Determining the Vulnerability of Wolves to Harvest

A Thesis

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Abstract

Individual behaviors are influenced by environmental, genetic, and demographic factors. Some animals choose to live in groups and cooperatively breed, and their behaviors can change depending on dynamic factors such as group size and composition that affect group persistence. Extensive research on cooperative breeding species has shown that the lethal removal of breeders from a group has direct and indirect effects on the persistence of the group. In Idaho, USA, gray wolves (*Canis lupus*) are harvested annually, this has provided an opportunity to investigate the effects of harvest on a population of cooperative breeders. These annual hunting and trapping seasons overlap with the dispersal and breeding periods for wolves. Currently, we know little about how many breeders, dispersing aged adults (>22 months), yearlings, and pups are harvested each year via hunting and trapping or when they are harvested each season.

In the first chapter, we applied 10 years of genetic and metadata collected from harvested wolves to investigate how behaviors and ecological drivers might influence the vulnerability of wolves throughout the harvest season. We created pedigrees from non-invasive genetic scat sampling to create expected proportions of three different age classes (pup, yearling, and sexually mature or >22 months old) of wolves and compared them to the observed number of those cohorts harvested during ecologically significant periods (i.e., dispersal and breeding). We found that pups are more vulnerable to harvest in December when wolf harvest transitions largely to trapping (66%). We compared the expected and observed proportions of wolves ≥2 years old during peak dispersal season (December) and breeding season (January – February) as well as yearlings from September to October when the group moves out of rendezvous sites and found no overall trend. However, there was considerable annual variation suggesting there is more to learn about how the vulnerability of different sex and age classes of cooperative breeders varies throughout the harvest season.

In the second chapter, we estimated the frequency of breeders in harvest and whether breeders were more vulnerable to harvest during the breeding season. We demonstrate a novel approach for using genetic data collected opportunistically from harvested wolves to determine if/when breeders are more vulnerable to harvest and to estimate the minimum number of breeders harvested annually in Idaho, USA, using pedigree analyses. We genotyped and aged 229 adult wolves and 203 pups using tissue and tooth samples, respectively, from wolves harvested between 2014 and 2016. We identified a minimum count of 36 breeders (n = 18 in 2014 and 18 in 2015) and found that breeders were disproportionately harvested (*P* = 0.08) during the breeding season (January; 25% of all breeders harvested during 2014 and 2015 harvest seasons). We estimate that a minimum of 16% of adult wolves harvested annually are breeders, or roughly 1 in 6. Our estimate of the number of breeders harvested annually is conservative because the pedigree analysis is dependent on both a pup and breeder from the same group having been harvested in the same year, and samples were excluded from the analysis if they were missing age and harvest month data or had <16 confirmed loci. Our results demonstrate that breeders are routinely harvested and that their behavior during breeding season may increase their vulnerability to harvest.

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Dedication

There are many I thank for support and cooperation. To all of my Milwaukee family; Rebholz, Licato, Correa, Cieslak, and Maternowski clans thanks for the support and keeping me humble by constantly roasting me for following my dream. Thank you to the Enderis Park Affiliation for giving me a place to sleep and always keeping me with a full plate and bowl full through these years. Nate, AJ, and Conner thanks for keeping things groovy. Molly, Anne, Matt, Kaitlyn, Nolan and all the rad folks from UI, thanks for all the help getting this thesis to this point.

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Chapter 1: Linking age and social status of cooperative breeders to vulnerability throughout the harvest season

Introduction

Cooperative breeding generally refers to the shared care of related, or even unrelated, young by helpers (i.e., non-breeding individuals) within a group (Solomon et al. 1997). Behavioral ecologists have defined three hallmarks of cooperative breeding that concentrate on the distinctive attributes of care-giving individuals: (1) delayed dispersal from the natal group, (2) reproductive suppression, and (3) alloparenting (i.e., the care of infants by individuals other than the mother; Solomon et al. 1997). The type of care given to individuals and timing of these "hallmark" attributes have evolved across a wide range of taxa and varies among species. Many species that live in groups display cooperative breeding behavior, and previous studies have recognized the vital role that non-parental group members play in a groups' persistence and reproductive success (Solomon et al. 1997, Packer, 2004, Gobush et al. 2008). Further investigation has provided evidence that who dies in the group and when may matter for group persistence and reproductive success (Ausband et al. 2017; Ausband 2019).

In groups of cooperative breeding vertebrates such as suricates (*Suricata suricatta),* cichlids (*Neolamprologus obscurus*), and African wild dogs (*Lycaon pictus*) an increased number of helpers (nonbreeding members of the group) has been shown to increase individual survival and fitness (Clutton-Brock et al. 1998, Downing et al. 2021). Helpers in these groups both directly and indirectly benefit themselves through group protection, rearing young that may be related to them, and aid the breeders by reducing their workload provisioning food and defending resources (Clutton-Brock et al. 1998, Downing et al. 2021). Though all members of the group benefit from this relationship the amount of aid and participation given by everyone in the group is not equal (Ausband et al. 2017, Ausband 2019).

Research has shown (Ausband et al. 2017) that group composition can impact how an individual decides to operate within their group; periods of ecological significance (i.e., breeding season, rearing young, peak dispersal time) can further change how that individual might behave. Gray wolves (*Canis lupus*) roam widely across large territories; how far and often individuals travel from the homesite (locations where wolf group members will congregate for several weeks) is dependent upon the age, time of year, sex, and the role of an individual in their group. As the pups mature and become more independent, the care given by certain individuals can increase or diminish (Ausband et al. 2016). After the breeding female dens, the group will then move pups to a homesite(s) or “rendezvous sites” for the next 8 to 20 weeks (June - August). Wolf pups, like other cooperative breeding species such as meerkats, spend their time at the homesite developing fundamental skills for predator avoidance and obtaining food through social learning and teaching within the group (Clutton-Brock et al. 2011). Other members of the Group will aid in alloparental care, though not all will participate equally. Non-breeders and less experienced hunters will remain at the homesite to guard and provision the pups while the breeders and other adult’s forage. By mid-September the pups will have reached adult size and can travel with the adults, leaving their rendezvous sites and joining the pack on hunts (Packard 2003). However, at this time they may still be highly vulnerable to predation because they are still naïve to their environment and the risks outside of the homesite. Adult wolves may be more aware of these hazards, but they might also increase their vulnerability during ecologically significant times of year. Adults will increase daily movement when dispersing (i.e., leaving their natal population of individuals) and might be more inclined to uses riskier avenues of travel and investigate foreign sounds and scents during breeding season.

In cooperative breeders, individuals can perform different roles and such roles may affect the decision they make throughout the year. There is evidence that social, and environmental factors such as group size, group composition, food abundance, and predation risk can influence the individual’s decision to help or stay within the group (Clutton-Brock et al. 2006). Dispersing animals are the primary route to reproductive success in cooperative breeding species and commonly observed in individuals of both sexes in most group-living mammal species (Girman et al. 1993). Male and female wolves can disperse > 850 km to find a mate or receptive pack with an opportunity to become a breeder (Fritts et al. 1983; Mech et al. 2003). However, leaving the group and traveling alone is risky; exposure to unfamiliar landscapes can increase the chances of starvation and predation (Pusey et al. 1987). These risks may increase in areas with annual harvest, low population sizes, and fragmented habitat.

Wolves in Idaho, USA are a cooperative breeding species that are managed as a big game species, where they are harvested presumably opportunistically across all sex and age classes. Additionally, harvest seasons overlap breeding, dispersal, and pup-rearing periods. This creates an ideal opportunity to investigate how vulnerability relates to annual harvest in a population of cooperative breeders. Wolves are defensive of their established territories, group, and breeding positions (Mech 2003). Such behaviors (e.g., howling) can be exploited via methods used by hunters and trappers and may be biased toward certain sex and age class of wolves. For example, a dominant male or female breeder might be more enticed or responsive to auditory or olfactory lures displayed by hunters and trappers during their breeding season. Dispersing adult males in search of a mate may react in a similar manner at this time; while focused on encountering a mate they ignore more risks than other age and social groups of wolves in the area. How the timing of a harvest season coincides with these ecological periods and changes in individual behavior as it relates to their vulnerability is currently unknown. Typically, males choose to disperse between late fall and the start of the breeding season (mid-February) in search of a breeding position within another group, but more commonly create their own pack (Mech 2003). The discussion on the best method for management of gray wolves in the American Northwest has high public interest and the debate is fierce but empirical data regarding the effects of hunting and trapping on wolf behavior are rare (Gude et al. 2009).

We wanted to know how complex wolf social structures and behaviors affect their vulnerability to harvest. We used long-term genetic data to create pedigrees of wolves and tested how different age classes and cohort's vulnerability might be affected by different harvest time periods throughout the year. We hypothesized that sexually mature wolves (>22 months old) would be more vulnerable to harvest (trapping and hunting) during peak periods of dispersal and breeding. We predicted that i) sexually mature wolves (>22 months old) would be disproportionately represented in harvest during January-February, and ii) sexually mature wolves (>22 months old) would be disproportionately represented in harvest (trapping and hunting) during December while dispersal is high, trapping season begins (accounting for 66% of harvest), and breeding season is near (Table 1).

We also hypothesized that pups and yearlings (<16 months old) would be more vulnerable to harvest during autumn because they are naïve to dangers presented by hunters from September to October in their environment outside of the homesites. We predicted that pups and yearlings would be disproportionately represented in harvest from September to October. We hypothesized that pups would be more vulnerable in December because they would be more naïve to dangers presented by trappers at the start of trapping season. We predicted that pups would be disproportionately represented in harvest in December (Fig.1, Table 1).

Table 1. Predictions for vulnerability of different ages of wolves to various harvest methods and seasons in Idaho, USA. Predictions correspond to Idaho hunting and trapping seasons as well as ecologically significant times of year for wolves.

Time Period	Age class with increased vulnerability
January-February	Sexually mature (>22 mo.)
December	Pups and sexually mature
September- October 30	Pups and yearlings (<16 mo.)

Study Area

We collected scats in three study areas (North, South, East) encompassing five Idaho Department of Fish and Game Management Units (GMUs) within Idaho, U.S.A. (GMUs 4, 28, 33–35; Fig. 1). All study areas were in mountainous regions of primarily United States Forest Service (USFS) lands. Yearly temperatures ranged from -13 °C in the winter to 36 °C in the summer (Western Regional Climate Center 2020). Annual precipitation ranges from 30 cm to 130 cm (Western Regional Climate Center 2020). Elevation ranges from 646 m to 3219 m. The northern study area (GMU 4; 3,189 km^2) has a maritime climate and is dominated by western red cedar (*Thuja plicata)*, Douglas fir (*Pseudotsuga menziesii)*, Engelmann spruce (*Picea engelmannii)*, and lodgepole pine (*Pinus contorta)*. The eastern (GMU 28; 3,388 km^2) and southern (GMUs 33–35; 3,861 km^2) study areas have a continental climate and are dominated by ponderosa pine (*P. ponderosa)*, lodgepole pine, spruce mixed forests and sagebrush (*Artemisia tridentate)*, steppe (Mack et al. 2010). Public harvest of wolves began in Idaho in 2009, temporarily ceased in 2010, and began again in 2011. Most harvest occurred during September–March with a peak during the big-game rifle hunting season (Ausband 2016); we used samples from all wolves harvested throughout the harvest season in the state of Idaho.

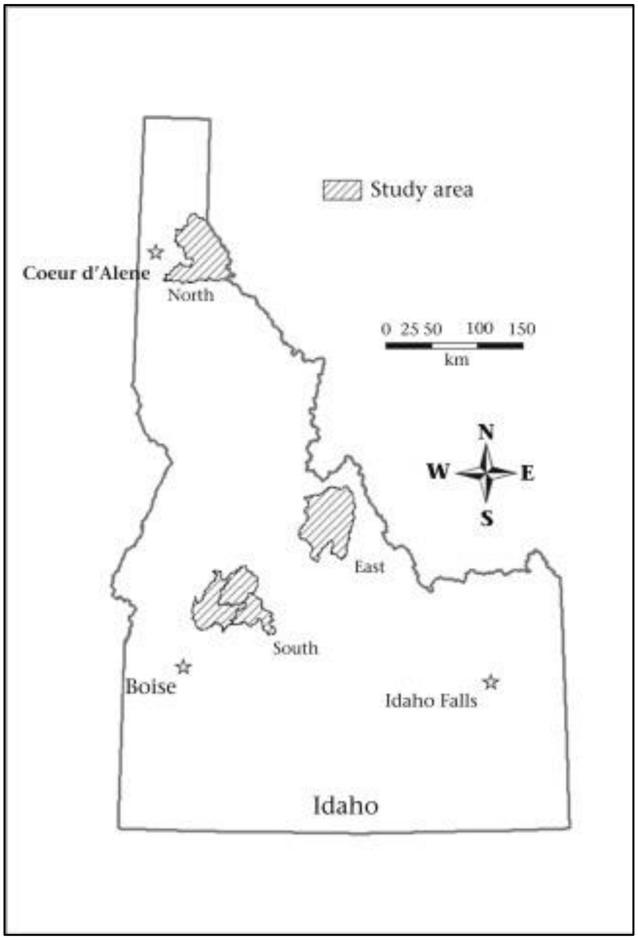

Figure 1. Long-term gray wolf study areas in Idaho, USA used to assess the expected proportions of wolves in harvest.

Field Methods

In summer, six technicians survived for 10-18 wolf packs residing in three study areas in Idaho. We survived for wolves using a predicted habitat model and sampled wolf packs genetically using noninvasive methods developed through our project. Briefly, we collect scats for genetic analysis at rendezvous sites of reproductively active wolf groups. In these areas, technicians surveyed historic and highly suitable rendezvous sites predicted by a peer-reviewed habitat model (Ausband et al. 2010). This model uses NDVI (greenness), roughness, and curvature to predict areas highly probable (≥70% suitability) of being a pup-rearing site Once the pup-rearing site was located, we attempted to find an activity center (area where pups congregate) through howl surveys and back tracking wolf sign (tracks, scat, hair, chew toys; Mech et al. 1982). Technicians typically gathered 125-200 samples (~60% adult samples, ~40% pup) per group per year and attempted to locate and resample each group annually. For more detailed information on field procedures reference (Ausband et al. 2010). During harvest seasons 2009-2010, 2011-2018, IDFG personnel collected tissue samples and tooth samples during mandatory check-in of harvested wolves from across the state of Idaho. Tooth samples collected by IDFG personnel were sent to Matson's Laboratory, Manhattan, MT for cementum analysis to age harvested wolves. IDFG personnel also recorded sex, date of harvest, means of take, location, animal condition, and affixed a pelt tag to the animal hide. The data on sampled wolves was recorded by IDFG personnel which we separated into harvest year, harvest month, and age cohorts. Climate change is occurring globally and altering climates for disease. With climate change, it is of ever-increasing importance to understand the interactions of the microbiome with its larger system. The geographic range of trees and fungi are delineated by factors such as temperature, moisture, elevation, and wind (Lonsdale and Gibbs, 1996). Species will only occur in an area if these factors create a suitable habitat for its reproduction and dispersal. Pathogens occur in ranges where the habitat is suitable and where there is a potential host.

Laboratory Methods

We extracted DNA from scat samples using Qiagen kits (Qiagen, Inc.,Valencia, CA) in a facility dedicated to low quality DNA at the University of Idaho's Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) in Moscow, ID,USA. For every set of scat samples that DNA is extracted from, we used negative controls to monitor for contamination. We used nine microsatellite loci and sex identification primes to identify individuals and gender (Stansbury et al. 2014). To verify matches or mismatches we generated nine additional microsatellite loci on the best sample from each unique individual (i.e., total = 18 loci) and samples that differed at only one locus out of the original nine loci (Stansbury et al. 2014). Each unique individual was assigned with a unique wolf ID number. We used an Applied Biosystems 3130xl capillary machine to separate PCR products; we conducted two independent amplifications for a consensus of a heterozygotes and at least three independent times for a consensus of a homozygote each locus. We included a positive and negative control for each PCR amplification. We analyzed 40 adult and 25 pup scats from each pack; if a group had >2 individuals detected only once, we analyzed additional samples to obtain 10 more consensus genotypes (if additional samples are available). For additional details on laboratory methods that were used please regard (Stenglein et al. 2010; Stenglein et al. 2011; Stansbury et al. 2014).

Determining Observed Numbers Harvested

We used ten years of harvest data (meta data and tooth samples; 2008-2018) from gray wolves harvested throughout the state of Idaho. For each year, we separated individuals by sex and age class; pups, yearlings, and sexually mature adults (≥2-years-old) and calculated proportions for each class. We used the metadata from harvest, to calculate our "observed" age classes in harvest. Individual wolves that were harvested illegally or lethally removed from the population by a professional agency for predator control were removed from the analysis.

Determining Expected Numbers Harvested

We used pack pedigrees from genetic sampling in summer (2008-2018) to calculate an "expected" number of sex and age classes in harvest. We first separated the metadata by year and month and calculated totals for each and age cohort. If the age of an individual was unknown (due to having newly sampled adult individuals in a pack or a newly sampled packs) we estimated age using the existing proportions of adult age classes in that year's population for the individuals sex class. We multiplied the proportions for each cohort from summer sampled pack pedigrees by the total number of harvested wolves from that year to generate the monthly expected number of wolves for each cohort. We applied a monthly survival rate of 0.98 (for wolves ≥1 year old; Smith et al. 2010) and 0.96 (for pups; Ausband et al. 2015, Ausband, 2016) and subtracted all harvested individuals from the prior months to get the "corrected expected" for each cohort every month.

Using the age cohorts created from harvest meta data and tooth samples, we were able to compare the expected to the observed for each temporal period and identify if an age cohort was disproportionately harvested. We used a Chi-square test of independence in Program R (R Core Team 2021) to compare expected versus observed numbers for all cohorts in each month's harvest to test our hypotheses about the relationship between vulnerability of age class to ecologically significant time periods (breeding; January-February, peak dispersal; December, and September–October, when the whole pack moves together outside of their rendezvous sites). We considered differences significant when $P \leq 0.05$ and used this measure of significance for each cohort each year as well as a combination of all years that harvest took place during the ecologically significant time periods (2009, 2011-2018).

Results

There were six ecologically significant periods between January and February in 2009 and 2011 and December of 2010 where there was not any public hunting and trapping of wolves throughout Idaho. Out of the remaining 49 possible comparisons between years, ecologically significant periods, and age classes we documented 12 times where wolves were ($P \leq 0.05$) harvested more than the expected, four times for wolves ≥2 years, three for yearling, and five for pups (Table 2). We observed 3 instances where wolves were harvested less than expected (2018 ≥2 years old in January – February, $\chi^2 = 7.9$, df = 1, $P = 0.005$; 2015 yearling in September – October, $\chi^2 = 4.5$, df = 1, $P = 0.033$; 2013 ≥2 years old in January – February, $\chi^2 = 4.6$, df = 1, $P = 0.032$) in all three age classes; and 34 instances where the expected and observed number of wolves was considered "equal" ($P > 0.05$).

Table 2. Results for the number of wolves harvested (observed) and expected by age group during ecologically significant periods in Idaho, USA 2009-2018. When expected and observed number of wolves was considered "equal" (P > 0.05), when observed was "more" than expected (P ≤ 0.05), and when wolves were harvested "less" than the expected (P ≤ 0.05).

>2YO	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	All Years
Jan - Feb	No Harvest	Equal	No Harvest	Equal	Less	Equal	More	More	Equal	Less	Equal
December	More	No Harvest	More	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal
YEARLING											
Sep-Oct	More	No Harvest	Equal	Equal	Equal	More	Less	Equal	More	Equal	Equal
PUP											
Sep-Oct	Equal	No Harvest	Equal	More	Equal	Equal	Equal	Equal	Equal	Equal	Equal
December	Equal	No Harvest	More	Equal	More	Equal	Equal	Equal	Equal	More	More

There was an association with pups being vulnerable to harvest during December, the start of trapping season. We found three years pups were harvested more than the expected (2013, 2016, and 2018) in December, and the combination of all ten years of December harvest data showed they were harvested more than expected (χ^2 = 26. 9, df = 1, P <0.0001), and one year (2012) between September and October.

There was no association with yearling vulnerability and the start of the harvest season, between September and October. However, yearlings were disproportionately harvested more than the expected three times (2009, 2014, 2017) between September and October, the most out of any other age class. Wolves ≥ 2 years old were not associated with vulnerability during the breeding season and were only harvested more than expected twice in each of the ecologically significant periods across all years. Additionally, there was no difference between the 10-year total corrected expected (n = 120.2) for wolves ≥2 years old harvested during the breeding season, with <1 individual compared to the observed (n = 209.5; Table 3). Additionally, there was no association with sexually mature wolves (≥2 years) being harvested disproportionally during December (peak dispersal).

Table 3. Estimated total counts of wolves harvested (observed) and expected by age group and ecologically significant periods in Idaho, USA 2009-2018. Decimals were rounded up from the tenth decimal point. Results were only calculated for groups associated with hypotheses.

Results for All Years	Observed >2YO	Expected >2YO	Observed Yearlings	Expected Yearlings	Observed Pups	Expected Pups
Jan - Feb	210	210	-	-	-	-
Sep - Oct	-	-	182	162	327	294
December	118	101	-	-	190	101

Discussion

Human caused mortality can have compounding effects on group living species, because all individuals within a group directly and indirectly aid the group. Removing individuals can affect group performance and the decisions that individuals will make throughout the year (Ausband et al. 2017; Clutton-Brock et al. 2006). Age class and ecologically significant periods had marked effects on the vulnerability to hunting for certain cohorts of wolves. Transitioning from standard opportunistic rifle and bow hunting to trapping proved to be a contributing factor in a shift in the proportion of pups harvested during December. Results for other age cohorts were highly variable across the ten years and may be dependent on annual variation in hunter effort and participation, changes in harvest regulations, and perhaps litter size.

Our hypothesis that wolves ≥2 years old would be more vulnerable between January and February was not supported. Out of the three different age classes considered wolves ≥2 years old comprised the highest number of wolves harvested between January and February across all ten years. This suggests that these numbers reflect the number of sexually mature wolves harvested but not the number of individuals actively expressing breeding behaviors and responding to hunter induced stimuli. Accounts of reproductive suppression within our study area is unknown (Ausband 2017) and constraints on breeding by subordinates that are sexually mature may affect how individuals are actively participating in breeding during this time. Identifying the number of breeders in annual harvest (P. Rebholz, Chapter 2) may better predict how hunting and trapping stimuli during breeding season can affect their vulnerability.

Contrary to our hypothesis, wolves ≥2 years old were not more vulnerable to harvest during December when trapping season begins and dispersal is high. This trend was true for all subsequent years following 2011, but results from 2009 and 2011 agreed with our hypothesis perhaps because the resident wolf population had not been previously exposed to annual harvest. During 2009, wolf populations were at their greatest (Ausband 2017; Bangs & Fritts 1996) following their reintroduction into Idaho in 1995-1996 (Bangs & Fritts 1996) and annual statewide harvest season provided hunters with a saturated population of wolves that may have been unaccustomed to being hunted. Our findings show that vulnerability for all wolves ≥2 years old was not influenced by the transitioning from opportunistic hunting into trapping season during peak dispersal season, however, not all wolves harvested during that period would have been dispersing individuals. Wolves in the Rocky Mountains, USA are reproductively mature at 22 months, and typically do not disperse from the natal group until 3 years of age but have been recorded dispersing as early as 12 months (Jimenez et al. 2017). Female wolves are generally more philopatric and more likely than males to replace breeders within their natal packs (Jimenez et al. 2017). Typically, males choose to disperse between late fall and the start of the breeding season (mid-February) in search of a breeding position within another group, but more commonly create their own pack (Mech 2003). Our analysis grouped both males and female wolves ≥2 years old and may have missed the more common group of dispersing wolves during this time, future studies may better isolate dispersal behaviors by individual cohorts by refining the scope to males ages 2-4.

Our hypothesis that pups and yearlings would be more vulnerable between September and October after they leave the summer rendezvous sites was not supported. However, our hypothesis that pups would be more vulnerable in December because they would be more naïve to dangers presented by hunters at the start of trapping season was supported. Both hypotheses had a notable amount of variation among years suggesting that there is still a gap in knowledge in what is causing these cohorts to be over and underrepresented in harvest. Sufficient data on annual litter size across the state is unknown but may be a driving factor for why there was so much variation among years. Pair bond duration has been shown (Ausband 2019) to effect pup recruitment as well. It is possible that pups in a group with long duration pair bonds may be less vulnerable to harvest in these months because more experienced pairs will better be able to teach their offspring to avoid dangers associated with hunting and trapping.

Overall, harvest seemed to be opportunistic but there are years for each age class and ecologically significant period where we found wolves were harvested more than expected. This finding suggests there is still much to learn about what management actions most strongly affect wolf vulnerability to harvest. Our analysis and methods for determining the expected number of wolves harvested each year proved to be accurate for the majority of years and months. This technique could benefit future studies, with the goal of identifying how interannual variation effects groups of wolves and help determine thresholds where managers could manipulate harvest seasons and better isolate groups of wolves and regulate populations.

Chapter 2: Using harvest samples to identify timing and frequency of breeders in a population of gray wolves

Introduction

Some animals have evolved to live in groups in part because it lessens the workload of rearing young and increases fitness. Such cooperatively breeding species live in groups wherein one or more of the nonbreeding members (helpers) aid the breeders in rearing and protecting their young through territory defense, food provisioning, and teaching fundamental skills for long-term survival (Clutton-Brock, 2006; Solomon et al. 1997). These behavioral strategies vary among species and are influenced by environmental, genetic, and demographic factors. For example, group size and composition are dynamic factors that can affect a group's persistence because the amount of effort an individual contributes to the group may vary as a function of the different roles they perform, their sex, and age (Ausband, 2015; Ausband, Mitchell, and Waits 2017).

Breeders have been shown to have a disproportionate influence on group persistence and population growth. In some cooperatively breeding species, the breeding males and females spend more time than other group members directly and indirectly aiding in rearing young. For example, in packs of gray wolves (*Canis lupus*) the male and female breeders show increased care for pups compared to other pack members in the first month after they are born (Mech et al. 2003). Females will directly care for and feed the pups during the early months after birth, and the male breeder will contribute indirectly to pup care by provisioning the lactating mother with food, by hunting, and territory defense. Studies of gray and red wolves (*C. rufus)* show that the removal of certain sex and age classes from a group can have direct and indirect consequences for a population by decreasing recruitment and group size through breeder turnover (i.e., death or expulsion of a breeder; Ausband et al. 2017; Sparkman et al. 2017).

Group-living canids have been established as model species for studying the role that each individual plays within a social cooperative breeding group and how each role is critical to breeder fitness and group persistence (Ausband et al. 2017; Sparkman et al. 2017). In many of these species, breeders are vital to perpetuating the group, and the death or removal of an individual breeder can greatly affect a group's composition, genetic content, and shortterm population growth (Ausband et al. 2015; Ausband et al. 2017). Harvest in African lions (*Panthera*…) increased the frequency of breeder turnover, because harvest was disproportionally targeting large males that typically sired cubs of multiples resident females in the group (Loveridge et al. 2010). Human-caused mortality can have compounding effects on group-living species such as wolves. Studies have shown that harvest has both direct (removing an individual from a population) and indirect effects on fitness and recruitment of wolves at the individual and group levels (Ausband, Mitchell, and Waits 2017). The indirect effects of harvest can reduce fitness by limiting pair-bond duration, group size, and breeder turnover, all factors that have been correlated with a group's performance in alloparenting, predator avoidance, group hunting, and pup recruitment (Clutton-Brock 2006).

In Idaho, USA gray wolves are harvested annually, and harvest seasons overlap breeding, dispersal, and pup-rearing periods. Despite their importance to population growth, we often do not know how many breeders are harvested or when. Wolves are defensive of their established territories, groups, and breeding positions (Mech 2003). These innate behaviors (e.g., howling) can be exploited by hunters and trappers and may lead to bias toward certain sex and age classes in the wolf harvest. For example, a dominant male or female breeder might be more responsive to auditory or olfactory lures used by hunters and trappers during the breeding season. Similarly, dispersing adult males in search of a mate may also be especially vulnerable while focused on encountering a mate they ignore more risks than other age classes and social groups of wolves in the area. How the timing of harvest coincides with these life-history stages and their influence on individual behavior and vulnerability to harvest is poorly understood. Although gray wolf management in the American Northwest is hotly debated, empirical data on the effects of hunting and trapping on wolf behavior are rare (Gude et al. 2009). Additionally, despite our current understanding of how removing breeders from a group affects the population, little is known about temporal patterns of breeder vulnerability and what time of year they are likely to be harvested in established populations.

We sought to estimate the relative frequency of breeders in the wolf harvest in Idaho, and to test whether breeders were disproportionately harvested during the breeding season. In Idaho, the state requires wolves to be checked in after they are harvested, at which time Idaho Department of Fish and Game (IDFG) personnel can record the sex of the individual and collect samples to subsequently determine the age and obtain a DNA sample. However, these data are insufficient for determining whether harvested adult wolves are breeders. Fortunately, data on genetic relatedness and diversity can be used to reconstruct pedigrees, which can then be used to investigate mating systems, connectivity, and relationships among harvested individuals (Clendenin et al. 2020; Stenglein et al. 2011). Pedigree reconstruction can also facilitate more accurate genetic mark-recapture estimates from closely related individuals, which is vital for understanding population demographics of cooperatively breeding species. The application of this technique has given researchers a useful tool for studying whether who dies in a group and when may matter for group persistence and reproductive success (Ausband et al., 2017; Ausband 2019). In this study, we used genetic data and pedigree analysis to develop a new method for estimating the number of breeders in the wolf harvest and to determine what months breeders were most likely to be represented in the harvest.

We hypothesize that using pedigrees from harvest tissue samples we can estimate the frequency of breeders in harvest because wolves of all age classes are harvested each year during annual harvest and there will be multiple individuals harvested from the same family group. We predicted a sufficient number of packs will have both breeders and pups harvested to determine the frequency of breeders in harvest. We hypothesized that breeders will be more vulnerable to harvest during breeding season because they will be focused finding a mate or defending their spot as a breeder and less focused on threats presented by hunters and more likely to investigate auditory and olfactory cues displayed by hunters. We predicted breeders would be most likely to be harvested during the breeding season (i.e., January – early February in Idaho).

Study Area

Our study area comprised Idaho, USA (216,632 km^2) and included a wide variety of different landscapes, including mountainous forests, desert shrub, prairies, and open valleys. Elevations in the state range from 217 m to >3,859 m. Public forests and private timber holdings dominated by western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) comprised most areas. Public harvest of wolves began in Idaho in 2009, temporarily ceased in 2010, and began again in 2011. In 2014 through 2016 most harvest occurred during September–March, with a peak during the big-game rifle hunting season (Ausband 2016). Wolf trapping (foothold and snare; accounts for 66% of harvest in December) also occurred during the three study years in all 13 Wolf Management Zones created by IDFG.

Sampling

During the harvest seasons of 2014-2016, IDFG personnel collected tissue samples during mandatory check-in of harvested wolves. IDFG personnel also recorded location, date of harvest, means of take, animal condition, and affixed a pelt tag to the animal hide. Data on sampled wolves were separated into harvest year 2014-2015 and 2015-2016 to incorporate both the wolf's harvest year.

Laboratory Methods

We extracted DNA from tissue samples using Qiagen kits (Qiagen, Inc., Valencia, CA) in a facility dedicated to low-quality DNA at the University of Idaho's Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) in Moscow, ID, USA. For every set of tissue samples, we used negative controls to monitor for contamination. We used nine microsatellite loci and sex identification primers to identify individuals and sex (Stansbury et al. 2014). To verify matches or mismatches for samples different by only locus and to obtain sufficient data for parentage analyses, we generated nine additional microsatellite loci on each individual's sample (total = 18 loci) (Stansbury et al. 2014). All samples were sequenced at 10multiplex loci, if the individual was sampled and sequenced for the first time and no prior pedigree information was recorded, we sequenced an additional eight microsatellite loci. If the individual was sampled previously but the sample was missing or differed at one locus, we sequenced an additional eight loci. Each individual was assigned a unique wolf identification number that could be matched to the unique pelt tag number given by IDFG at the time of the harvest report. We used an Applied Biosystems 3130xl capillary machine to separate PCR products and conducted two independent amplifications for a consensus of heterozygotes and at least three independent times for a consensus of a homozygote at each locus. We included a positive and negative control for each PCR amplification. Additional details on laboratory methods are provided by Stenglein et al. (2010, 2011), and Stansbury et al. (2014).

Analysis Methods – Pedigrees

Once consensus genotypes were obtained at 17-18 loci, we imported them into the Program COLONY to calculate allele frequencies and run pedigree analyses. We took a conservative approach and only used samples that had >16 confirmed loci. After importing allele frequencies and genotypes for each individual, we allowed for polygamy in both males and females, assumed an allelic dropout rate of 0.01, and determined resulting pedigrees using maximum likelihood. When parentage was undetermined in COLONY, we further examined offspring genotypes against the likely parents of the remaining offspring in the group to allow for a two-allele mismatch owing to allelic dropout between parent and offspring to verify parentage across the 18 loci (Allendorf et al. 2013).

We extended the sample set and techniques of Clendenin et al. (2020) for sibship reconstruction by estimating a minimum number of breeders using only tissue and tooth samples from harvested wolves. We used program COLONY to combine the genetic and demographic data (genetics, age, harvest date, and harvest location) to generate a minimum population size of breeders that were harvested in each year's annual harvest season (Clendenin et al. 2020). We used this same method on all adult harvested wolves to genotype (using DNA from tissue samples) and age (using tooth samples and cementum analysis; Matson's Laboratory, Manhattan, MT) wolves from each year's harvest. We excluded all one-year-old wolves from the analysis, because wolves <2 years old are highly unlikely to have acquired a position as a breeder in the group (Ausband 2022). We included all males and females as potential parents and all sampled pups as potential offspring; samples were excluded if they were missing age data. We only accepted relationships produced by COLONY when $P \geq 0.90$. We divided the total number of breeders detected by the total number of successfully genotyped adults to estimate the minimum percent of breeders in annual harvest.

To test the validity of our genetic assignments, we compared the harvest location from the breeder and pup to the breeder and pup(s) relationships from the output assigned by COLONY. We used the metadata provided by hunters to assess the timing of harvest and separated the breeders from all other age classes of wolves. Samples with missing harvest dates were excluded from the analysis. We compared the observed frequency of breeders found in the harvest each month to the frequency of all wolves harvested throughout the state. We used a Chi-square test of independence in Program R to compare expected versus observed numbers of breeders in each month's harvest to test our hypothesis about the relationship between vulnerability of breeders and harvest month.

Results

We genotyped 229 harvested, breeding-age adults (>1 year old) and 203 young of the year (pups) from the 2014 to 2016 harvest season throughout the state of Idaho (Table 4). We documented 36 breeders in the harvest between the two-harvest years, probability of parentage was 0.98 (SD = 0.01). The minimum number of breeders harvested each year was 18 individuals in both the 2014-2015 and the 2015–2016 seasons. In the 2014 harvest year, nine females and nine males were harvested, and 11 females and nine males were harvested in 2015 (Table 5). Using the total number of breeders detected (36) and the total number of successfully genotyped and aged wolves (229), we estimated that roughly 1 in 6 (15.8%) harvested adult wolves were breeders.

Table 4. Counts of harvested gray wolves in Idaho, USA, 2014-2016. Wolves were assigned by age class using cementum aging from tooth samples collected by Idaho Department of Fish and Game. Harvest year is from metadata collected from hunters at mandatory harvest check-in by Idaho Department of Fish and Game.

	Total harvested 2014	Total harvested 2015
Young of the year	98	105
Adults in harvest	276	314

Table 5. Estimated counts from pedigree analyses of harvested gray wolves in Idaho, USA, 2014-2016. Estimates of breeding status are based on the relationship assignments in

Program COLONY and harvest month is from metadata collected from hunters at mandatory harvest check-in by Idaho Department of Fish and Game.

	Total 2014	Total 2015
Males successfully genotyped	46	71
Females successfully genotyped	61	51
Male breeders	9	7
Female breeders	9	11

Of the observed matched pairs of breeders and their offspring, 97.2% (35 out of 36) of the breeders were harvested in the same GMU as their offspring, whereas one pup was harvested in an adjacent GMU. Of the 36 breeders harvested, nine were harvested in January (25% of all identified breeders in the harvest; Fig. 2). There was a weak trend toward breeders being disproportionately harvested in January over the two years ($\chi^2 = 3$, df = 1, P = 0.08). We did not detect a trend in vulnerability to harvest during any other month.

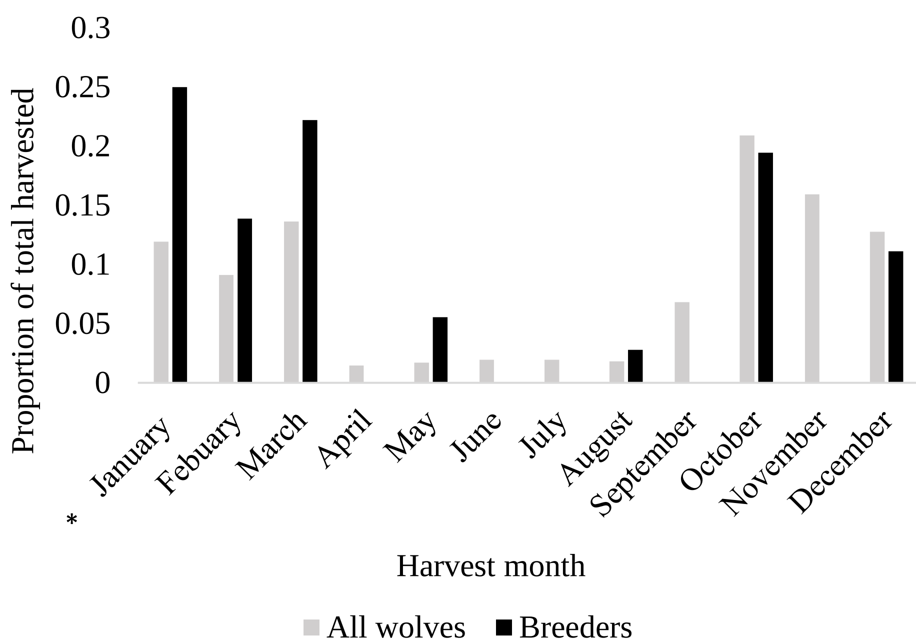

Figure 2. Estimated proportion of wolves from pedigree analyses of harvested gray wolves in Idaho, USA, 2014-2016. Wolves were assigned by breeding status and month they were harvested. Estimates of breeding status are from relationship assignments in Program

COLONY and harvest month is from metadata collected from hunters at mandatory harvest check-in by Idaho Department of Fish and Game. In January there was a weak trend toward breeding wolves being more vulnerable to harvest during breeding season (P = 0.08).

Discussion

Breeders are important for group persistence and recruitment in cooperatively breeding species, but the social status of harvested wolves is often unknown. We show that breeders are routinely harvested in Idaho, and there was an increase, albeit a weak one, in harvest vulnerability during the breeding season (i.e., January). We answered these questions using data made readily available to wildlife managers through mandatory harvest check-in (IDFG, state wildlife agency); and used a novel combination of harvest samples, genotyping and pedigree analyses using free software. We show that the minimum number of breeders harvested can be accurately and reliably identified from pedigrees using harvested samples. Our approach is useful for identifying the minimum number of breeders harvested in a population and for assisting with identifying when breeders are more likely to be harvested during the harvest season.

We found some evidence that breeders were more vulnerable to harvest in January during the two harvest seasons. Given our conservative approach, additional breeders may have been removed from the analysis because they did not meet our criteria. Research has shown that breeders in group-living species such as wolves have a disproportionate influence on group persistence and population growth (Ausband et al. 2017; Sparkman et al. 2017). Surprisingly, no prior studies have used the approach we described to determine the number of breeders harvested annually or to quantify temporal variation in vulnerability to harvest. Breeders may be more vulnerable during ecologically significant periods like the breeding season due to innate behaviors that are exploited by hunting and trapping techniques. This information could be useful for wildlife managers because nonbreeding and breeding adult wolves are nondescript, our research can give managers a tool to identify the breeding and social status of adult wolves harvested in a population.

Gray wolves are continuing to recolonize their historic range in the lower 48 states; state and federal agencies are and will be tasked with setting harvest quotas for wolf trapping and hunting seasons. Agencies might consider establishing mandatory harvest checks where state agency personnel can collect demographic data (genetic samples, tooth samples for age, harvest date, and harvest location). Collecting these data and using our method to create pedigrees from the harvested wolves will give wildlife agencies the ability to use opportunistic data and inform management and conservation of wolf populations. Creating pedigrees from harvest data can give agencies the potential to locate areas with newly established breeding packs in addition to identifying a minimum count of breeders in harvest, and when breeders are likely to be harvested. Knowing how many breeders are harvested and when, agencies can adjust regulations and craft seasons to alter the hunting or trapping pressure for cohorts like breeders. Breeder turnover in a population of cooperative breeders can influence population growth and affect offspring recruitment (Ausband et al. 2017; Clutton-Brock 2006). Thorough consideration of the factors influencing cooperative breeding strategies is timely for regions where wolves are beginning to recolonize and harvest seasons are not yet in place (e.g., California, Colorado).

It should be noted that we generated a minimum count of breeders because we chose to take a conservative approach to developing this new method. We successfully estimated the minimum percent of breeders harvested annually in Idaho (approx. 16%), and our methods can be used to determine how many breeders are harvested in other populations as long as the total number of individuals harvested is known. Nearly 1 in 6 adult wolves that are harvested annually in Idaho are breeders, and this ratio is higher during the breeding season. With 17% of our samples failing due to degradation, dependency on both the pup and the breeder having been harvested in the same year for breeder identification, samples being excluded because of missing age and harvest month data, and only accepting samples from wolves <2 years old with >16 confirmed loci there is a strong possibility that we underestimated the number of breeders harvested each year. Future research might better predict breeder vulnerability to harvest and how many breeders are harvested each year by using more recent tissue samples as well as lowering the conservative thresholds for acceptable samples.

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