Total-accounting method for reconstructing missing deaths of gray wolves (*Canis lupus*) in Wisconsin from wolf-year 1980 to 2012

Our aim in using a total-accounting method was to reconstruct the causes of death for missing wolves from the unmonitored but collared subset (*methods and Materials*) and estimate the number of missing deaths in the non-radiocollared subset.

Had we estimated mortality patterns and rates from the actively monitored subset *only,* we would have estimated relative risk with a bias for older, female, territorial residents in core counties, which suffer differential hazard rates in other regions (Schmidt et al. 2015) and a bias against poached wolves whose transmitters were inactivated. An alternative approach is to estimate the number of missing wolves and then reconstruct their fates, a total accounting approach.

Reconstructing the missing, radiocollared wolves

Over the study period, 486 wolves were radiocollared by the State of Wisconsin, but 15 of those died in the *P* age class so we excluded them from consideration below (Eq. 1 first set of parentheses). Of the 471 in age class *A* that died, disappeared, or remained on the air, 251 (53%) deaths were recorded (noting that 28 Wisconsin-collared wolves died out of-state and 27 non-Wisconsin collared wolves died in-state, reflected in the second set of parentheses in Eq. 1 below; and see *Supporting information SD1, SD2*). Also 38 living wolves had actively monitored radiocollars (WDNR 2015 in ER report 143) and an unknown number of unmonitored but collared radiocollars were left on living wolves at the end of our study (L_i); these living wolves are represented in Eq. 1 by the third set of parentheses.

Eq. 1: $M_i = (486 - 15) - (251 + 27 - 28) - (38 + L_i) = 183 - L_i$

That left 183 - L_i missing deaths in the unmonitored but collared subset for which we could not account. We estimated L_i, using the distribution of time-to-death, T_m, for radiocollared wolves with reported deaths (mean 631 ± 674 days, n = 251, *Supporting Information SD2*). The best-fit distribution for T_m was the exponential (Akaike Information Criterion *AICc* = 3977 versus next-best fit *AICc* = 4011, SAS Corp. 2013). Thus L_i was the sum of probabilities that each unmonitored but collared wolf of unknown fate might have survived to 14 April 2012 considering the expected value of T_m, the time elapsed between capture and disappearance, $T_{disappear}$, and the remaining time until the end of our study, T_{end} . We began by estimating Tc as the sum of two known times, using Eq. 2.

Eq. 2: $T_c = T_{disappear} + T_{end}$

where $T_{disappear}$ had an observed mean of 534 ± 767 days, n = 179 and T_{end} 3878 ± 2976 days, n = 179 (*Supporting Information SD2*). The precision of dates of disappearance is unlikely to be better than ± 15 days because of monthly aerial radio-telemetry flights. Then we estimated Li by adding the probabilities that each missing wolf outlasted the expected survival time Tm using Eq. 3.

Eq. 3: $L_i = \sum [e^{-(Tc \div Tm)}] \div T_m$

From Eq. 3, we estimated L_i = 1.5 ± 0.001 unmonitored but collared wolves presumed alive by the end of our study period. This number was robust to relaxing the assumptions. For example, making the most liberal assumption that time on the air before disappearance did not count towards future survival time yielded L_i = 2.6 wolves. Thus L_i gave us our final estimate for Eq. 1 of M_i = 180–182 missing deaths in the unmonitored but collared subset which we sought to reconstruct (Table 2).

Migration.—To reconstruct cause of death for M_i, we had to consider migration. Migrants to and from Wisconsin died of similar causes as those that died in-state (*Results*), therefore we assumed that missing deaths of migrants out-of-state resembled missing deaths in-state and undertook no correction here to estimate M_i. Note that non-radiocollared wolves cannot be treated the same way because neighboring states would not know these were originally Wisconsin wolves hence reporting bias would affect estimates for all causes of death.

Legal causes.—We did not reconstruct any missing deaths from legal causes because the missing, unmonitored but collared wolves had Wisconsin-labeled collars and legal causes were perfectly reported in in the neighboring states (*Methods and Materials*).

Nonhuman causes and collisions.—Following the logic of detection bias in the *Methods and Materials*, we assumed that nonhuman causes of death in the actively monitored subset provided an unbiased estimate of relative risk of nonhuman causes of death in the unmonitored but collared subset because the former was unaffected by detection bias. We also assumed collisions in the actively monitored subset provided an unbiased estimate of the relative risk of collisions in the unmonitored but collared subset. We assumed collisions only trigger reporting bias in general because drivers would detect a collision but might not report it for various reasons (*Methods and Materials*). Accordingly, we estimated relative risk for nonhuman causes or collisions in the actively monitored subset by Eq. 4.

Eq. 4: $R_{a,cause} = O_{a,cause} \div O_a$

where $O_{a,cause}$ was the reported number of deaths for the given cause in the actively monitored subset, and O_a was the reported number of deaths from all causes n the entire actively monitored subset. Note we estimated relative risk to reconstruct missing deaths – not mortality hazard rate that would take into account the time exposed to a hazard. Eq. 4 assumed the same real relative risk applied when a wolf was monitored or unmonitored. Departures from that assumption for nonhuman causes of death would be most likely in the direction of higher human-caused mortality, especially poaching (Liberg et al. 2012), rather than higher nonhuman causes, because the unmonitored but collared subset was found more often in peripheral counties (*Results*) where human-caused mortality predominated (Wydeven et al. 2001). Hence Eq. 4 was an over-estimate for nonhuman causes and collisions and thereby conservative for estimating poaching risk below.

By Eq. 4, the relative risk of nonhuman causes of death for the actively monitored subset only was 30.4–40.2% (Table 2), where the lower bound omitted the unknown causes and the upper bound treated unknown causes as nonhuman causes, which is an over-estimate. A relative risk of 30.4–40.2%, was much higher than the 4% calculated for the unmonitored but collared deaths reported in Table 2 ($O_{i,nonhuman}$), as expected when wolves were unmonitored. We also calculated Eq. 4 for collisions, yielding 10.8% relative risk. With the above two estimates of relative risk, we used a Monte Carlo simulation to estimate variation in the missing deaths from nonhuman and collision causes among unmonitored but collared wolves (described below).

The reconstruction steps described above are conservative for estimating poaching risk because poached wolves might die then have their collars inactivated, but we assumed inactivation preceded death by estimating nonhuman causes and collisions before reconstructing poaching.

Reconstructing missing deaths from nonhuman causes and collisions.—Because Eq. 4 provided no estimate of variability such as *SD*, we employed a Monte Carlo simulation with 5080 iterations for each of the upper and lower bounds to recreate the credible interval for $M_{i,nonhuman}$ and $M_{i,collision}$ among the M_i = 182 missing deaths from Eq. 1. We chose to stop at

5080 iterations after the *SD* of nonhuman causes reached an asymptote. We used a uniform random number generator to reconstruct nonhuman causes and collisions. Legal causes were not reconstructed because none were missing (perfect reporting) for radiocollared wolves (*Materials and Methods*) and we did not need to reconstruct unknown causes because the missing deaths were by definition unknown. In the final step of the simulation, we assigned all remaining, missing deaths from M_i as poached wolves. The output of the simulation was a lower bound and an upper bound on the number of deaths missing from the unmonitored but collared subset for nonhuman causes, collisions, and poaching (Table 2).

The averages of 5080 iterations for $M_{i,nonhuman}$ were 55.5 ± 6.3 for a lower bound and 84.1 ± 7.0 for an upper bound (Table 2). Note the upper bound reconstructs all observed unknown deaths as nonhuman causes, which was certainly an over-estimate. The range of values for $M_{i,nonhuman}$ provided an estimate for detection bias alone, by Eq. 5.

Eq. 5: $DETECTION_{nonhuman} = M_{i,nonhuman} \div (O_{nonhuman} + M_{i,nohuman})$

We estimated $DETECTION_{nonhuman}$ at 83 ± 2%.

Recall that estimates of M_i were subject only to one component of bias each; nonhuman causes were affected only by detection bias and collisions were affected only by reporting bias (*Materials and Methods*, Table 2).

As above, we reconstructed 19.5 ± 4.3 collisions among the missing deaths of unmonitored but collared wolves (Table 2). Using Eq. 5 for collisions instead of nonhuman causes, we estimated reporting bias alone at 58%. As predicted in *Methods and Materials*, reporting bias was lower (for collisions) than detection bias (for nonhuman causes of death).

Poached.—We were left with 77.9 to 105.5 missing deaths in the unmonitored but collared subset, which could not be assigned to any but the last remaining cause of death, which was poaching. Hence $M_{i,poached}$ was simply calculated by Eq .6,

Eq. 6: $M_{i,poached} = 1 - M_{i,nonhuman} - M_{i,collision}$

not by recalculating Eq. 4 for poached wolves because the relative risk of being poached for a wolf in the actively monitored subset required correction for both detection bias (accidental poaching that led to undetected death) and reporting bias (cryptic poaching).

Because 'cryptic poachers' inactivated radiocollars after killing a wolf in our model, the assumption that monitored and unmonitored wolves were equally likely to be poached was false by definition – poaching ended monitoring sometimes (*Materials and Methods*). When we calculated reporting bias for poached wolves that were unmonitored but collared, we used Eq. 5 again. That yielded 80–84% reporting bias, which represented the percentage of radiocollared wolves that were poached but not reported and were either unmonitored at the time of death, or had their transmitters inactivated after death. Alternately, we recalculated Eq. 5 including the subset being actively monitored after death (yielding 46–54% reporting bias). That can be interpreted as the percentage of unreported, poached wolves after radiotelemetry detected 30–34% (the difference between the former and the latter estimates).

Reconstructing the number of missing, non-radiocollared wolves

The relative risk estimates presented in Table 3 for radiocollared wolf deaths combined (reported and reconstructed) offered insight into the non-radiocollared subset. The non-radiocollared subset experienced more detection bias we surmised. For example legal causes were 30.9% of the reported deaths in the non-radiocollared subset compared to 6.3% in the reconstructed, radiocollared subsets (Table 2), yet legal causes affected radiocollared and non-radiocollared subsets equally (*Materials and Methods*). Therefore we knew a large number of non-radiocollared wolf deaths were missing from the dataset (Table 2), which if reported would have reduced the relative risk of legal causes closer to 6%.

Furthermore the above difference of 24% allowed us to estimate the absolute number of unreported deaths among non-radiocollared wolves, for a total or $M_n = 1,986 \pm 3$ missing deaths in age class *A* from wolf-year 1980–2012. Note the precision of this estimate reflects the low variation in the estimates of legal causes in the radiocollared subsets (Table 2).

We made no effort to reconstruct cause of death further among the non-radiocollared wolves because of the sampling biases that differentiated the radiocollared from the non-radiocollared subset (*Materials and Methods*).

Annual unreported deaths.—We apportioned the missing deaths by wolf-year with a single assumption. We assumed that missing deaths accumulated at an annual rate proportional to the population size. Thus we applied the logistic curve that fit the wolf population growth to allocate the missing deaths, given M_n and M_i from Table 2 and the minimum and maximum population estimates from Table 1. This assumption is consistent with the observation that disappearances of radiocollared wolves correlated closely to the wolf population estimates annually (Table 1–Pearson $r = 0.76$, $P < 0.01$).

By fitting the number of unreported deaths ($M_i + M_n$) to the population size each wolf-year, we estimated that 33–36% (*SD* 15%) of the estimated population died unreported annually (Table 1). Reported deaths comprised 10–11% (*SD* 4%) annually of the estimated population (*Results*) so only 31% of mortality was reported annually. Nor did the missing deaths correlate closely with reported deaths, when both were expressed as an annual percent of the minimum population estimate (Pearson $r = 0.16$), which suggests reporting was not a random process. That finding is consistent with suggestions in Chapron and Treves 2016 that policy changes were followed by changes in poaching that was more likely to be cryptic).

Annual mortality hazard rates.—We calculated *per capita* wolf mortality hazard rates, or the average time to death for each radiocollared wolf of age class *A*, divided by its "time on the air" exposed to mortality factors (Table 1, *Supporting information SD*2). We estimated the mean mortality hazard rate at 18% ± 10% for monitored, radiocollared wolves. Treating disappearances as deaths changed little (mean 19% ± 9%, *Supporting Information SD2*). Computing the mortality hazard rate simply as the number of deaths in the actively monitored subset divided by the number of radiocollared wolves annually from Table 1 also yielded an annual average of 18% ± 12%, therefore calculating exposure or "time on the air" for each wolf only improved the precision without changing the accuracy of the average. These estimates are all consistent with official estimates (*Discussion*).

Next we compared the mortality hazard rate faced by the radiocollared subsets and the non-radiocollared subset. Although the exposure time for non-radiocollared wolves was unknown, the above result about precision of the estimate gave us confidence in assuming a full year of exposure for each living wolf each wolf-year. This step assumed that the hazard posed by exposure for non-radiocollared wolves was the same as the hazard posed by "time on the air" for radiocollared wolves. Our assumption was conservative because radiocollared wolves tended to have lower exposure to mortality hazards because they used areas with lower road density and fewer people (*Results*).

Then, we divided the annual number of unmonitored wolves dying each wolf-year (reported + missing, Table 1) by the annual number of unmonitored wolves estimated alive at the start of the wolf-year (subtracting the number radiocollared from the minimum population estimate, Table 1), to estimate the annual mortality hazard rate for all unmonitored wolves. Using he lower bound divided by the minimum population estimate and the upper bound divided by the maximum population estimate, we estimated a mean of 47% to 51% (*SD* 18 to 20%) annual mortality hazard rate for unmonitored wolves. That mean was 29–33% higher than for monitored, radiocollared wolves calculated in the same way (*Results)*.

We then added the number of wolves that were reported + reconstructed to have died each wolf-year and divided by all wolves alive at the start of that wolf-year (Table 1), to estimate the overall annual hazard rate as a weighted mean equaling 38 to 41% (*SD* 10%). That mean is an under-estimate because it assumed all wolves present at start were present the whole wolf-year.

LITERATURE CITED

Liberg, O., G. Chapron, P. Wabakken, H. C. Pedersen, N. T. Hobbs, and H. Sand. 2012. Shoot, shovel and shut up: cryptic poaching slows restoration of a large carnivore in Europe. Proceedings of the Royal Society of London Series B 270:91-98.

Natural Resources Board. 2012. Adoption of Board Order WM-09012(E) relating to wolf hunting and trapping regulations, establishment of a depredation program, and approval of a harvest quota and permit level. Wisconsin Department of Natural Resources.

Schmidt, J. H., D. S. Johnson, M. S. Lindberg, and L. G. Adams. 2015. Estimating demographic parameters using a combination of known-fate and open n-mixture models. Ecology http://dx.doi.org/10.1890/15-0385.1. accessed 17 Decemebr 2015.

Wydeven, A. P., D. J. Mladenoff, T. A. Sickley, B. E. Kohn, R. P. Thiel, and J. L. Hansen. 2001. Road density as a factor in habitat selection by wolves and other carnivores in the Great Lakes region. Endangered Species Update 18:110-114.

Chapron, G., and A. Treves. 2016. Blood does not buy goodwill: Allowing culling increases poaching of a large carnivore. Proceedings of the Royal Society B http://dx.doi.org/10.1098/rspb.2015.2939