates, litter sizes, and the proportion of females with no litter were calculated using historic data for wolves in Idaho (1996-2002) and Yellowstone National Park (YNP; 1996-2012). We then estimated average reproductive vital rates for individuals in small ($\leq$4 adults) and large ($\geq$8 adults) groups. We considered 1996-2002 to be characterized by

relatively low wolf densities (Idaho and YNP data) and unsaturated habitat and 2003-2012 to be

high wolf density characterized by saturated habitat and more stable territories (YNP data only).

We incorporated the variance around these vital rates into our model to simulate environmental

stochasticity in the metapopulation. Pup mortality rates were calculated to Dec 31 and then

multiplied by the winter mortality rate provided in Smith et al. (2010) and Massey et al.

(unpublished data) to obtain an annual mortality rate for pups at low and high densities,

respectively. Mortality rates for yearlings and adults at low wolf densities were derived from

Smith et al. (2010) and Massey et al. (unpublished data) at high wolf densities. We calculated the

percentage the SD was of the reported survival rate in Smith et al. (2010) and Massey et al.

(unpublished data) and allowed the mortality rate for pups to fluctuate by that amount. Yearling

and adult mortality rates were allowed to vary by a percentage equivalent to 2 SE's reported in

Smith et al. (2010) and Massey et al. (unpublished data). We did not have separate estimates of

mortality rates for yearling and adults in small versus large groups.

Results

No immigration

Vital rates in Idaho and Yellowstone were lower for wolves in small groups than large groups

and this difference was most pronounced at high wolf densities (Table 3). The only exception to

this difference was pup mortality which was 7% lower in small groups at low densities. Wolves

in both small and large groups experienced higher mortality rates at high metapopulation

densities, but the difference in mortality rates and decreased litter sizes was more pronounced for

wolves in small groups (Table 3). Probabilities of group extinction estimated through simulations

were greater for small than large groups, particularly at high densities (Figs. 1A-B). At low

densities, metapopulations beginning with 10 and 25 groups grew to 12 and 30 groups,

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respectively, regardless of the initial group size distribution. In metapopulations composed of small groups, colonization probabilities for new groups was 0.45 (SD = 0.05) whereas in metapopulations composed of large groups it was 0.90 (SD = 0.01) at low densities. At high densities, metapopulations initially consisting of all small groups failed to add groups to the metapopulation whereas those consisting of $\geq$50% large groups had a 20% increase in the total number of groups in the metapopulation over 5 years. Colonization probabilities for new groups was >5 times higher in metapopulations composed of large groups than small groups (0.63 vs. 0.12, SD = 0.01, 0.02, respectively). All metapopulations declined at high densities in the absence of immigration although metapopulations with $\geq$50% large groups declined less than those initially consisting of all small groups (Figs. 3B). The net gain ($N_{t+5} - N_t$) in number of individuals was greater, or net loss lower, when the metapopulation contained $\geq$50% large groups (Figs. 3A-B).

Our focal simulations using altered age distributions yielded similar patterns in number of groups present at $t_{(5)}$. Abundance of wolves at $t_{(5)}$ was different by 3-8 individuals compared to simulations using our initial age distributions (Table 2).

Mortality rates of pups were higher and more variable, litter sizes were smaller, and the proportion of females with no litter was higher for wolves in small groups than large groups. Therefore, for focal simulations assessing the influence of K, we allowed vital rates to return to levels measured for small groups when group size was >15 and >20. Such simulations where K was reached, in part, when group size was >15 and >20 differed by 0.1-5.7 individuals compared to our simulations where K was reached at 30 individuals and survival was subsequently truncated.

Low immigration

For simulated metapopulations under the influence of low rates of immigration, probabilities of group extinction were again greater for small than for large groups, particularly at high densities (Figs. 1A-B). At low densities, metapopulations beginning with 10 and 25 groups grew to 12 and 30 groups, respectively, regardless of the initial group size distribution. In metapopulations composed of small groups, colonization probabilities for new groups was 0.71 (SD = 0.03) whereas in metapopulations composed of large groups it was 0.95 (SD = 0.01) at low densities. At high densities, metapopulations beginning with 10 and 25 groups grew to 12 and 30 groups only when the metapopulation initially consisted of $\geq$50% large groups. Colonization probabilities for new groups was higher in metapopulations composed of large groups than small groups (0.78 vs. 0.32, SD = 0.01, 0.05, respectively). Metapopulations containing $\geq$50% large groups had lower growth rates than metapopulations comprised of small groups at low densities but all metapopulations had similar growth rates at high densities (Figs. 2A-B). The net gain ($N_{t+5} - N_t$) in number of individuals was generally greater when the metapopulation contained $\geq$50% large groups (Figs. 3A-B).

High immigration

Under the influence of high rates of immigration, probabilities of group extinction were low and similar among all simulated metapopulations (Figs. 1A-B). At both low and high densities, the number of groups in all metapopulations grew 20% through colonization of new groups. In all metapopulations, colonization probabilities for new groups was 1.0 (SD = 0.00). Metapopulations containing $\geq$50% large groups had lower growth rates than metapopulations composed of small groups (Figs. 2A-B). The net gain ($N_{t+5} - N_t$) in number of individuals was greater for metapopulations initially comprised of small groups (Figs. 3A-B).

Discussion

We show that group sizes within a metapopulation can affect population growth, but the strength of that effect depends on density and immigration. Group size had marked effects on individual vital rates, colonization of new groups, and ultimately metapopulation growth. The influence of group size on fitness and metapopulation growth weakened as immigration increased and density declined. We show marked effects of density and immigration on metapopulation growth in cooperative breeders who are reliant on limited, patchy resources. Thus, studies examining the importance of group size on fitness can benefit by simultaneously considering the influences of density and immigration.

The distribution of group sizes as well as the interactions between groups in a metapopulation can influence population trajectory and thus management and conservation decisions. Our findings have implications for harvested cooperative breeders (e.g., gray wolves in the U.S.). For example, metapopulations at low densities containing small groups of generally fecund pairs, such as gray wolves, can harbor the potential for marked growth whereas a metapopulation of large groups has a higher total abundance, more nonbreeding individuals, and will not increase its per capita abundance as rapidly. At high densities, however, large groups can absorb increased mortality rates yet still colonize new groups even as overall individual abundance declines ($r = -0.01$). Metapopulations that began with as few as 5 large groups added more groups and had much lower group extinction probabilities than metapopulations with all small groups. A harvest regime that maintains $\geq 50\%$ large groups could allow for replenishment of wolves to nearby areas where mortality is higher and related group sizes are smaller.

When estimating vital rates empirically, we considered groups with ≥ 8 adults as large (range = 8-26). Very large groups could have decreased vital rates because there may be a threshold group size where some phenomena (e.g., daily caloric intake) are negatively influenced

by increasing group size (Creel and Creel 1995). Despite this potential negative bias in our empirical estimates, vital rates for individuals in groups with $\geq$8 adults were generally still higher than those estimated for individuals in small groups. Additionally, our results are likely optimistic for metapopulations of small groups because, although pup survival and female reproductive rates were lower for wolves in small groups, we did not have separate estimates of mortality rates for yearlings and adults in small and large groups.

Immigration from outside the metapopulation added individuals to groups and also established new groups. As predicted, immigration generally weakened the positive effect that large group size had on metapopulation growth. We hypothesized that the effect of immigration would be stronger for large groups but we found the opposite was true. We suspect that this could be because immigration of a few individuals into groups within a small metapopulation is a higher proportional contribution to that metapopulation than they would be to a relatively larger metapopulation. Although metapopulations of small groups grew faster, even with low immigration rates, metapopulations of large groups added more individuals (i.e., net gain, N_{t+5} − N_t), had lower group extinction rates, and were generally less variable. We did not allow individuals to disperse from groups until group size was >3, thus small group sizes may have been bolstered somewhat and our results for metapopulations of small groups are slightly optimistic. High rates of immigration made metapopulations of both small and large groups perform equally well. Imposing carrying capacity when groups were >30 likely affected these results for metapopulations of large groups at low densities. Immigration, even low rates, strongly influenced both small and large group metapopulation growth rates at high densities. Courchamp et al. (1999) found that immigration lowered the extinction rate of groups but it required that dispersing individuals be available from nearby groups which may not always be

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true. In some populations of social carnivores, (i.e., South Africa's wild dogs, Mexican wolves; *C. l. baileyi*) there is no immigration or it is human-induced and quite low thus the effect of group size distribution on metapopulation growth is likely strong.

We modeled small metapopulations of cooperative breeders (n = 10 and 25 groups) over relatively short time intervals (5 years). While we permitted immigration we did not model the effect of wolves leaving the metapopulation (i.e., emigration) other than through death. We might expect, over longer time intervals than what we considered, that as populations persist group sizes would enlarge, breeding opportunities would be scarce, and some individuals would eventually emigrate from the metapopulation to attempt to find breeding opportunities elsewhere. Such emigration should weaken the ability of large groups to repopulate nearby smaller groups and as a result small group extinction rates would be higher than what we observed. Furthermore, the net gain in number of individuals and growth rate for metapopulations of large groups may decline although we indirectly accounted for the loss of some such individuals (i.e., emigrates) when we truncated survival as group sizes approached K.

Group social structure (i.e., social learning, dominance hierarchies) may influence group success (Gobush et al. 2008). Our proposed model and subsequent analyses did not consider aspects of social behavior although any such effects should have been captured in our empirical estimates of vital rates. The link between complex factors such as group social structure and fitness has not been demonstrated for wolves. We posit that group size, immigration, and conspecific density have stronger influences than social structure on group success and ultimately metapopulation growth. For example, group extinction events can occur after the loss of even 1 important individual however, recruitment of young is generally greater and group extinction rates lower in large groups (Brainerd at al. 2008; Gobush et al. 2008; Borg et al.

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2014). Given this, we expect larger groups would absorb changes to group social structure more readily than small groups.

Wolves in small groups can have higher mortality rates (i.e., hazard ratios) than wolves in large groups, although perhaps only marginally so (Smith et al. 2010). We measured markedly higher vital rates for wolves living in large groups over those in small groups, however. An increased ability to successfully compete with conspecifics as well as other species may be one explanation for the markedly higher vital rates we measured for wolves living in large groups than small groups. Gray wolves in the northern Rocky Mountains coexist with conspecific competitors as well as other competitors such as grizzly bears (*Ursus arctos*), black bears (*U. americanus*), mountain lions (*Felis concolor*), and humans (*Homo sapiens*). The presence of natural enemies (Courchamp et al. 2000b) can increase the probability of extinction in group-living species where minimum group size thresholds exist. Under the influence of competition from conspecifics and others, small groups of African wild dogs had difficulty provisioning young and also guarding them from predation (Courchamp et al. 2002). Ruprecht et al. (2012) found that gray wolves living in small groups spent more of their time guarding young than those living in larger groups. Ausband et al. (In Review) also found a strong influence of predation risk on guarding rates in wolves; where predation risk was high individuals spent more time guarding young. Adequately guarding and provisioning young in a predator rich environment may be difficult for small groups and could contribute to the depressed vital rates we measured for such individuals.

Wolf populations in the northern Rocky Mountains of the U.S. have increased dramatically during the last 3 decades, due in large part to reintroductions in Idaho and Yellowstone National Park in 1995-1996 (Bangs and Fritts 1996). Our simulations estimated

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rapidly growing metapopulations of wolves at low densities regardless of initial group size distribution ($\bar{x}$ (r) = 0.17, range = 0.06-0.40). Such estimated growth rates compare favorably to those reported during the early colonization period after wolf reintroduction in the Rockies and natural wolf recovery in the Midwest U.S. (USFWS et al. 2000; Beyer, Jr. et al. 2009; Van Deelen 2009; Wydeven et al. 2009). In recent years, as suitable habitat has become saturated, population growth in the northern Rockies as well as the Midwest U.S. has slowed (Beyer, Jr. et al. 2009; Van Deelen 2009; Wydeven et al. 2009; USFWS et al. 2010). At such high densities, our models predicted relatively stable to slightly decreasing metapopulations, particularly when group sizes within metapopulations were small. Beginning in 2009, wolves in Montana and Idaho have been harvested and group sizes have declined in recent years (IDFG 2014). Our simulations indicate that individual wolf abundance can decline yet the number of groups and distribution can increase in a metapopulation initially consisting of mostly large groups. Thus, population monitoring programs that focus on individual abundance may underestimate the abundance and distribution of groups when assessing population health.

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Table 1. Initial subpopulation (i.e. group) size distribution of metapopulations used to model gray wolf population growth.

Subpopulations n = 10 at $t_{(0)}$			
Group size	Number of subpopulations when all groups were small	Number of subpopulations when 50% of groups were small	Number of subpopulations when all groups were large
2	3	2	
3	2	1	
4	5	2	
8		2	4
10		2	3
12		1	3

Subpopulations n = 25 at $t_{(0)}$			
2	7	4	
3	6	4	
4	12	5	

8	4	9
10	4	8
12	4	8

Table 2. Initial age distribution of groups used to simulate wolf metapopulation growth.

Age	N = 2	N = 3	N = 4	N = 8	N = 10	N = 12
1	0	1	2	4	4	4
2	0	0	0	2	4	4
3	2	0	0	0	0	2
4	0	2	2	2	2	0
5	0	0	0	0	0	2

Table 3. Vital rates and variances estimated from gray wolves in Idaho and Yellowstone National Park, USA. Vital rates were used to populate metapopulation model to simulate wolf population growth at low (1996-2002) and high densities (2003-2012).

Low density				
Vital rate	Group size $\leq$4	Variance	Group size $\geq$8	Variance
Mortality$_{(pup)}$	0.28	0.02	0.35	0.09
Mortality$_{(adult)}$	0.23	0.02	0.23	0.02
Prop. females breeding	1.0, F=1; 0.50, F=2; 0.33, F=3; 0.25, F$\geq$4; 0.50, F$\geq$5	N/A	1.0, F=1; 0.50, F=2; 0.33, F=3; 0.25, F$\geq$4; 0.50, F$\geq$5	N/A
Prop. females litter = 0	0.15	N/A	0.06	N/A
Litter size	4.41	0.82	4.85	1.20
Dispersal rate	0.12	N/A	0.12	N/A
High density				
Mortality$_{(pup)}$	0.45	0.25	0.38	0.15
Mortality$_{(adult)}$	0.28	0.02	0.28	0.02
Prop. females breeding	1.0, F=1; 0.50, F=2; 0.33, F=3; 0.25,	N/A	1.0, F=1; 0.50, F=2; 0.33, F=3; 0.25, F$\geq$4;	N/A

	F$\geq$4; 0.50, F$\geq$5		0.50, F$\geq$5	
Prop. females litter = 0	0.19	N/A	0.07	N/A
Litter size	3.20	1.31	5.36	0.61
Dispersal rate	0.09	N/A	0.09	N/A

Figure 1. Probability of group extinction from simulations of gray wolf metapopulation model at

a.) low densities and b.) high densities using various initial group size distributions and

immigration rates. Error bars represent SE.

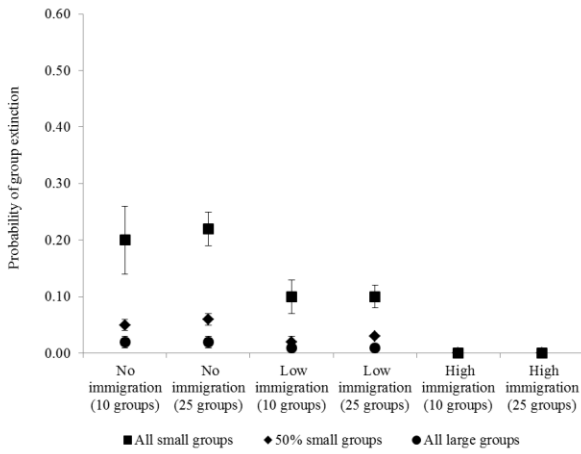Fig. 1a   Fig. 1b

Figure 2. Stochastic growth rate (r) estimated from simulations of gray wolf metapopulation model at a.) low densities and b.) high densities given various initial group size distributions and immigration rates. Error bars represent SE.

Fig. 2a

Fig. 2b

Figure 3. Net gain ($N_{t+5} - N_t$) in number of individuals from simulations of gray wolf metapopulation model at a.) low densities and b.) high densities using various initial group size distributions and rates of immigration. Error bars represent SD.
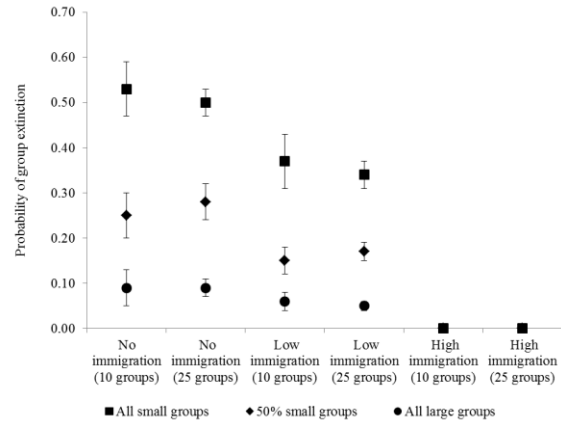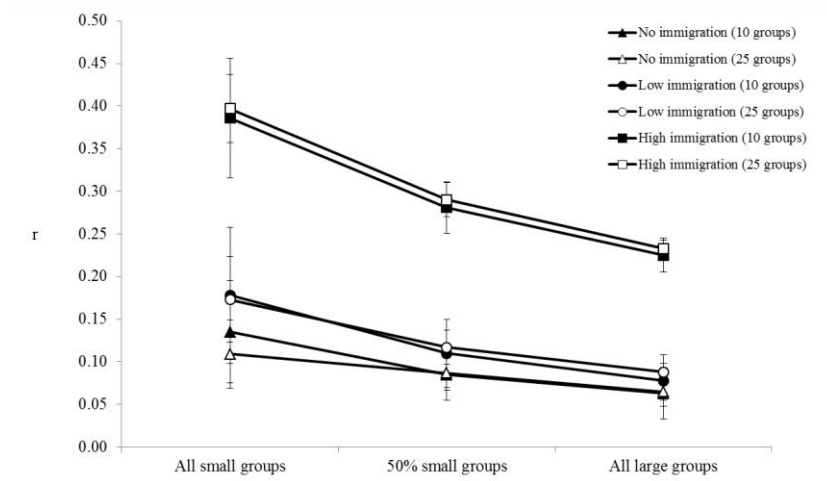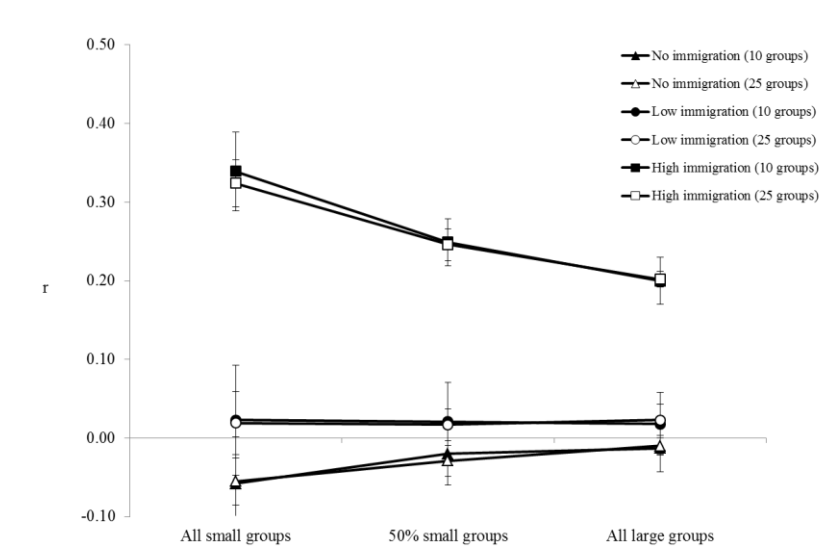
Fig. 3a
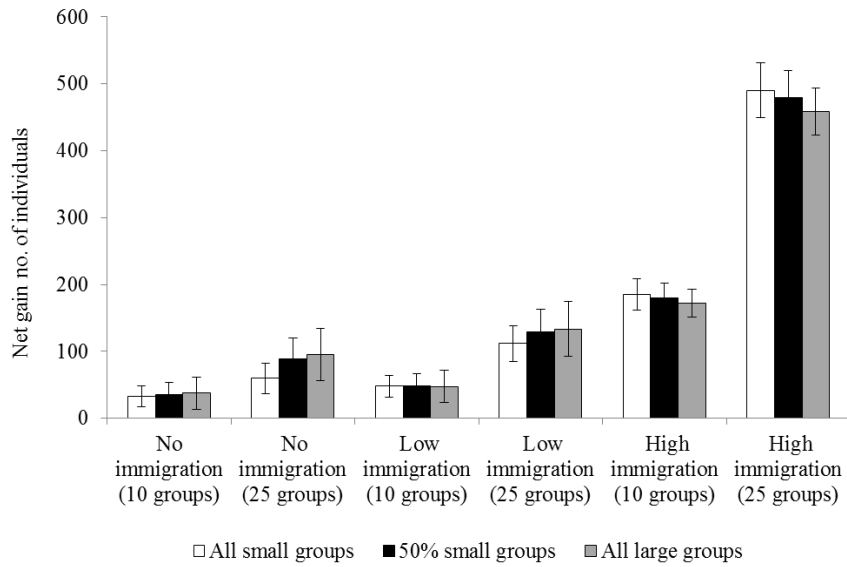
Fig. 3b
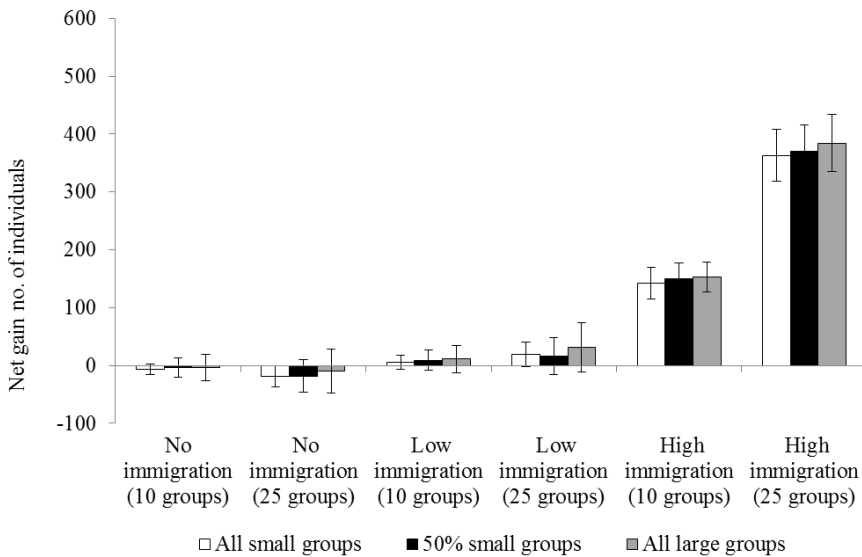
Title: Recruitment in a social carnivore before and after harvest

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Running Title: Recruitment before and after harvest

Abstract

Knowledge about recruitment in a population can be critical when making conservation decisions, particularly for harvested species. Harvest can affect population demography in complex ways and this may be particularly true for cooperatively breeding species whose successful reproduction is often linked with complex social dynamics. We currently have a poor understanding of how harvest affects recruitment in cooperatively breeding species. We used noninvasive genetic sampling and a natural experiment to estimate recruitment in a population of gray wolves (*Canis lupus*) before and after harvest in the northern Rocky Mountains, USA (2008-2013). We hypothesized that recruitment would decline after hunting and trapping began and that the decline in recruitment would be attributable to the harvest of pups and not subtler mechanisms associated with group dynamics and reduced reproductive success. We collected fecal samples wolves in 10 packs for 6 consecutive years, extracted DNA, and genotyped 154 individual pups across 18 microsatellite loci. Population harvest rates averaged 23.8% (SD = 9.2). Our hypothesis that recruitment would decline was supported; survival from $3 - 15$ months of age decreased from 0.60 (95% CI: 0.48-0.72) without harvest to 0.38 (95% CI: 0.28-0.48) with harvest and recruitment declined from 3.2 (95% CI: 2.1-4.3) to 1.6 (95% CI: 1.1-2.1) pups per pack after harvest was initiated. We cannot unequivocally dismiss other factors that could have reduced recruitment, however, an increase in recruitment when harvest temporarily ceased lends support to our conclusion that harvest reduced recruitment. We attributed just 18-38% of pup mortality directly to harvest and suggest that there are indirect effects of harvest on recruitment that may be associated with changes in group size and structure. Harvest models that do not include both direct and indirect effects of harvest on recruitment may underestimate the potential impact of harvest on population growth in social species.

Keywords: carnivore, *Canis lupus*, gray wolf, groups, harvest, hunting, social, survival**,** trapping

Introduction

Knowledge about recruitment (i.e., the number of surviving young to a given age) within a
population can be critical when making conservation decisions, particularly for harvested
species. Harvest can affect population demography in complex ways and this may be particularly
true for cooperatively breeding species whose successful reproduction is often linked with
complex social dynamics (Malcolm and Marten 1982; Whitman *et al.* 2004; Maldonado-
Chapparo and Blumstein 2008). Several studies have documented the positive influence of group
size on survival and recruitment of young in group-living carnivores (Malcolm and Marten 1982;
Courchamp and Macdonald 2001; Sparkman *et al.* 2011; Stahler *et al.* 2013). Decreases in group
size can lead to a reduction in the ability to adequately guard and provision young (Courchamp,
Rasmussen and MacDonald 2002).

Some simulation studies have provided needed insights into how harvest affects population
growth in social species. For example, Whitman *et al.* (2004) simulated selective harvest of
trophy male African lions (*Panthera leo*) and found that resultant increases in infanticide rates
lead to an increased risk of extinction. Maldonado-Chapparo and Blumstein (2008) found the
selective harvest of females and reproductive suppression were influential factors affecting
population growth in simulated populations of capybaras (*Hydrochoerus hydrochaeris*).
Although we have a rich literature and theoretical basis for understanding cooperative breeding
in mammals (Solomon and French 1997; Russell 2004) we currently have a poor understanding
of how widespread public harvest affects recruitment in such social species.

Estimating recruitment, or net production, in the wild can be challenging because young have
often grown to adult size making accurate visual discrimination of age classes difficult. Despite

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this difficulty, recruitment is a vital rate routinely used by wildlife managers (e.g., elk, *Cervus elaphus*; Peek 2003) and is required to assess the status of some endangered species in the United States (USFWS 1994, 1996a, 1996b). Furthermore, recruitment (along with survival and immigration) is a key component in determining whether harvest is an additional source of mortality or is compensated by increases in other population vital rates (Mills 2013). Recently, some have argued the relative contributions of recruitment and immigration to population growth as well as the overall effect of harvest on populations of social canids (Creel and Rotella 2010; Gude *et al.* 2012). Adams *et al.* (2008) stated that dispersal, resulting in both immigration and emigration, were key components to growth and persistence of a harvested population of gray wolves (*Canis lupus*) in Alaska. These studies did not assess recruitment within groups however, thus the effects of harvest on recruitment in groups of cooperatively breeding canids are not well understood.

Wolves in the U.S. northern Rocky Mountains (NRM) were reintroduced in 1995-1996, with the exception of a small remnant population in northwest Montana (Bangs and Fritts 1996). Since reintroductions, the U.S. Fish and Wildlife Service (USFWS) and states in the NRM have documented recruitment in the population by reporting the number of breeding pairs each year (USFWS 1994). The USFWS defines a breeding pair as an adult male and female and ≥ 2 pups on 31 December of each year (USFWS 1994, 2009). Biologists in the NRM have largely used capture, radiotelemetry, and visual observations (aerial) to document the number of wolf breeding pairs annually (Bangs and Fritts 1996; Mitchell *et al.* 2008). Such an approach relies on: 1) having at least one member of a pack radiocollared, 2) the collared animal travelling with the pups and breeders at the time of the survey, and 3) weather conditions that permit visual observations. Each of these conditions may not always be met and can make estimates

inaccurate. Additionally, gray wolf pups are sufficiently large at eight months of age making accurate visual discrimination from an aircraft challenging for all but the most experienced observers. Recruitment could potentially be inferred using estimates from the literature. Studies reporting estimates of wolf pup survival, however, are typically based on samples of pups collared at 4-6 months of age (Hayes and Harestad 2000) and often in unharvested populations (Mech 1977; Smith *et al.* 2010) leaving a lack of knowledge about early pup survival (i.e., from den emergence to late summer). Biologists could potentially obtain visual counts of young at dens and compare them to late autumn/early winter counts via aerial telemetry (Mech *et al.* 1998) although such estimates of recruitment can be skewed by incomplete counts either at the beginning or end of the time period. Alternatively, biologists could mark pups during denning season (April-May) when pups are very young and relatively immobile (Mills, Patterson, and Murray 2008). This procedure could provide early season estimates of reproduction but is only possible during a relatively short timeframe (i.e., 2-3 weeks), relies on knowledge of active den locations, and must be conducted at a time of year when wolves are most sensitive to disturbance (Frame, Cluff, and Hik 2005).

Once reliable estimates of recruitment are obtained, however, one can begin to determine what factors are driving this important vital rate. For example, declines in prey abundance and outbreaks of disease have been shown to reduce recruitment in wolves (Harrington *et al.* 1983; Mech and Goyal 1993; Johnson, Boyd, Pletscher 1994). Additionally, reductions in group size can lead to decreased recruitment within groups of cooperatively breeding canids (Malcolm and Marten 1982; Courchamp and Macdonald 2001; Sparkman *et al.* 2011; Stahler *et al.* 2012). States in the NRM face the challenge of documenting recruitment (i.e., breeding pairs) in a recovered and harvested population of wolves. The USFWS first removed Endangered Species

Act protections for wolves in the NRM in 2008 but they were subsequently relisted that year. Wolves were delisted again in 2009 at which time states initiated harvest. Subsequent litigation and relisting precluded fall harvest in 2010. Congress removed Endangered Species Act protections for wolves in the NRM with the exception of Wyoming (USFWS 2011). Idaho and Montana resumed public hunting in fall 2011 and Idaho instituted a trapping season in November 2011. U.S. States in the NRM must document recruitment during the required five-year post-delisting monitoring period. In a harvested population of wolves, however, traditional capture and radiocollaring techniques may lose effectiveness because marked animals are harvested requiring a nearly constant effort to capture and radiocollar new individuals. Furthermore, if the population is large, marking a sufficient number of individuals to generate reliable population metrics may not be feasible. Although required for the continued documentation of recovery goals, estimates of recruitment are also important for understanding the effects of the newly reinstated wolf harvest in the NRM.

In this study we used an alternative approach for estimating recruitment based on noninvasive genetic sampling. We did not rely on radiocollared wolves but instead used genetic sampling to estimate first-year survival (from approx. 3 – 15 months of age) of wolf pups and recruitment. We then used a natural experiment and asked whether recruitment had changed in the wolf population after harvest was initiated. An increase in recruitment could occur at lower densities (Stahler *et al.* 2013) possibly because of increased food availability at lower densities as Knowlton (1972) determined for coyotes (*Canis latrans*). Alternatively, harvest may reduce recruitment through direct mortality or indirect effects that are more difficult to measure such as those associated with group size and composition (Courchamp and MacDonald 2001). We hypothesized recruitment (i.e., pup survival to 15 months) would decline after harvest was

initiated. We also hypothesized that the majority of pup mortality would be directly due to harvest.

Materials and Methods

We conducted annual surveys for wolves between mid-June and mid-August for six years (2008-2013) in two study areas in central Idaho, USA (Fig. 1). The east study area was Idaho Department of Fish and Game (IDFG) Game Management Unit (GMU) 28 (3,388 km^2) and the west study area was GMUs 33, 34, and 35 (3,861 km^2). Both areas were dominated by ponderosa pine (*Pinus ponderosa*), lodgepole pine (*P. contorta*), and spruce (*Picea englemannii*) mixed forests and sagebrush (*Artemisia tridentata*) steppe. Annual precipitation ranged from 89-178 cm and temperatures range from -34° C in winter to 38° C in summer (Western Regional Climate Center 2012).

Detailed field sampling and laboratory analysis methods have been published elsewhere (Ausband *et al.* 2010, Stenglein *et al.* 2010, 2011, Stansbury *et al.* 2014), and we provide a summary here. We used radiotelemetry locations of wolves collared as part of annual state monitoring efforts to locate and sample rendezvous sites. In areas that did not contain radiocollared individuals as part of IDFG annual monitoring efforts we surveyed for wolves at historic and predicted rendezvous sites on approx. 15 July. We applied the predictive rendezvous site habitat model described by Ausband *et al.* (2010) and surveyed highly probable (≥70% suitability) rendezvous sites at dawn and dusk when wolves were active and likely to respond to howls (Harrington and Mech 1982). After howling, two technicians separated and surveyed the site for 30-45 minutes looking for wolf signs. At occupied or recently occupied sites, we located the activity center and collected scat samples for 3-4 hours radiating out from the activity center on trails to ensure we collected scats from all available adults in the pack (Joslin 1967; Ausband

et al. 2010; Stenglein *et al.* 2010). We considered scats <2.5 cm diameter to be pup scats

(Ausband *et al.* 2010; Stenglein *et al.* 2010) and those >2.5 cm to be adult wolf scats (Weaver

and Fritts 1979). Pup counts using genotypes resulting from the 2.5 cm discrimination rule for

pup vs adult scats were tested against pup counts from intensively monitored radiocollared wolf

packs and were found to be accurate (Stenglein *et al.* 2010; Stansbury *et al.* 2014). This sampling

approach generated 125-200 samples per pack and could provide genotypes for each animal in

the pack (Stenglein *et al.* 2011). Each site was surveyed and sampled one time. After an active

site was detected and sampled, we excluded other probable rendezvous sites within a 6.4 km

radius to avoid duplicate sampling of packs (Ausband *et al.* 2010). We located and resampled

each pack (n = 10) in the study areas every year. One pack had 2 years (2008 and 2009) removed

from analyses because we were unable to locate the rendezvous site in 2009.

We extracted DNA from samples using Qiagen stool kits (Qiagen Inc., Valencia, CA) in

a room dedicated to low quantity DNA samples and using negative controls to monitor for

contamination. We initially screened all samples in a mitochondrial DNA species-identification

test to remove non-target species and low-quality samples (De Barba *et al*. In Press). We used

nine nuclear microsatellite loci and sex identification primers to identify individuals and gender

as described in Stenglein et al. (2010). We generated an additional nine microsatellite loci on the

best sample for each unique individual (i.e. for a total of 18 genotyped loci) and for samples that

differed at only one locus out of initial nine loci to verify matches or mismatches (Stenglein et al.

2011, Stansbury *et al*. 2014). We used an Applied Biosystems 3130xl capillary machine

(Applied Biosystems Inc., Foster City, CA)) to separate PCR products by size and verified peaks

individually by eye with GENEMAPPER 3.7 (Applied Biosystems Inc., Foster City, CA). We

used Program Genalex v. 6.5 (Peakall and Smouse 2012) to match genotypes from scat samples

and we required >8 loci to confirm detections of the same individual. We initially amplified all samples twice and required successful amplification of alleles at >5 loci for the sample to continue for an additional 1–3 replications. We discarded samples that amplified at <5 loci. For each locus, we required >2 independent PCR amplifications for consensus of a heterozygote and >3 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 program STRUCTURE v.2.3.3 (Pritchard, Stephens, and Donnelly 2000) with reference samples of known wolves (n=66), domestic dogs (*C. l. familiaris*, n=17), and coyotes (*C. latrans*, 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 (MCMC) iterations and 10 iterations to estimate individual ancestry and remove samples highly probable as dogs or coyotes from the dataset. We used RELIOTYPE (Miller, Joyce, and Waits 2002) to test the accuracy of unique genotypes detected in only one sample (i.e. single captures) by ensuring the genotype attained a 95% accuracy threshold. In 2008 and 2009 we analyzed all collected samples. After 2010, we analyzed 40 adult and 25 pup scats from each pack based in part on rarefaction results regarding sampling effort (Stenglein *et al.* 2011). We analyzed additional samples to obtain 10 more consensus genotypes if a pack had >2 individuals detected only once. The actual number of additional samples analyzed in such instances varied because of differences in nuclear DNA amplification success rates.

We estimated population harvest rates using summer counts of wolves from ongoing population monitoring (USFWS 2010, 2012, 2013) and research (Ausband *et al.* 2010) and the spatial locations of harvested wolves in our study areas. To determine the percent of direct pup mortality due to harvest, we obtained tissue samples from harvested wolves and generated

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genotypes for each across the same 18 microsatellite loci. We then matched genotypes of wolf

pups sampled in summer to genotypes generated from harvested wolf pups using Program

Genalex v. 6.5. We allowed for one allele mismatch between matching samples to account for

allelic drop-out in noninvasive samples.

We used the Kaplan-Meier survival model (Kaplan and Meier 1958) adjusted for a single

time period to estimate wolf pup survival from 3-15 months (15 July$_{(t)}$ − 15 July$_{(t+1)}$). We let

y be the number of genotyped pups in one year, and $y - d$ be the number of genotyped pups that

survived until the next year where d was the number of pups that died. The first year survival of

pups was $S = 1 - (d/y)$ and we used Greenwood's formula to estimate the variance $\sigma^2 = S^2 \times$

$d/(y \times (y - d))$ (Klein and Moeschberger 2003). We estimated first-year pup survival by year,

study area, and sex and determined that two survival estimates were different if the 95%

confidence intervals did not overlap. We calculated mean recruitment and standard error by year

and study area. We used a t-test to calculate the average difference in recruitment for years with

harvest and years without harvest and paired by pack.

Results

We collected fecal samples from wolves at 117 potential or known to be occupied (via

radiotelemetry) wolf rendezvous sites in 2 study areas during summers 2008-2013. We collected

and successfully genotyped DNA from fecal samples of 154 wolf pups (Table 1) in 10 wolf

packs. The probability of identity for siblings (i.e., chance that 2 individuals would have the

same genotype) ranged from 3.54 x 10^{-4} to 1.18 x 10^{-3} across study areas. In 8 cases (5.2%)

wolves detected as pups survived to the next year (determined from radiotelemetry and harvest),

but were not detected during summer sampling; we updated their detection histories to represent

the fact that they were alive and were simply not detected by our sampling. Average date of

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sampling was 15 July and average time to sampling the following year was 361.6 (SE = 4.5) days. Harvest mortality rates were approximately 8.0% and 35.4% in 2009/2010, 22.0% and 22.2% in 2011/2012, and 27.1% and 28.1% in 2012/2013 in the east and west study areas, respectively (Table 1).

Wolf pups in the east study area had higher survival rates than the west study area even in years without harvest (0.77, SE = 0.08 vs. 0.47, SE = 0.08). The number of pups born was also slightly higher in the east study area (4.8, SE = 0.6) than the west study area (3.9, SE = 0.5). Across all years, the east study area (0.58 [95% CI: 0.46 – 0.70]) had 20% higher survival than the west study area (0.38 [95% CI: 0.27 – 0.48]). Average recruitment in the east study area was 2.9 (95% CI: 1.9 – 3.8) pups compared to 1.5 (95% CI: 0.8 – 2.0) pups in the west study area.

The average number of pups alive on 15 July was 4.0 (SE = 0.68) in years with harvest and 4.8 (SE = 0.65) in years without harvest. There was no difference in pup survival by sex (males: 0.46 [95% CI: 0.35 – 0.57], females: 0.48 [95% CI: 0.37 – 0.59]. On average, pup survival was 0.60 (95% CI: 0.48 - 0.72) in years without harvest and 0.38 (95% CI: 0.28 - 0.48) in years with harvest (Table 1, Figs. 2, 3). For a given pack, recruitment of pups fell from an average of 3.2 (95% CI: 2.1 – 4.3) pups surviving to 15 months in years without harvest to 1.6 (95% CI: 1.1 – 2.1) in years with harvest ($P = 0.017$; Figs. 4, 5). Approximately 95% of harvested wolves in our study areas had tissue samples collected and DNA extracted. The proportion of pup mortality that was directly attributable to hunting and trapping increased each year and ranged 18-38% ($\bar{x} = 27.4\%$; SE = 6.0%; Table 1).

Discussion

We failed to reject our hypothesis that survival and recruitment of young would decline after harvest was initiated, although we cannot unequivocally dismiss other possible factors that could

have also reduced recruitment. During years with harvest survival decreased the least in 2009-2010; the year with the lowest quotas and lowest overall harvest mortality rates, particularly in our east study area (8%). Some of the decrease we estimated in recruitment can be attributed to decreased reproduction in years of harvest (4.0 vs. 4.8) but not all of the decrease can be accounted for this way. Current levels of recruitment are unlikely to compensate for mortality in other age classes of wolves in the pack and thus we predict pack sizes will continue to decline (IDFG 2014). Immigration into our study areas particularly the west study area, may be required if the management goal were to maintain current population size and levels of harvest. Immigration can play a large and important role in population persistence in some wolf populations (Adams *et al.* 2008), but we currently do not have estimates of immigration into our study areas. Future work could attempt to estimate immigration rates in the population using our sampling approach to determine the number of unrelated wolves adopted by packs each year (Rutledge *et al.* 2010). The resulting immigration rates would allow for the development of population models that include empirically-based estimates of both recruitment within packs and immigration into packs. Such a model could assess population viability over time in light of continued harvest. Currently, average pup survival to 7.5 months is 0.57 ($0.32^{(1/2)}$) and managers could use this to estimate recruitment (i.e., the number of breeding pairs) on 31 Dec assuming a constant mortality rate and the harvest levels we observed.

We rejected our hypothesis that most pup mortality would be directly due to harvest. The relatively low proportion of pup mortality that could be attributed to harvest (approx. 27%) suggests effects on recruitment beyond simply the number of young harvested. Harvest can decrease recruitment and group size simultaneously thus it is difficult to disentangle the direct (i.e., pups harvested) and indirect effects (i.e., fewer helpers leading to decreased recruitment) of

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harvest. Harvest can reduce group size which in turn may reduce recruitment but harvest would still remain the ultimate cause of the decline in recruitment. Decreases in group size can lead to a reduction in pup-guarding ability (Courchamp, Rasmussen and MacDonald 2002) and increased predation of young and reductions in provisioning rates, particularly at low prey densities (Harrington, Mech, and Fritts 1983). Average group size has decreased since harvest began in Idaho (IDFG 2014) and this may have had indirect effects on survival and recruitment of young. Although harvest and group size can be correlated, disentangling the effects of harvest on group size and composition and how they in turn affect recruitment is fertile ground for future research. Harvest models that do not include both direct and indirect effects of harvest on recruitment may underestimate the potential impacts of harvest on population growth in social species. Several studies have documented the positive influence of group size on pup survival and recruitment in group-living carnivores (Malcolm and Marten 1982; Courchamp and Macdonald 2001; Sparkman *et al.* 2011; Stahler *et al.* 2013).

In addition to detecting declines in recruitment after harvest, we also found differences in survival and recruitment between study areas. Food availability may explain differences in survival and recruitment between the study areas. We did not have prey abundance estimates for our east study area but it is possible that prey exists at higher densities in the east than the west area. Litter sizes in the east study area were slightly higher (4.8, SE = 0.6) than the west study area (3.9, SE = 0.5) suggesting that prey and subsequent reproductive output was greater in the east study area. We considered 2 other potential influences to explain differences in survival and recruitment between the 2 study areas; poaching and intraspecific (i.e., wolf on wolf) mortality. Wolves in the west study area may have experienced increased poaching mortality because of their relatively close proximity to a large urban area with a high human density (Boise, ID).

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Using data from 98 radiocollared wolves in our study areas, we found no evidence that poaching

rates were higher in the west (11.0%) than east (12.5%) study area. We did, however, find some

evidence of intraspecific mortality in the west (6.1%) yet no evidence in the east study area.

Wolves in the west study area congregate along a river corridor that is elk range in winter.

Monitoring flights have found wolf packs just 1.6-3.2 km apart in the west study area while on

the winter range (J. Struthers, IDFG, unpublished data). We did not observe such high densities

and potential for intraspecific killing in the east study area (J. Husseman, IDFG, unpublished

data). The available data suggests a small difference in intraspecific mortality rates, but does not

fully explain differences in survival and recruitment between the study areas.

Generally, fecal DNA of carnivores degrades rapidly in the natural environment (Piggott

2004, Santini *et al.* 2007, Murphy *et al.* 2007); however, we have found that some scats may

contain DNA that persists longer in our environment than previously thought (D. Ausband,

unpublished data). If DNA does persist >1 year in our study areas it may have biased our

survival rates higher than the true value. Lastly, not detecting an animal at year 2 (i.e., false

negative) may negatively bias our survival estimates, although Stenglein *et al.* (2011)

demonstrated that each animal in a pack can be detected with our sampling technique. By

sampling packs multiple years we found false negatives (i.e., alive at 15 months but not detected)

5% (n = 154) of the time allowing us to correct these animals' detection histories. Despite the

demonstrated low probability that we missed individuals more often at year 1 such a bias would

be found in all years of our data. Thus, although our survival estimates would be biased low, our

results and conclusions would remain unchanged. Jimenez *et al.* (In Review) estimated that 4%

of wolves in the NRM during 1993-2008 disperse in their first year. Therefore, our data may

include animals that dispersed from their natal pack in their first year and did not die. The

number of such animals would be quite low (~1 wolf in our sample each year), however, and would not likely affect our survival rates appreciably.

We documented a correlation between harvest and recruitment. Control sites would be necessary to unequivocally exclude other variables that may have reduced recruitment, however, there is no such area available in Idaho because harvest is statewide. While declines in prey abundance and disease outbreaks have been shown to reduce recruitment in wolves (Harrington *et al.* 1983; Mech and Goyal 1993; Johnson, Boyd, Pletscher 1994) neither were observed during our study (USFWS 2010, 2011, 2012, 2013; IDFG unpublished data). Additionally, we note that if recruitment declined because of some unmeasured external factor it is unlikely we would have documented an increase in recruitment during the year that harvest temporarily ceased (2010-2011; Figs 2, 3). Although it appears that prey abundance and disease were not influential during our study future work should attempt to control for these potentially confounding variables.

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Table 1. Mean survival from 3-15 months, number of gray wolf pups recruited into packs, and percent of mortality attributable to harvest before and after harvest in Idaho, USA, 2008-2013.

Year	Population harvest rate (%)	N	Mean pup survival (SE)	Mean pups recruited (SE)	Mortality directly attributable to harvest (%)
2008-2009	0.0	20	0.60 (0.11)	2.4 (0.5)	N/A
2009-2010	21.7	23	0.50 (0.11)	1.8 (0.8)	18.2
2010-2011	0.0	42	0.60 (0.08)	3.1 (0.7)	N/A
2011-2012	22.1	38	0.36 (0.08)	1.8 (0.7)	25.9
2012-2013	27.6	31	0.32 (0.08)	1.1 (0.4)	38.1

Figure 1. Study areas in Idaho, USA where wolves were sampled genetically to estimate pup survival and recruitment before and after harvest, 2008-2013.

Figure 2. Mean wolf pup survival from 3-15 months by year before and after harvest in Idaho, USA, 2008-2013. Errors bars represent SE.

Figure 3. Mean wolf pup survival from 3-15 months before and after harvest in Idaho, USA, 2008-2013. Errors bars represent 95% CI.

Figure 4. Mean wolf pups recruited by year before and after harvest in Idaho, USA, 2008-2013. Errors bars represent SE.

Figure 5. Mean wolf pups recruited before and after harvest in Idaho, USA, 2008-2013. Errors bars represent 95% CI.

Figure 1.
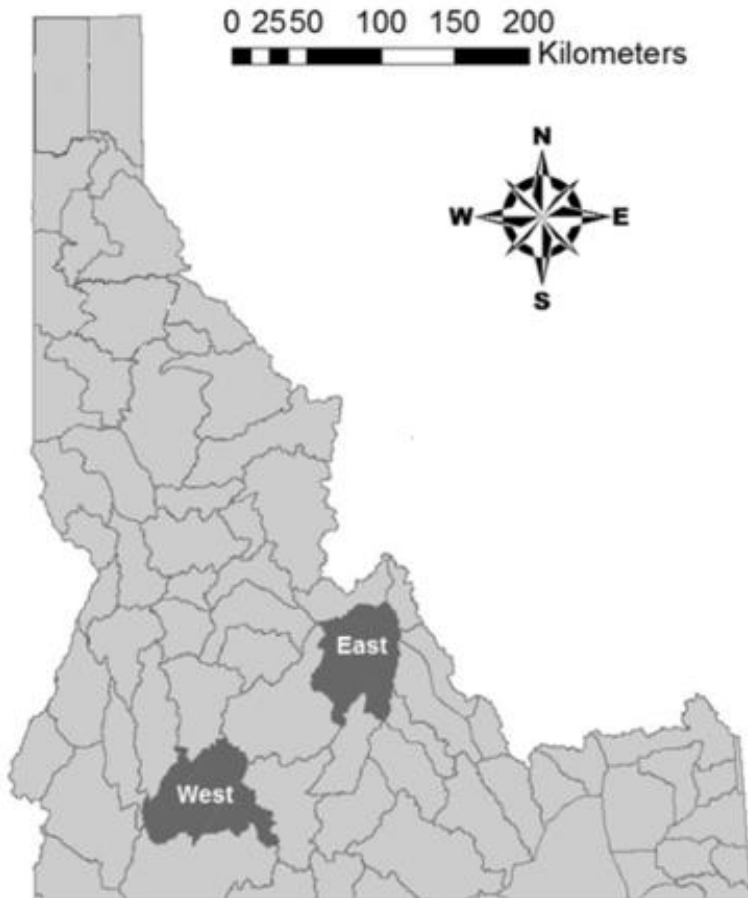
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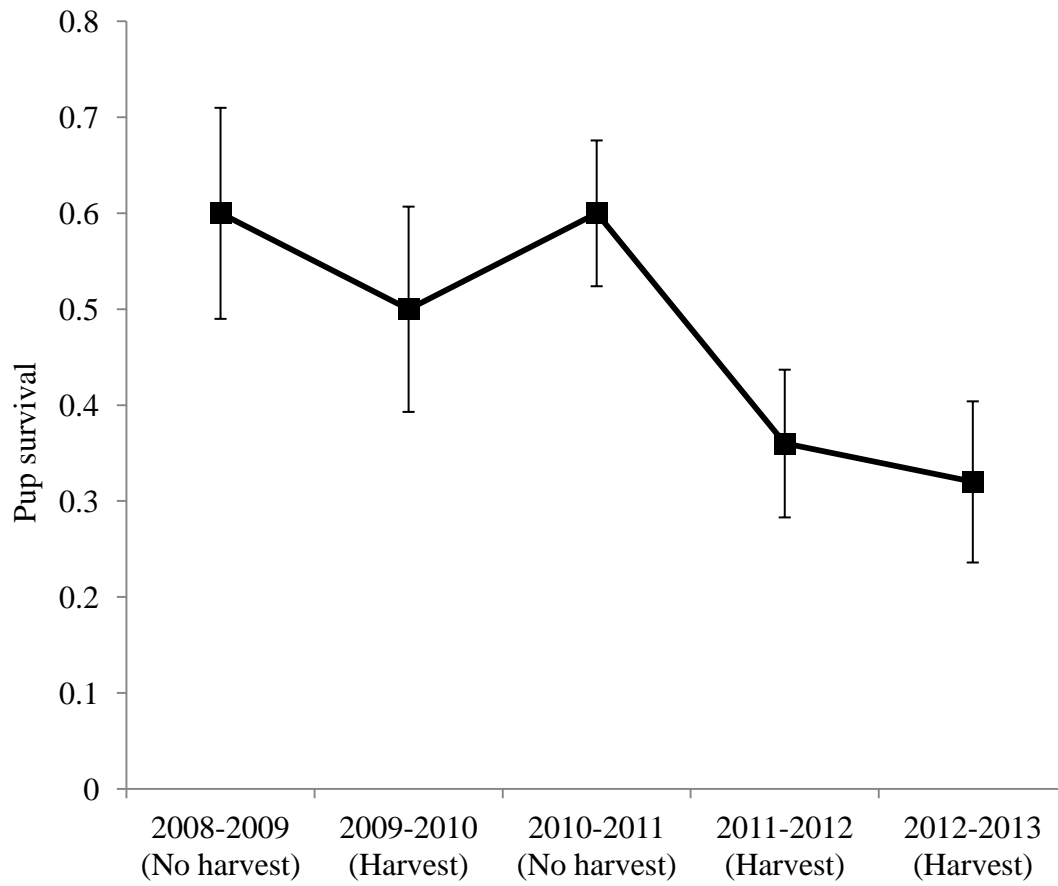
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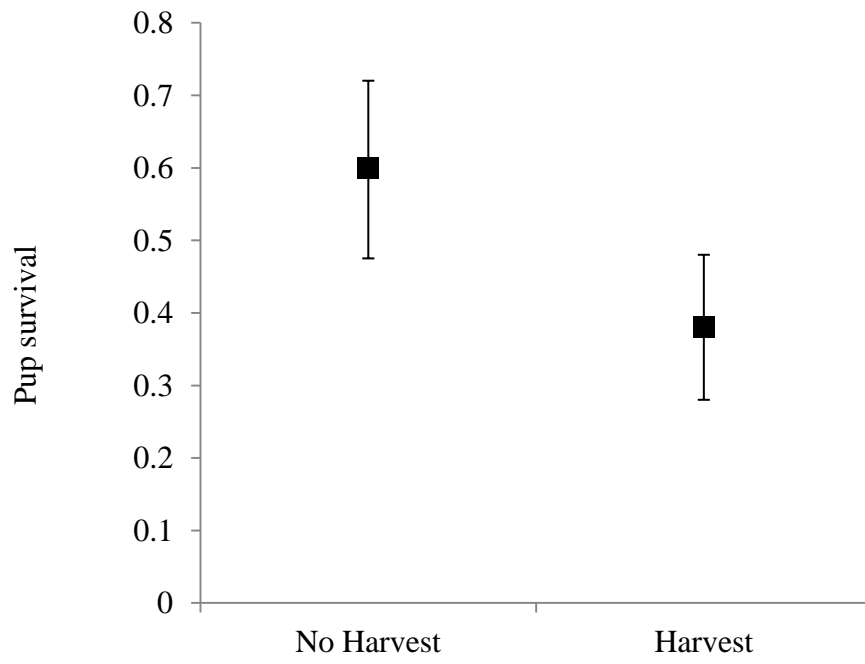
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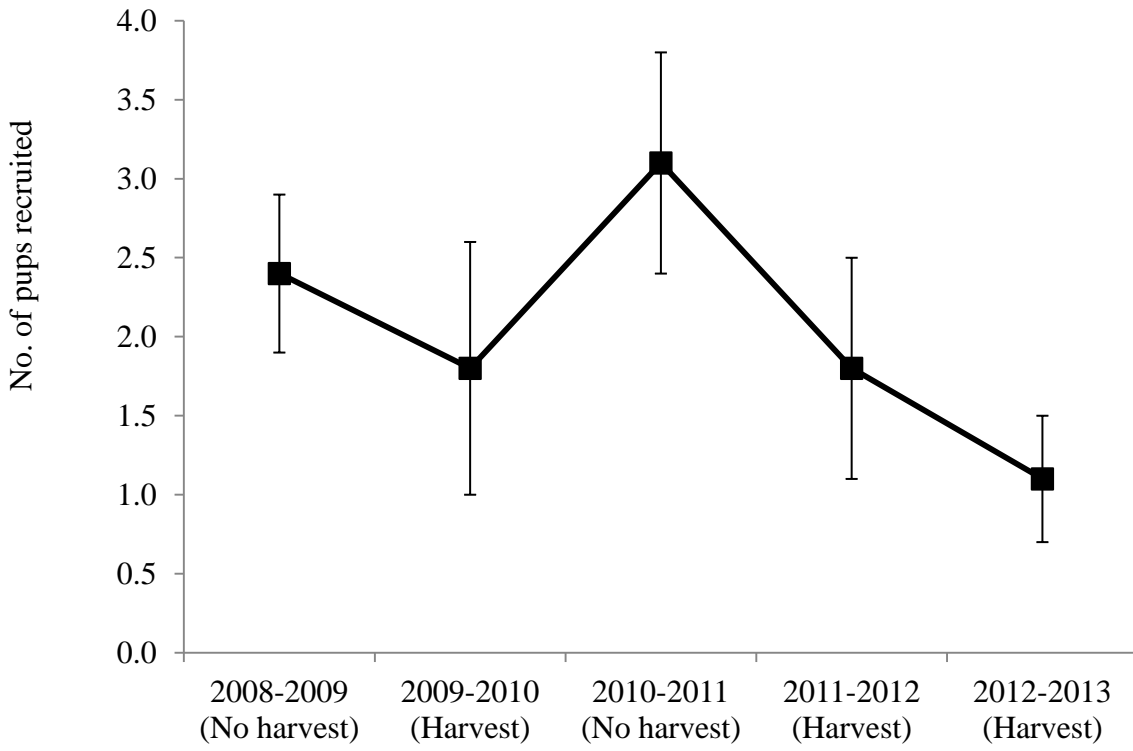
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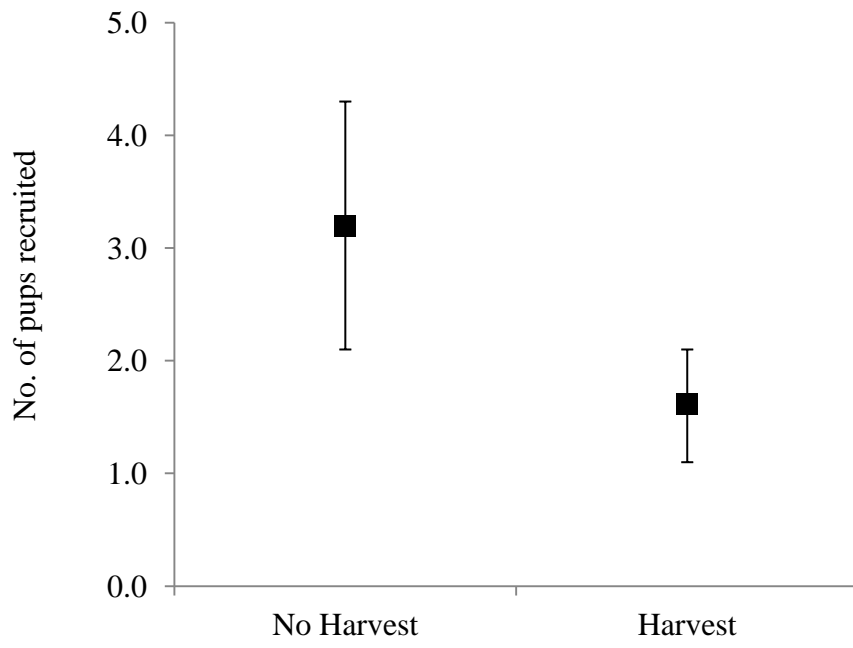
Title: Effects of mortality on recruitment and groups of cooperative breeders

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Abstract

Recruitment in cooperative breeders can be negatively affected by reductions in group size, changes to group composition and breeder turnover. We wanted to know how mortality, in the form of persistent harvest, affects group size, composition, and ultimately recruitment (i.e., pup survival to 15 months) in a cooperative breeder. We used noninvasive genetic sampling and 18 microsatellite loci to construct group pedigrees and estimate recruitment for gray wolves (*Canis lupus*) under 3 different harvest regimes ranging from heavily harvested to fully protected in Alberta, Canada, and Idaho and Yellowstone National Park (YNP), USA. We hypothesized that harvest reduces recruitment because of reduced group size, reduced intragroup diversity (i.e., fewer adults of varied sex and age classes), and breeder turnover. Alternatively, harvest increases recruitment possibly due to increased food availability or harvest mortality does not affect recruitment differently than natural mortality.

Harvest reduced recruitment and group size, intragroup diversity, breeder turnover, and the potential to inherit a breeding position all affected recruitment as well. Group size, and related metrics (number of breeders present), weakened the negative effects of harvest on recruitment. Not all additions to group size had positive effects, however. The presence of older nonbreeding males reduced recruitment. Given this, selection should favor female-biased sex ratios and relatively early dispersal (or expulsion) for males; we observed both albeit over a limited timeframe. We show that ameliorating the negative effects of harvest on recruitment is one benefit of group-living but individuals are not equal in their contributions to recruitment within groups.

Introduction

Group living has evolved across a wide range of taxa and species. Many group living species display cooperative breeding behavior. Cooperative breeding generally refers to the cooperative care of related, or even unrelated, young by helpers (i.e., nonbreeding individuals in the group) within a group (Solomon and French 1997). In mammals, both manipulative and observational studies have shown that the presence of helpers can be critical to breeder fitness and group persistence (Solomon and French 1997; Courchamp et al. 2000; Courchamp and Macdonald 2001; Courchamp et al. 2002; Stahler et al. 2013).

The number of helpers in a group can positively influence recruitment (Tardif et al. 1984; Solomon and French 1997; Clutton-Brock 2006), but group composition may also have an important influence on recruitment and population growth in cooperative breeders. For example, selective removal of male African lions (*Panthera leo*) results in instances of infanticide and reduced population viability (Whitman et al. 2004). Breeder turnover and reduced genetic relatedness within groups can affect both recruitment and group survival in other cooperatively

breeding mammals as well (Solomon and French 1997; Pope 2000; Brainerd et al. 2008; Gobush et al. 2008; Borg et al. 2014). Group composition may also be important because not all age and sex classes help equally within a group. Individuals in groups of gray wolves (*Canis lupus*), for example, vary widely in the amount of pup-guarding behavior they display (Thurston 2002; Ruprecht et al. 2012; Ausband et al. In Review). Considering the importance of pup-guarding to recruitment in African wild dogs (Courchamp et al. 2002), groups of wolves that have diverse sex and age classes may also have experienced adult helpers that contribute more to rearing young (Lawton and Guindon 1981; Tardif 1997) 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, as found in gray wolves (Cassidy 2013) and African wild dogs (Creel and Creel 1995). Given their complex social structures, territorial defense that relies in part on large group size, and persistent harvest regimes, gray wolves are an ideal species for studying the relationships between mortality, group size and composition, and recruitment in cooperative breeders.

Unharvested wolf groups are typically composed of a breeding pair and 2-3 generations of offspring where young remain in their natal group and care for subsequent years' offspring. In the Rocky Mountains of the U.S., wolves generally do not disperse from their natal group until 3 years of age even though they are reproductively mature at 22 months (Jimenez et al. In Review). If selection has favored breeding wolves that retain mature offspring and diverse group structures then recruitment may be negatively affected by events that simplify intragroup diversity (i.e., number of different sex and age classes). Groups of gray wolves in Idaho, USA, had significantly lower recruitment after public harvest was initiated but the number of pups harvested could not entirely account for the decline in recruitment (Ausband et al. 2015). Indirect effects of harvest

on recruitment, perhaps because of reduced group size or altered group composition may form the mechanism underlying observed changes in recruitment. Generally, we have a poor understanding of how persistent mortality affects group characteristics and recruitment in cooperative breeders because most studies of cooperative breeding have not encompassed human harvest.

We examined how varying levels of mortality, in the form of persistent public harvest, affects group size, composition, and ultimately recruitment in a cooperatively breeding mammal. We used noninvasive genetic sampling and 18 microsatellite loci to construct group pedigrees and estimate the probability of recruitment for gray wolves under 3 different harvest regimes ranging from heavily harvested to fully protected. We posited that harvest would negatively affect group size and composition and as a result would reduce a group's ability to successfully rear young and grow in number of individuals. Specifically, we hypothesized that harvest, 1) reduces the probability of recruitment because of reduced group size, 2) reduces the probability of recruitment because of reduced intragroup diversity (i.e., fewer adults of varied sex and age classes), 3) reduces the probability of recruitment because of breeder turnover, 4) alternatively, harvest increases the probability of recruitment possibly due to increased food availability, or 5) harvest does not affect the probability of recruitment differently than natural mortality.

Study Areas

We had 3 study areas in Idaho, southwest Alberta, Canada, and Yellowstone National Park (YNP), Wyoming. The 3 study areas represented a wide range of human-caused mortality from heavily harvested and agency-controlled (southwest Alberta and central Idaho) to fully protected (YNP).

From 2008-2014, 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. Idaho is mountainous and dominated by a mix of ponderosa pine (*Pinus ponderosa*), lodgepole pine (*P. contorta*), and spruce (*Picea englemannii*) forests and sagebrush (*Artemisia tridentata*) steppe. Annual precipitation ranges from 89-178 cm and temperatures range from -34° C in winter to 38° C in summer (Western Regional Climate Center 2014). Public harvest of wolves began in Idaho in 2009, temporarily ceased in 2010 and began again in 2011. Population harvest rates in our Idaho study areas average 24% (Ausband et al. 2015). Control actions to address livestock depredations are rare in our study groups in Idaho.

During summers 2012-2014 we also sampled wolves in 5-6 groups in YNP. YNP is dominated by lodgepole pine forests and expansive meadow systems. YNP is relatively dry and precipitation averages 47 cm annually and temperature fluctuations range from -39°C in winter to 30°C in summer at Yellowstone Lake (Western Regional Climate Center 2014). Wolves exist at relatively high densities and there is no human hunting inside YNP.

Lastly, during summers 2012-2014 we also sampled wolves in 2 groups in southwest Alberta. Southwest Alberta is a highly contrasted landscape where mountainous forests meet the dry short-grass prairie region. Mountain forests are dominated by Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine forests. Where forest meets prairie there are expansive aspen (*Populus tremuloides*) forests dominated by livestock grazing. Temperatures range from -32°C to 23°C and precipitation averages 40 cm annually on the prairie (Alberta Agriculture and Rural Development, 2014). Wolf densities are thought to be managed at low levels in southwest Alberta and wolf control actions, bounties, and human harvest are presumed to be higher than the Idaho study areas.

Methods

Field methods

When available, we used radiotelemetry locations of wolves to locate rendezvous sites and collect wolf scat samples for subsequent DNA analyses. In areas that did not contain radiocollared individuals as part of agency monitoring we surveyed for wolves at historic and predicted rendezvous sites. We applied a predictive rendezvous site habitat model (Ausband et al. 2010) and surveyed highly probable (≥70% suitability) rendezvous sites at dawn and dusk (Harrington and Mech 1982). After howling, 2 technicians separated and surveyed the site for 30-45 minutes looking for wolf signs. At occupied or recently occupied sites, we located the activity center and collected pup and adult scat samples for 3-4 hours radiating out from the activity center on trails to ensure we collected scats from all available adults in the pack (Joslin 1967; Ausband et al. 2010; Stenglein et al. 2010). We considered scats <2.5 cm diameter to be pup scats (Ausband et al. 2010; Stenglein et al. 2010) and those >2.5 cm to be adult wolf scats (Weaver and Fritts 1979). Pup counts using genotypes resulting from the 2.5 cm discrimination rule for pup vs. adult scats were tested against pup counts from intensively monitored radiocollared wolf groups and were found to be accurate (Stenglein et al. 2010; Stansbury et al. 2014). This sampling approach generated 125-200 samples per pack and could provide genotypes for each animal in the pack (Stenglein et al. 2011). We used data only from reproductively active groups because we could not be sure we sampled every animal in the group if their movements were not centered at a pup-rearing site. Each site was surveyed and sampled one time. After an active site was detected and sampled, we excluded other probable rendezvous sites within a 6.4 km radius to avoid duplicate sampling (Ausband et al. 2010). We attempted to locate and resample each group every year. Additional detail on field and laboratory methods

have been published elsewhere (Ausband et al. 2010, Stenglein et al. 2010, 2011, Stansbury et al. 2014).

Laboratory methods

DNA analyses on collected scat samples were performed at the University of Idaho's Conservation Genetics Laboratory (Moscow, ID). We extracted DNA from samples using Qiagen stool kits (Qiagen Inc., Valencia, CA) in a room dedicated to low quantity DNA samples and using negative controls to monitor for contamination. We initially screened all samples in a mitochondrial DNA species-identification test to remove non-target species and low-quality samples (De Barba et al. 2014). We used nine nuclear microsatellite loci and sex identification primers to identify individuals and gender as described in Stenglein et al. (2010). 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 (Stenglein et al. 2011, Stansbury et al. 2014). We used an Applied Biosystems 3130xl capillary machine (Applied Biosystems Inc., Foster City, CA) to separate PCR products by size and verified peaks individually by eye with GENEMAPPER 3.7 (Applied Biosystems Inc., Foster City, CA). We used Program Genalex v. 6.5 (Peakall and Smouse 2012) to match genotypes from scat samples and we required >8 loci to confirm detections of the same individual. We initially amplified all samples twice and required successful amplification of alleles at >5 loci for the sample to continue for an additional 1–3 replications. We discarded samples that amplified at <5 loci. For each locus, we required >2 independent PCR amplifications for consensus of a heterozygote and >3 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 program STRUCTURE v.2.3.3 (Pritchard et

al. 2000) with reference samples of known wolves (n=66), domestic dogs (*C. l. familiaris*, n=17), and coyotes (*C. latrans*, 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 (MCMC) iterations and 10 iterations to estimate individual ancestry and remove samples highly probable as dogs or coyotes from the dataset. We used RELIOTYPE (Miller et al. 2002) to test the accuracy of unique genotypes detected in only one sample (i.e. single captures) by ensuring the genotype attained a 95% accuracy threshold. In 2008 and 2009 we analyzed all collected samples. After 2010, we analyzed 40 adult and 25 pup scats from each pack based in part on rarefaction results regarding sampling effort (Stenglein et al. 2011). We analyzed additional samples to obtain 10 more consensus genotypes if a pack had >2 individuals detected only once. The actual number of additional samples analyzed in such instances varied because of differences in nuclear DNA amplification success rates.

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 Program COLONY version 2.0.5.5 (Jones and Wang 2009). 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 Program COANCESTRY version 1.0.1.5 (Wang 2011) and then imported those into Program 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 2 allele

mismatch owing to allelic dropout between parent and offspring to verify parentage across the 18 loci. We sampled 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 nonbreeding females, ≥ 2 year old nonbreeding females, 1 year old nonbreeding males, ≥ 2 year old nonbreeding males, unknown age females, unknown age males, female pups, male pups) and recruitment (pups reared to 15 months). We obtained such detailed group compositions before and after harvest in Idaho as well as in Alberta and YNP. We defined intragroup diversity as the number of individuals in the group multiplied by number of sex and age classes represented. We documented breeder turnover between years and estimated the number of helpers (nonbreeding females and nonbreeding males) and breeders present at $t = 3$ months (i.e., pups 3 months old) and $t = 15$ months (i.e., pups 15 months old; only helpers ≥ 2 years old at $t = 15$ months because 1 year old helpers at $t = 15$ months are not independent with the response variable, recruitment).

We used logistic regression with recruitment as the response variable to look for potential differences in recruitment before and after harvest in Idaho. We treated each sampled pup as a case, considered whether they were alive or dead at 15 months a binary response, and defined recruitment as the probability of surviving to 15 months of age. We also used logistic regression to ask whether recruitment before harvest in Idaho resembled recruitment levels we measured in YNP and whether recruitment after harvest in Idaho resembled levels measured in southwest Alberta. We used a generalized linear model with a Poisson distribution to assess whether litter sizes at 3 months were different among the 3 study areas. We used multiple logistic regression with recruitment as the response variable to assess the relative influence of harvest, intragroup diversity, group size (at $t = 3$ months and $t = 15$ months), and breeder turnover on recruitment.

Additionally, we used multiple logistic regression to assess the influence of each sex and age class and study area on recruitment. We used all data from all study areas to first assess the influence of harvest on recruitment. We then constructed models using data from Idaho to examine the influence of group composition and size on recruitment in years when there was harvest. We used Akaike's Information Criteria (AIC) to evaluate the relative support for each model and assessed the likelihood of the model given the data using model weight (w_i; Burnham and Anderson 2002). We used the receiver operating characteristic (ROC) to assess model fit and assumed reasonable fit when the area under curve was >0.70.

Results

We genotyped 279 adults and 193 pups in 10 groups in Idaho during 2008-2014. We genotyped 31 adults and 35 pups in 2 groups in Alberta, and 85 adults and 47 pups in 4 groups in YNP during 2012-2014 (Table 1). Litter sizes at 3 months of age were 5.0 (SE = 0.47), 5.8 (SE = 0.95), and 2.6 (SE = 0.32) for Idaho, Alberta, and YNP, respectively. YNP had significantly fewer pups at 3 months than Alberta (p = 0.02) or Idaho (p = 0.001).

Harvest was the most influential variable that negatively affected the probability of recruitment for pups across the 3 study areas (Table 2). Recruitment of pups to 15 months of age declined significantly in Idaho in years when wolves were harvested (3.69 vs 1.65 pups/group; logit = 0.56 − 1.26 (harvest); p < 0.0001). The probability of a pup being recruited did not differ between YNP and Idaho before harvest (0.62 vs. 0.67; p = 0.66) but differed significantly after harvest began in Idaho (0.67 before vs. 0.37 after; p = 0.003). The probability a pup was recruited in Alberta (0.13) was significantly lower than Idaho (0.37) even after harvest began (p = 0.04).

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Across all study areas and years, the number of breeders in a group when pups reached 15 months of age had a significant positive effect on the probability of recruitment. The number of nonbreeding males ≥2 years old when pups reached 15 months of age had a negative effect on the probability of recruitment although the number of >1 year old nonbreeding males in a group initially had a slight positive effect on the probability of recruitment when pups were young (Table 2).

In years with harvest in Idaho, a global model that included group size, intragroup diversity (relative abundance of sex and age classes), and breeder turnover had the most support for predicting wolf pup recruitment (Table 3). Group size when pups reached 15 months of age had a positive effect on the probability of recruitment during harvest. Intragroup diversity and breeding male turnover when pups reached 15 months of age had a negative effect on the probability of recruitment during years with harvest (Table 4). Similar to the model across all study areas and years (Table 2), the number of ≥2 year old nonbreeding males present when pups reached 15 months had a significant negative effect on the probability of recruitment (odds ratio = 0.34; 0.12-0.96, 95% CI) whereas the number of breeders present at 15 months had a significant positive effect on the probability of recruitment (odds ratio = 3.88; 1.33-11.28, 95% CI) during years with harvest. For discussion purposes, we use the term "recruitment" hereafter to represent the probability of recruitment.

Discussion

We show that a benefit of group-living is that the negative effects of harvest on recruitment can be weakened by group size. The odds of recruitment increased >5 times for each additional adult in a group when pups reached 15 months of age (Table 4). Not all additions to group size had positive effects, however. The presence of older nonbreeding males particularly reduced recruitment. Increases in intragroup diversity (number of sex and age classes in a group

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when pups reached 15 months of age) had negative effects on recruitment and we posit this is related to the effect of older nonbreeding males and sample size limitations. Three groups had ≥ 2 nonbreeding males and none of these groups recruited pups.

Individuals such as older nonbreeding males may cheat (i.e., not help) to increase the benefits of group-living for themselves and such behavior has been widely documented (Wenseleers and Ratnieks 2006; Crofoot and Gilby 2012). Older nonbreeding male helpers may not participate as much as female helpers in provisioning or guarding young, at least during portions of the pup-rearing season (Ausband et al. In Review). Older nonbreeding male helpers may have increased fitness by dispersing rather than waiting to inherit a breeding position in their natal group; a strategy female wolves appear to use more often than males (Von Holdt et al. 2008). Conversely, adult males may be involuntarily expelled from the group because of the negative effects that sexually mature males have on the fitness of breeders. Given the lower likelihood of inheriting a breeding position in their natal group, one might expect males to be selfish, grow large, and help less. Additionally, although they may help increase prey acquisition rates (MacNulty et al. 2009a) older nonbreeding adult males may also consume more at kills due to their larger body size (MacNulty et al. 2009b). Clearly, several plausible hypotheses exist to explain the negative effect older nonbreeding males have on pup recruitment.

Our model predicts that during years with harvest pups in groups with 2 breeders and 2 adult male helpers had a 0.25 probability of surviving to 15 months whereas pups in groups with 2 breeders and 2 adult female helpers had a 0.43 probability of surviving to 15 months. Given the disparate probabilities of recruitment for groups with adult females compared to adult male helpers one might expect selection to favor groups that expel adult males and recruit adult females perhaps through skewed sex ratios of litters. We found evidence of both earlier dispersal

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for males (perhaps due to expulsion) and sex ratios that were biased toward females. Of the helpers that stayed with their natal pack for ≥ 3 years, only 29% were males yet 71% were females. This sex-biased philopatry allowed female helpers to obtain a breeding position in 10 cases whereas male helpers only bred in their natal pack 4 times. Helper males may have been expelled from the group or died at an earlier date than female helpers. Harvest began in Idaho in 2009, rates have gradually increased (22% in 2009 to 28% in 2013; Ausband et al. 2015), and in our study groups we observed an increase in the proportion of females in litters in recent years (Fig. 1). Pen and Weissing (2000) predicted that groups with few helpers would produce the helping sex whereas groups with helpers would produce the opposite sex. We found no such trend in our data. Sex ratios varied annually to some degree and we caution against inferences about biased sex ratios and selection without further study. The harvested wolf population in Alberta showed no trend toward female-biased litters (0.50:0.50). Only 3 of 23 pups were recruited in Alberta and the effect of harvest may be strong enough to overwhelm the potential benefits of skewed helper sex ratios for increasing recruitment.

Breeder turnover has been found to reduce recruitment in cooperative breeders (Whitman et al. 2004; Brainerd et al. 2008; Maldonado-Chapparo and Blumstein 2008; Borg et al. 2014) and we found that turnover of breeding males in particular had negative effects on recruitment in years with harvest. Male vacancies were often filled by males adopted from outside the group (71.4%, n = 14). Such individuals may not have been as effective as 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. In contrast (78.9%, n=19), vacancies caused by losses of breeding females were filled by nonbreeding females within the group.

The number of breeders present at 15 months (which may or may not have included the initial breeders when pups were 3 months old) was a strong predictor of recruitment across all study areas and years and also only in years with harvest. Maintaining breeders in the group, even if they are new individuals, can increase recruitment. Additionally, in some cases adult 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. Under group augmentation theory, such wolves would be expected to help more and thus potentially increase recruitment (Kokko et al. 2001).

Genetic relatedness within groups can influence recruitment because helpers preferentially direct care to related young (Tardif 1997). We did not find a significant reduction in genetic relatedness between helpers and pups after harvest began ($r = 0.31$ vs. 0.29; $p = 0.23$) thus we did not include relatedness in our analyses. Relatedness between helpers and young in the protected population of wolves in YNP was 0.20 yet 0.29 for a heavily harvested population of wolves in Alberta. Genetic relatedness may be a poor measure to look for effects on recruitment in gray wolves because relatedness within a group can be quite high (e.g., $r = 0.50$) even when there is just one helper and its sibling young. Given our results showing that nonbreeding adult males are associated with decreased recruitment, if the lone helper is an adult male then genetic relatedness to the sibling young may be inconsequential in such cases.

Recruitment in Idaho before harvest was similar to levels measured in unharvested YNP but was significantly lower after harvest was initiated. Therefore, we reject our hypothesis that harvest and increasing mortality do not affect recruitment differently than natural mortality in

unharvested wolf populations. Alberta had much lower pup recruitment rates than either Idaho (after harvest) or YNP.

Sample sizes are limited for Alberta (2 groups over 3 years) thus an Alberta-specific model is not appropriate, 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. 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 recruitment in Alberta. Given the very low levels of recruitment we measured in southwest Alberta, it appears this population of wolves is likely dependent on immigration for population persistence.

Studies have found increased food availability after high human-caused mortality events (e.g., control) can lead to increases in recruitment in some canids (i.e., coyotes, *Canis latrans*; Knowlton et al. 1999). We found no evidence that recruitment increased after harvest. Hypothetically, harvest could create breeding vacancies and areas where groups are no longer extant thus small reproductive pairs and groups could proliferate. Such a scenario could lead to increased recruitment in the population via more breeding pairs. We have not found this to be true in our study areas nor has the number of groups increased statewide in Idaho since harvest began (USFWS 2012, 2013). Our analyses focused on reproductive groups because they could be adequately sampled with confidence, thus years when groups did not have pups were not included in our analyses. As a result, it is likely that we underestimate the effect of harvest on recruitment at the population level.

Recruitment, although important, is just one component for measuring fitness in cooperatively breeding carnivores. Behaviors 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 fulfill different roles during foraging (MacNulty et al. 2009a, b). Maintaining diverse sex and age classes in a group may enhance foraging success and lead to larger body size in breeders, thus positively affecting fitness. Group size can also influence territory maintenance and defense (Creel and Creel 1995; Cassidy 2013) leading to increased fitness for breeders. Mortality can influence both group size and composition which in turn affect recruitment, territory maintenance and defense, and foraging success. Determining how persistent mortality due to harvest also influences group-living benefits such as territory defense and foraging success can enhance our understanding of the evolution and maintenance of group living in managed populations of cooperative breeders.

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Table 1. Group composition and number of pups recruited in groups of wolves in Alberta, Canada, and Idaho and Yellowstone National Park, USA.

Study area	Year	Group	No. of breeders	No. of nonbreeding females	No. of nonbreeding males	No. of pups at 3 months	No. of pups at 15 months
Alberta	2012-2013	Castle River	2	1	2	7	0
	2012-2013	Willow Creek	2	0	3	7	0
	2013-2014	Castle River	2	2	0	6	2
	2013-2014	Willow Creek	1	0	5	3	1
Idaho	2008-2009	Bear Valley	2	8	6	4	2
	2008-2009	Casner Creek	2	2	1	3	2
	2008-2009	Jureano Mtn	4	3	3	6	3
	2008-2009	Moyer Basin	2	4	5	5	4
	2008-2009	Scott Mtn	2	2	0	1	1
	2009-2010	Casner Creek	2	4	1	4	3
	2009-2010	Hoodoo	2	5	2	4	0
	2009-2010	Jureano Mtn	3	1	7	3	2
	2009-2010	Moyer Basin	1	6	7	6	5
	2009-2010	Wapiti	2	4	4	6	2

2010-2011	Archie Mtn	2	1	2	9	0
2010-2011	Bear Valley	2	1	0	6	6
2010-2011	Casner Creek	2	6	0	2	2
2010-2011	Hoodoo	3	2	2	12	9
2010-2011	Jureano Mtn	2	1	2	4	3
2010-2011	Moyer Basin	4	2	4	9	9
2010-2011	Timberline	2	0	2	7	4
2010-2011	Wapiti	2	5	1	9	3
2011-2012	Bear Valley	2	3	4	5	0
2011-2012	Hoodoo	3	5	4	14	8
2011-2012	Jureano Mtn	3	2	3	2	0
2011-2012	Little Anderson	2	1	4	1	0
2011-2012	Moyer Basin	2	4	6	6	2
2011-2012	Scott Mtn	2	0	0	3	2
2011-2012	Timberline	2	2	3	4	1
2011-2012	Wapiti	2	7	2	8	2
2012-2013	Bear Valley	2	3	2	2	0
2012-2013	Casner Creek	2	1	0	3	0
2012-2013	Hoodoo	2	8	3	4	4

	2012-2013	Jureano Mtn	2	1	1	5	2
	2012-2013	Little Anderson	2	0	0	7	0
	2012-2013	Moyer Basin	2	0	2	5	3
	2012-2013	Scott Mtn	2	1	1	1	0
	2012-2013	Timberline	2	1	2	4	0
	2012-2013	Wapiti	2	3	2	3	1
	2013-2014	Jureano Mtn	2	1	2	7	3
	2013-2014	Scott Mtn	2	1	1	3	3
	2013-2014	Timberline	1	0	1	1	0
	2013-2014	Wapiti	2	2	0	5	0
YNP	2012-2013	Cougar Creek	4	1	2	4	5
	2012-2013	Junction Butte	0	8	3	2	3
	2012-2013	Bechler	4	3	1	2	5
	2012-2013	Snake River	4	0	2	2	3
	2013-2014	Cougar Creek	3	3	3	2	6
	2013-2014	Junction Butte	2	4	1	4	4
	2013-2014	Bechler	3	1	4	2	4
	2013-2014	Snake River	2	1	2	3	3

Table 2. Logistic regression parameters (SE) and odds ratios from model (AUC = 0.78) predicting the probability of wolf pup recruitment (i.e., survival to 15 months) in Alberta (2012-2014), Idaho (2008-2014), and Yellowstone National Park (2012-2014). NBF = nonbreeding female, NBM = nonbreeding male, AF = adult female, AM = adult male, BF = breeding female, BM = breeding male.

Parameter	Estimate	SE	Odds ratio	Lower 95% CI	Upper 95% CI
harvest	-1.89	0.42	0.15	0.07	0.35
1 yr old NBFs$_{(t=3\ months)}$	-0.04	0.19	0.97	0.67	1.39
1 yr old NBMs$_{(t=3\ months)}$	0.35	0.17	1.42	1.02	1.99
≥2 yr old NBFs$_{(t=3\ months)}$	0.06	0.16	1.06	0.78	1.44
≥2 yr old NBMs$_{(t=3\ months)}$	-0.08	0.17	0.93	0.67	1.29
unk AFs$_{(t=3\ months)}$	-0.03	0.16	0.98	0.71	1.35
unk AMs$_{(t=3\ months)}$	-0.26	0.25	0.77	0.47	1.24
breeders$_{(t=3\ months)}$	-0.27	0.23	0.76	0.49	1.21
≥2 yr old NBFs$_{(t=15\ months)}$	0.01	0.16	1.01	0.73	1.38
≥2 yr old NBMs$_{(t=15\ months)}$	-0.40	0.19	0.67	0.47	0.97
breeders$_{(t=15\ months)}$	0.82	0.24	2.27	1.41	3.64
area (Alberta)	-0.42	0.90	0.66	0.11	3.83

area (Idaho)	0.18	0.55	1.20	0.41	3.49
constant	0.04	0.88			

Table 3. Log-likelihood (-2LL), number of parameters (K), Akaike's Information Criterion Value (AIC), change in (Δ) AIC value, and Akaike weight (w_i) of multiple logistic regression models predicting the probability of wolf pup recruitment (i.e., survival to 15 months) in years when there was public harvest, Idaho (2009, 2011–2014). BF = breeding female, BM = breeding male.

Model	-2LL	K	AIC	ΔAIC	w_i
Global: (diversity $_{(t=3\ months)}$ + diversity $_{(t=15\ months)}$ + group size$_{(t=3\ months)}$ + group size$_{(t=15\ months)}$ + BF turnover + BM turnover)	123.2	7	137.2	0	0.98
Breeder turnover: (BF turnover + BM turnover)	140.5	3	146.5	9.3	0.01
Group size: (group size$_{(t=3\ months)}$ + group size$_{(t=15\ months)}$)	141.8	3	147.8	10.6	0.00
Intragroup diversity: (diversity $_{(t=3\ months)}$ + diversity $_{(t=15\ months)}$)	145.4	3	151.4	14.2	0.00

1 Table 4. Logistic regression parameters (SE) and odds ratios from the highest-ranked

2 model (Akaike weight = 0.98; AUC = 0.71) predicting the probability of wolf pup

3 recruitment (i.e., survival to 15 months) in years of harvest in Idaho (2009, 2011-2014).

4 BF = breeding female, BM = breeding male.

Parameter	Estimate	SE	Odds ratio	Lower 95% CI	Upper 95% CI
diversity$_{(t=3 \text{ months})}$	0.05	0.03	1.05	1.00	1.12
diversity$_{(t=15 \text{ months})}$	-0.46	0.16	0.63	0.47	0.86
group size$_{(t=3 \text{ months})}$	-0.18	0.17	0.83	0.60	1.16
group size $_{(t=15 \text{ months})}$	1.71	0.61	5.52	1.66	18.33
BF turnover	-0.54	0.60	0.58	0.18	1.88
BM turnover	-1.50	0.68	0.25	0.06	0.85
constant	-2.71	0.91			

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8   Table 5. Logistic regression parameters (SE) and odds ratios from model (AUC = 0.66)

9   predicting the probability of wolf pup recruitment (i.e., survival to 15 months) in Idaho

10   during years with harvest (2009, 2011-2014). NBF = nonbreeding female, NBM =

11   nonbreeding male, AF = adult female, AM = adult male, BF = breeding female, BM =

12   breeding male.

13

Parameter	Estimate	SE	Odds ratio	Lower 95% CI	Upper 95% CI
1 yr old NBFs$_{(t=3\ months)}$	-0.13	0.27	0.88	.052	1.50
1 yr old NBMs$_{(t=3\ months)}$	0.10	0.32	1.10	0.59	2.08
$\geq$2 yr old NBFs$_{(t=3\ months)}$	-0.06	0.23	0.95	0.61	1.48
$\geq$2 yr old NBMs$_{(t=3\ months)}$	0.19	0.27	1.21	0.71	2.07
unk AFs$_{(t=3\ months)}$	-0.03	0.58	0.97	0.31	3.00
unk AMs$_{(t=3\ months)}$	-0.53	0.61	0.59	0.18	1.94
breeders$_{(t=3\ months)}$	-0.10	0.72	0.91	0.22	3.74
$\geq$2 yr old NBFs$_{(t=15\ months)}$	0.38	0.26	1.47	0.89	2.42
$\geq$2 yr old NBMs$_{(t=15\ months)}$	-1.08	0.53	0.34	0.12	0.96
breeders$_{(t=15\ months)}$	1.36	0.55	3.88	1.33	11.28
constant	-2.68	1.90			

14   Figure 1. Sex ratio of pups in study areas in Idaho, USA, 2008-2014.
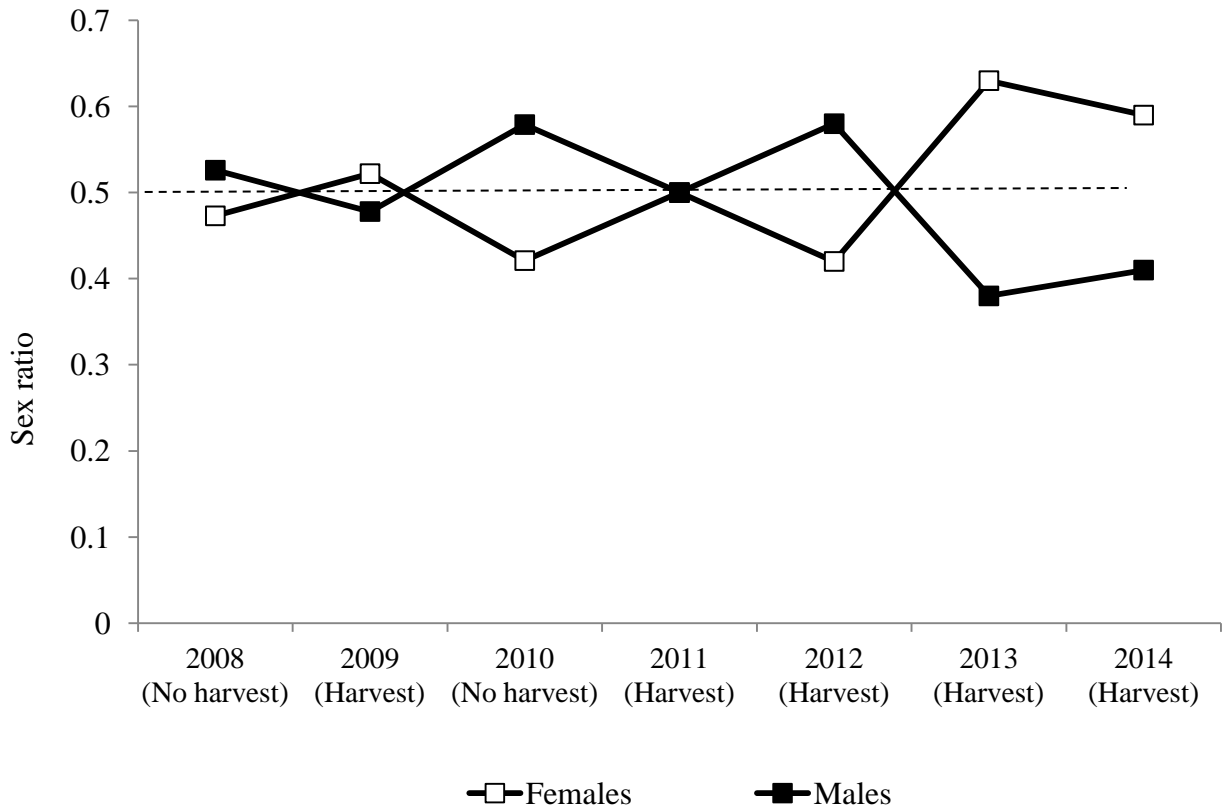
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17 **Title:** Individual, group, and environmental influences on helping behavior in a social

18 carnivore

19 **Abstract:** Variation in group composition and environment can affect helping behavior

20 in cooperative breeders. We do not, however, have a good understanding of how group

21 size, individual traits, food abundance, and predation risk simultaneously influence

22 helping behavior. We evaluated pup-guarding behavior in gray wolves (*Canis lupus*) to

23 assess how differences in individuals, groups, and environment affect helping behavior.

24 We used data from 92 satellite-collared wolves in North America (2001-2012) to estimate

25 individual pup-guarding rates. The presence of predators did not have a significant effect

26 on time spent guarding pups. Individuals in groups with low helper to pup ratios spent

27 more time guarding young than those in groups with more helpers, an indication of load-

28 lightening. Contrary to predictions from group augmentation theory, guarding rates

29 varied with sex of helpers only before pups were weaned. Helper age had no influence on

30 guarding rates. Prey density had a negative relationship with pup guarding rates after

31 weaning, suggesting pup-rearing sites may be places of information transfer between

32 individuals. We show that the interaction of individual, group, and environmental

33 variation can have strong influences on individual decision-making and cooperative

34 behavior.

35 **Key words:** *Canis lupus*, cooperative breeding, groups, helping, pup-guarding, wolves

36 **Introduction**

37   Cooperative breeding refers to the care of related or unrelated young by

38 nonbreeding individuals in a group (Solomon and French 1997). In cooperatively

39 breeding animals, both manipulative and observational studies have shown that the

40 presence of helpers is critical to fitness of the breeders and persistence of the group as

41 well (Mumme 1997; Solomon and French 1997; Courchamp, Clutton-Brock, Grenfell

42 2000; Courchamp and Macdonald 2001; Courchamp, Rasmussen, Macdonald 2002).

43 Variation in group composition and environment can affect helping behavior (Russell

44 2004; Clutton-Brock 2006) but we do not have a good understanding of how group size,

45 individual traits, food abundance, and predation risk simultaneously influence an

46 individual's decision to help.

47   In cooperatively breeding carnivores, foraging must often be done at great

48 distances from relatively immobile young. In such species the ability to adequately guard

49 young while other members in the group forage can be important for successful

50 reproduction in the group (Moehlman 1979; Pusey and Packer 1987; Courchamp and

51 Macdonald 2001). For example, when group size dropped below 5 animals in African

52 wild dogs (*Lycaon pictus*), groups reproduced less successfully than larger groups in part

53 because of increased predation on unguarded young (Courchamp and Macdonald 2001;

54 Courchamp et al. 2002). Group size can affect how much time an individual devotes to

55 guarding young (Courchamp and Macdonald 2001; Ruprecht, Ausband, Mitchell, Garton,

56 Zager 2012). Distributing the workload of rearing young among members of a group (i.e.,

57 load-lightening, (Crick 1992) has positive effects that have been documented across a

58 broad range of species including birds (Crick 1992), mongooses (Clutton-Brock et al.

59 2001), and monkeys (Sanchez, Pelaez, Gil-Burmann, Kaumanns 1999; Bales, O'Herron,

60 Baker, Dietz 2001). Load-lightening can lead to increased survival and growth of young

61 as well as increased fitness for female breeders because of increases in maternal

62 condition and survival (Sanchez et al. 1999; Bales et al. 2001; Russell, Sharpe,

63 Brotherton, Clutton-Brock 2003; Sparkman et al. 2011). Load-lightening can also allow

64 larger groups to both provision and guard young whereas individuals in smaller groups

65 may have to make costly tradeoffs between time spent guarding young and foraging

66 (Courchamp et al. 2002). The costs of such tradeoffs presumably increase when food is

67 scarce and individuals in small groups may help less when food availability is low

68 (Harrington, Mech, Fritts 1983).

69   Although group size can affect helping behavior, individual traits and

70 environmental variation can also be influential. In some primates, nonbreeding females

71 help more than nonbreeding males and may be learning to care for young giving them an

72 advantage once they initiate their own reproduction (Tardif, Richter, Carson 1984).

73 Helper age can also influence behavior because older helpers often assist more with

74 young than younger helpers (Lawton and Guindon 1981; Tardif 1997). Older helpers may

75 be gaining experience as they prepare for dispersal and subsequent breeding opportunities

76 of their own. Perception of predation risk on young can affect reproductive decision-

77 making (Martin 2011) and behaviors such as the guarding of young (Courchamp and

78 Macdonald 2001). Lastly, kin selection theory (Hamilton 1964) predicts that genetic

79 relatedness will have a positive influence on helping behavior. This is true for many

80 species but can vary as resources and conditions (e.g., territories, food abundance,

81 individual condition) change (Clutton-Brock 2006; Cornwallis, West, Davis, Griffin

82 2010).

83   Gray wolves (*Canis lupus*) often leave adults at den and rendezvous sites to guard

84   relatively sessile offspring while other adult wolves in the group forage or rest (Packard

85   2003). Pup-guarding behavior is crucial for group growth and stability in other species

86   with similar life history strategies to wolves (Courchamp and Macdonald 2001;

87   Moehlman 1979). Both grizzly bears (*Ursus arctos*) and other wolves prey on wolf young

88   (Hayes and Baer 1992; Smith et al. 2010) and wolves are commonly known to

89   aggressively chase grizzly bears and other wolves away from pup-rearing sites (Murie

90   1944; Peterson, Woolington, Bailey 1984; Hayes and Mossop 1987; Mech, Adams,

91   Meier, Burch, Dale 1998; Smith and Ferguson 2005; Smith et al. 2013). The breeding

92   female spends the most time of any group member guarding the young but this

93   diminishes markedly after weaning when guarding by nonbreeding (i.e., helper) wolves

94   increases (Ruprecht et al. 2012). Wolves within a group vary widely in how much time

95   they spend guarding young (Ruprecht et al. 2012; Thurston 2002) and we do not know

96   how individual, group, and environmental variation affect such behavior.

97   We studied guarding behavior to provide insights into how differences in

98   individual, group, and environmental factors affected helping behavior in gray wolves.

99   Specifically, we hypothesized that: 1) risk of pup predation positively influences helping

100   behavior and guarding rates, 2) individuals in groups with relatively more helpers than

101   young spend less time guarding pups because of load-lightening, 3) female helpers gain

102   experience rearing pups and thus spend more time guarding pups than male helpers, 4)

103   older helpers help more than younger helpers, and 5) helping behavior is contingent on

104   food availability and guarding of pups decreases as food becomes more scarce.

Study Areas

Our 4 study areas were in Alberta, Canada, and Idaho, Montana, and Yellowstone

National Park (YNP), Wyoming. Generally, Idaho and Montana are mountainous and

dominated by a mix of ponderosa pine (*Pinus ponderosa*), lodgepole pine (*P. contorta*),

and spruce (*Picea englemannii*) forests and sagebrush (*Artemisia tridentata*) steppe.

Annual precipitation ranges from 89-178 cm and temperatures range from -34° C in

winter to 38° C in summer (Western Regional Climate Center 2014). Wolves were

common and at moderate densities in both Idaho and Montana. Groups within our study

areas in Idaho did not overlap the range of grizzly bears while some, but not all, of our

groups in Montana did. Black bears (*U. americanus*), cougars (*Puma concolor*), coyotes

(*C. latrans*), and wolves were present in all of our study areas. Public harvest of wolves

began in both states in 2009 and control actions to address livestock depredations were

rare in our study groups. YNP is dominated by lodgepole pine forests and expansive

meadow systems. YNP is relatively dry and precipitation averages 47 cm annually and

temperature fluctuations range from -39°C in winter to 30°C in summer at Yellowstone

Lake (Western Regional Climate Center 2014). Wolves and grizzly bears both exist at

high densities and there is no human hunting inside YNP. Lastly, southwest Alberta is a

highly contrasted landscape where mountainous forests meet the dry short-grass prairie

region. Mountain forests are dominated by Douglas-fir (*Pseudotsuga menziesii*) and

lodgepole pine forests. Where forest meets prairie there are expansive aspen (*Populus*

tremuloides) forests dominated by livestock grazing. Temperatures range from -32°C to

23°C and precipitation averages 40 cm annually on the prairie (Alberta Agriculture and

127 Rural Development 2014). Wolf densities are thought to be low in southwest Alberta

128 while grizzly bears are abundant and wolf control actions and human harvest are

129 common.

130 **Methods**

131 Gray wolves were captured in foot-hold traps or by helicopter darting and were fitted

132 with Global Positioning System (GPS) collars from 2001-2012 (Alberta 2008-2009;

133 Idaho 2007-2012; Montana 2008-2010; YNP 2001-2012). Wolves were captured by

134 management agencies as part of monitoring and research efforts, and by University of

135 Montana personnel (Animal Use Protocol 008-09MMMCWRU and University of Alberta

136 Animal Care Protocol no. 565712). Wolves were sexed and aged via tooth wear at the

137 time of capture and breeding status was determined at time of capture or after subsequent

138 monitoring (USFWS 2002-2013). GPS collars were Lotek (Newmarket, Ontario, Canada)

139 and Telonics (Mesa, AZ) brand collars and were set to acquire 3-8 locations at evenly-

140 spaced intervals daily. Several collars in Alberta and YNP were deployed as part of

141 predation studies and acquired 48 or 24 locations daily spaced 0.5-1.0 hr apart.

142   We plotted wolf locations from 15 April – 1 September for each year. Because

143 there are marked shifts in guarding rates between age classes before and after weaning

144 (Ruprecht et al. 2012) we considered 15 April – 1 June the pre-weaning season (Kreeger

145 2003) and 2 June - 1 September the post-weaning period. We assumed distances >500 m

146 from pup-rearing sites would make detecting and alerting the pups to predators

147 ineffective. Thus we considered an individual wolf was guarding pups if its location fell

148 within a 500 m buffer of the group's den or rendezvous site location (Ruprecht et al.

149   2012). Additionally, pups move in areas around den and rendezvous sites and it is likely

150   that adults were closer than 500 m to pups when adults were within the 500 m buffer.

151   Where den and rendezvous site locations were not known from ground surveys and

152   monitoring work in the study areas we defined a cluster of GPS locations as a pup-rearing

153   site when $\geq$10 locations were within 500 m of one another for $\geq$6 days. Unsuccessful

154   GPS location attempts did not constitute abandonment of a site. Wolves may have

155   clusters of locations that are kill sites, but 85% of kills are abandoned after three days and

156   none have been found active after 5 days (Metz, Vucetich, Smith, Stahler, Peterson

157   2011).

158   The number of helpers and pups in each group were acquired via radiotelemetry

159   flights or ground surveys conducted in summer (USFWS 2002-2013). Some group counts

160   in Idaho were derived from noninvasive genetic sampling of scats at rendezvous sites

161   (Ausband et al. 2010; Stenglein, Waits, Ausband, Zager, Mack 2011; Stansbury et al.

162   2014). Scats <2.5 cm were considered pup and >2.5 cm adult (Stenglein et al. 2011;

163   Stansbury et al. 2014). We subtracted 2 (to represent the breeding pair) from the number

164   of adults in each group to estimate the number of helpers that were present.

165   The presence of grizzly bears varied among our study areas, thus we included the

166   presence or absence of grizzly bears for each group to represent predation risk for pups in

167   Alberta, portions of Montana, and YNP. As a second measure of predation risk, we

168   estimated wolf density (wolves/1,000 km^2) for groups in the northern range of YNP

169   where counts where nearly complete and constituted a census (D. Smith, YNP,

170   unpublished data). As a relative index of prey abundance, we estimated winter prey

171   density (elk/km^2) annually for 10 focal groups in the YNP northern range using aerial elk

172   counts from the prior winter (Northern Yellowstone Cooperative Wildlife Working

173   Group 2012).

174   We estimated the proportion of time spent guarding pups (number of locations

175   <500 m from den or rendezvous site/number of successful locations while site occupied)

176   before and after weaning for each individual in each year. We used locations that fell

177   within a 500 m buffer of a den or rendezvous site and did not use locations for fine-scale

178   habitat analysis, thus we assumed that any differences in collar brand and duty schedule

179   would not have biased our results.

180   We then arcsine-transformed the proportions to ensure normally distributed data.

181   We used a generalized linear mixed model (GLMM) with proportion of locations with

182   pups as the dependent variable and number of helpers (i.e., nonbreeding adults) to pups in

183   the group as a covariate and area, breeding status and sex of each wolf, and presence or

184   absence of grizzly bears as factors (SPSS 22; IBM Software NY, USA). We also included

185   a random effect for individuals. We did not have covariates of prey density, wolf density,

186   and helper age for every individual. Rather than impute these values we obtained data for

187   subsets of individuals where it was available and conducted 2 additional GLMM

188   analyses. These models included prey (log10) and wolf density (log10; YNP northern

189   range), and helper age (Montana, portions of Idaho, YNP). We used t-tests to look for

190   differences in guarding rates before and after weaning and considered differences

191   significant if p<0.05.

192   **Results**

193   We collected location data from 92 GPS-collared wolves for a total of 123 wolf summers

194   (Table 1). Breeding females spent the greatest amount of time guarding pups over the

195   course of the season although this declined from nearly 70% to 40% after pups were

196   weaned (p<0.001; Fig. 1). All other sex and age classes increased the time they spent

197   guarding pups after weaning although nonbreeding males showed the largest increase in

198   time spent guarding pups after weaning (p = 0.02; Fig. 1).

199   Before pups were weaned, breeding status and sex were the dominant predictors

200   of time spent guarding pups. Breeding females and nonbreeding females were significant

201   variables in the pre-weaning model while breeding males approached significance (p =

202   0.06; Table 2). The presence of grizzly bears, study area, and the ratio of helpers to pups

203   were not significant before pups were weaned. After pups were weaned, breeding status

204   and sex and number of helpers to pups were the dominant predictors of time spent

205   guarding pups (Table 2). The effect of grizzly bears and study area were not significant.

206   Nonbreeding females spent more time guarding pups than nonbreeding males before

207   weaning (p = 0.01) but the sexes did not differ after pups were weaned (p = 0.17; Fig. 1).

208   Helper age was not influential in models predicting guarding rates before or after pups

209   were weaned (Table 3). Although prey density varied widely (0.35-14.9 elk/km^2) it was

210   not important in models predicting an individual's time spent guarding pups before pups

211   were weaned but it had a significant negative effect after weaning (p = 0.04; Table 3).

212   Wolf density did not influence pup-guarding rates in YNP (Table 3).

213   **Discussion**

Guarding young from predation is an important behavior that enhances reproductive

success in group-living carnivores (Moehlman 1979; Courchamp et al. 2002). Our work

partially supports findings from previous studies of helping behavior in cooperatively

breeding species. Similar to other studies (Crick 1992; Clutton-Brock et al. 2001), we

observed load-lightening where individuals in large groups spent less time guarding

young than their counterparts in smaller groups. We discovered a lack of support,

however, for other findings, chiefly, that age and prey abundance have strong influences

on helping behavior (Tardif et al. 1984; Tardif 1997; Clutton-Brock 2006). Prey

abundance likely influences provisioning rates in wolves but we found that, after

accounting for the behavior of breeding females, characteristics associated with the group

influenced pup-guarding rates more than characteristics associated with individuals. A

helper's experience or ability may be less important than maintaining a large group size

in highly territorial species such as wolves that breed once a year. A group of experienced

helpers may not be as important to breeder fitness as maintaining an adequate number of

helpers to reduce workload.

Group composition influenced guarding behavior because individuals in groups

with fewer helpers per pup spent more time guarding pups than those in groups with

more helpers. For example, our model predicts that a nonbreeding female in YNP spends

nearly 10% more time (i.e., nearly 2.5 more hours each day) guarding pups if she is the

only helper in a group with 4 pups compared to being in a group with 3 other helpers and

just 2 pups. Individuals in small groups, or those with low helper to pup ratios, increase

their time spent guarding young and this presumably comes at the cost of obtaining food

236   for both themselves and pups. Our findings suggest that load-lightening occurs within

237   groups of wolves. The effects of such load-lightening on reproduction in wolves are not

238   known, but it may be one mechanism that explains why wolf pups have higher survival

239   and breeding females have increased fitness in larger groups than their counterparts in

240   smaller groups (Sparkman et al. 2011; Stahler, MacNulty, Wayne, vonHoldt, Smith

241   2013). Our counts of individuals in groups may be slightly conservative particularly for

242   larger groups where all individuals may not be visually or genetically detected during

243   sampling. Subsequently, we suspect the effect of group size on helping behavior may be

244   more marked than what we observed.

245   We found that breeding status and sex and the number of helpers relative to the

246   number of young in the group were important predictors of how much time an individual

247   devotes to guarding young. After weaning, breeding females spent less time guarding

248   young while all other age classes in the groups simultaneously increased the amount of

249   time guarding young. Breeding females may spend less time guarding pups after weaning

250   because of foraging demands related to the nutritional costs of recent lactation.

251   Group augmentation theory (i.e., helpers increase group productivity and thus

252   increase their own fitness) predicts that the sex which is most philopatric will help most

253   (Kokko, Johnstone, Clutton-Brock 2001). Our findings only partially support predictions

254   from group augmentation theory regarding which sex will help more. Females are

255   slightly philopatric in the U.S. Rockies (Jimenez et al. In Review) yet we found

256   nonbreeding females guarded pups more than nonbreeding males only before pups were

257   weaned and this trend was not evident after weaning. Males in some species may be

258   constrained to help through social coercion or face eviction from the group if they do not

259   help (Clutton-Brock 2006). Alternatively, female philopatry may not be marked enough

260   in U.S. Rockies' wolves to expect consistent differences in helping behavior between the

261   sexes. Group augmentation remains a viable theory to explain why both sexes remain and

262   help, however. Nonbreeding males and females guarded pups equally over the majority

263   of the pup-rearing season and thus both classes contributed to the reproductive success of

264   the group. Such helping behavior could ultimately enhance their individual success as

265   predicted by group augmentation theory.

266   In some cooperatively breeding species, older helpers assist more with young than

267   younger helpers (Lawton and Guindon 1981; Tardif 1997). We found no evidence,

268   however, that the age of helpers affected guarding rates in wolves. Hypothetically,

269   nonbreeding wolves may not be helping when attending pup-rearing sites but rather are

270   trying to obtain food and information on kill locations, particularly when prey densities

271   are low (Harrington et al. 1983). We found that prey density did not have a strong

272   influence on an individual's time spent guarding pups before weaning. After weaning,

273   however, there was a negative relationship (p = 0.04) between prey density and guarding

274   rates suggesting increased pup-guarding as prey became relatively more scarce. If true,

275   this would support the hypothesis that a benefit of helping behavior is acquiring

276   information on food. There was no evidence, however, that helping behavior was

277   contingent on food availability in black-backed jackals (*C. mesomelas*; Moehlman 1979)

278   and there was no relationship between time spent at den and rendezvous sites and prey

279   density for wolves in the Midwest U.S.(Potvin, Peterson, Vucetich 2004). Alternatively,

280 we may not have found a strong relationship between prey abundance and helping

281 behavior because previous winter's prey density was a poor index of prey availability

282 during pup-rearing season. We posit that years with low winter prey counts, however,

283 were likely to be indicative of low prey availability the following summer.

284   Wolf density was not an influential predictor of the amount of time an individual

285 spent guarding pups. One possible explanation may be that wolves decrease the chance

286 that neighboring wolves will encounter their pups by not placing pup-rearing sites near

287 the edges of their territories (Ciucci & Mech 1992). Additionally, territorial behavior

288 such as scent-marking and howling may further decrease aggressive wolf encounters with

289 a group's young. An alternative explanation for why wolf density was not influential

290 could be that wolf density in YNP was high enough during each year of our study that

291 individuals were spending the maximum amount of time available for pup-guarding

292 given foraging demands. Indeed, our lowest estimated wolf density in this model was

293 21.6 wolves/1,000 km^2 which is indicative of a healthy, saturated wolf population in this

294 region (Fuller, Mech, Cochrane 2003).

295   Helping behavior in wolves can take several forms; guarding, provisioning, social

296 development of pups. Although essential for a full understanding of the adaptive value

297 and evolution of helping behavior in this species, provisioning rates for gray wolves are

298 exceedingly difficult to obtain in the wild. We expect, however, that one of the main

299 factors driving guarding rates (i.e., group size) also influences provisioning rates. For

300 example, wolves in groups with fewer helpers spent more time guarding pups and we

301 presume this would lead to lowered provisioning rates as well.

302   The presence of load-lightening coupled with guarding rates that did not decline

303   when prey was relatively scarce suggests that individuals in small groups make

304   potentially costly tradeoffs (i.e., less time spent foraging) to adequately guard young. We

305   show that it is useful to simultaneously examine helping behavior in light of individual,

306   group, and environmental variation because the interaction of these variables can have

307   strong influences on individual decision-making and cooperative behavior.

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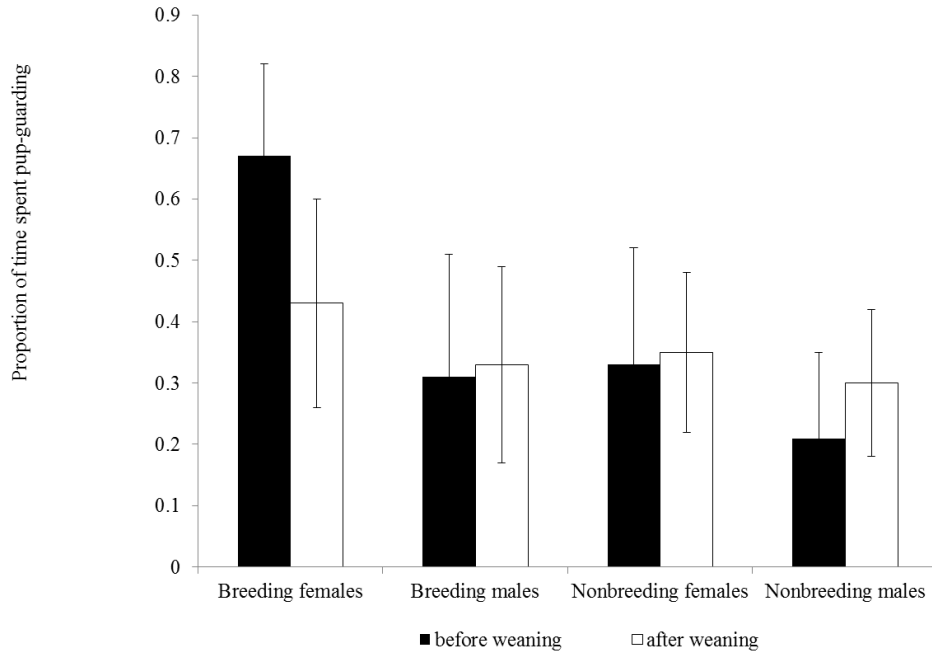
489

490   Figure 1. Pup-guarding rates for gray wolves before and after weaning in Alberta,

491   Canada, Idaho, Montana, and Yellowstone National Park, Wyoming, USA, 2001-2012.

492

493   Table 1. Number of GPS collared wolves used to estimate guarding rates of pups in

494   Alberta, Canada, Idaho, Montana, and Yellowstone National Park, Wyoming, USA,

495   2001-2012.

Study Area	No. breeding females	No. breeding males	No. nonbreeding females	No. nonbreeding males
Alberta	2	0	1	0
Idaho	10	9	26	11
Montana	4	3	4	1
Yellowstone	5	3	11	7
Total[1]	21	15	42	19

496   [1] n >92 wolves because 5 wolves changed breeding status over the course of the study

497

Table 2. Coefficients (p-values) for covariates from generalized linear mixed models (GLMM) predicting guarding rates of wolf pups before and after weaning in Alberta, Canada, Idaho, Montana, and Yellowstone National Park, Wyoming, 2001-2012.

	Intercept	Study area	Breeding status and sex[1]	Helper:pup ratio	Grizzly bears absent
Pre-weaning	0.08	0.20 (0.14; Yellowstone)	0.60 (<0.001; BF)	-0.003 (0.91)	-0.05 (0.78)
		0.10 (0.65; Idaho)	0.12 (0.06; BM)		
		0.21 (0.28; Montana)	0.15 (0.009; NBF)		
		0 (N/A; Alberta)	0 (N/A; NBM)		
Post-weaning	0.36	0.04 (0.61; Yellowstone)	0.18 (0.001; BF)	-0.05 (0.01)	-0.04 (0.67)
		-0.02 (0.89; Idaho)	0.04 (0.49; BM)		
		0.04 (0.69; Montana)	0.07 (0.13; NBF)		
		0 (N/A; Alberta)	0 (N/A; NBM)		

[1]BF = breeding females, BM = breeding males, NBF = nonbreeding females, NBM = nonbreeding males

1	Table 3. Coefficients (p-values) for covariates from subset analyses of generalized linear mixed models predicting

1 Table 3. Coefficients (p-values) for covariates from subset analyses of generalized linear mixed models predicting

2 guarding rates of wolf pups before and after weaning in Alberta, Canada, Idaho, Montana, and Yellowstone National

3 Park, Wyoming, 2001-2012. Subset analyses included independent variables of prey density, wolf density and helper

4 age.

	Intercept	Study area[1]	Breeding status and sex[2]	Helper:pup ratio	Prey density	Wolf density	Helper age
(Prey and wolf density)							
Pre-weaning	0.22	N/A	0.39 (<0.001; BF) 0.21 (0.06; BM) 0.13 (0.04; NBF) 0 (N/A; NBM)	-0.08 (0.02)	-0.02 (0.87)	0.14 (0.64)	N/A
Post-weaning	1.19	N/A	0.10 (0.11; BF) -0.05 (0.56; BM) 0.02 (0.76; NBF) 0 (N/A; NBM)	-0.06 (0.03)	-0.24 (0.04)	-0.38 (0.13)	N/A

(Helper age)

Pre-weaning	0.28	0.15 (0.01; YNP)	0.14 (0.02; NBF)	-0.03 (0.36)	N/A	N/A	-0.12 (0.12; age = 1)
		0 (N/A; ID)	0 (N/A; NBM)				-0.09 (0.25; age = 2)
							-0.25 (0.22; age = 3)
							0 (N/A; age = 4)
Post-weaning	0.31	0.089 (0.35; YNP)	0.05 (0.21; NBF)	-0.01 (0.50)	N/A	N/A	-0.02 (0.66; age = 1)
		-0.02 (0.80; ID)	0 (N/A; NBM)				-0.04 (0.43; age = 2)
		0 (N/A; MT)					0.09 (0.50; age = 3)
							0 (N/A; age = 4)

5   [1]YNP = Yellowstone National Park, ID = Idaho, MT = Montana

6   [2]BF = breeding females, BM = breeding males, NBF = nonbreeding females, NBM = nonbreeding males