Combining two non-lethal methods in crossover design randomized experiments

Abi R. Fergus 1 Samuel J. Hermanstorfer 1 Adrian Treves 1

1 Carnivore Coexistence Lab, Nelson Institute for Environmental Studies, University of Wisconsin, Madison, WI 53705, USA

Equal co-authors Corresponding authors: atreves@wisc.edu

Abstract

Wildlife control is the subfield pertaining to preventing threats to human property or safety from wild animals. Various research fields interact within wildlife control from animal behavior to agricultural management and other social sciences. We review understanding of the effectiveness of interventions using nighttime lights and fladry, both visual deterrents, to prevent carnivores from approaching livestock on working farms. We describe successes and failures in randomized, controlled trials (RCT) with crossover design and the limits to inference and generalizability of their results. We present quantitative and qualitative evidence -- from two published Masters theses presenting RCTs with crossover designs -- as a heuristic device to organize knowledge and highlight associated gaps. We focus on the sustainability of scientific wildlife control and collaborative research with farmers, material interventions, wildlife coexistence with livestock and people, and science communication. We conclude that small-scale field experiments in wildlife control will rarely achieve the statistical power to confidently generalize recommendations to use or avoid tested methods. Therefore, cautious experimental scientists who wish to avoid false discoveries or exaggerated claims may routinely experience

constraints on promoting methods for wildlife control. The same constraints may not be felt by farmers or their allies, creating a tension in the science-policy interface between personal testimonials and scientific generalizations. We recommend steps that may fortify the science and enhance the sustainability of robust experimental designs under field conditions.

Introduction

Many applied researchers and practitioners have long been searching for methods to protect domestic animals from predators. Among the oldest recommendations are to combine multiple methods and replace a method if wildlife habituate to it (1-4). The rationale for using multiple non-lethal methods simultaneously is ostensibly to slow habituation to one method and to address differential vulnerability to different deterrent methods that might arise among different wild animal species or individuals. While scientists seem to have consensus on those recommendations, we do not know of any peer-reviewed studies that evaluated two non-lethal methods simultaneously using a randomized, controlled trial (RCT).

RCTs are the so-called 'gold standard' from biomedical research (5, 6) and adopted by other disciplines as the best way to infer causal mechanisms (7, 8) including our own subfield of wildlife control (9-11). Randomly assigning subjects to treatment or control conditions should eliminate selection bias, a common barrier to strong inference and replication. Also, using some manner of control such as a placebo, should ensure that one can distinguish random, procedural effects from real treatment effects. But RCTs are not immune to bias (12). Among the other safe-guards recommended for RCTs and other studies are various types of blinding (researcher bias minimized by ignorance of which subjects received which condition); crossover designs (all subjects receive both the treatment and the control conditions in random order); and methods of peer-review or publishing that reduce publication biases. Some of these steps may prove infeasible, others practical.

Here we present the lessons we learned from two independent Master's theses reporting crossover design RCTs on the same two non-lethal deterrents: Foxlights® - a night-time lighting triggered at random intervals and fladry- a visual deterrent consisting of flags attached to nylon rope hanging from a fence line. In Chile, Ohrens et al. (13) completed a crossover design RCT in which they found the lights to reduce predation of llamas by pumas (*Puma concolor*), but increase in livestock approaches by Andean foxes (*Lycalopex culpaeus*). Another RCT found an increase of red fox (*Vulpes vulpes*) visits while testing the effectiveness of the lights on a free-range pig farm (14). Hall and Fleming (14) could not determine if this finding also equated to more predation but did find light treatments correlated with fewer piglets surviving per sow. A non-randomized trial found the lights to a multiple-use landscape (15). In the time since the two experiments took place, we suggested that lights may not deter carnivores accustomed to human-caused lighting, and may even attract those carnivores accustomed to lights in search of food (16).

Fladry also has a record of RCTs with and without crossover design and less robust studies (e.g., correlations). Building on anecdotes from eastern European hunters who reported gray wolves would avoid crossing fladry (17)), Dr. Marco Musiani began a program of field and captive studies. The earliest experiments took place in European zoos, where fladry significantly reduced captive wolves crossings of the flag lines (18). Experiments also took place in field settings, strengthening evidence for its use to keep wolves from approaching livestock (19-21). Shivik et al. (2003) used a RCT with crossover design to show that fladry significantly reduced wolf approaches to white-tailed deer (*Odocoileus virginianus*) carcasses in Wisconsin. We are not aware of a RCT evaluating both fladry and lights simultaneously.

Here, our aims are to review methodological lessons learned by prior investigators and add to them with our own difficulties and solutions to experimental evaluations of wildlife control methods in the field. We address the operational challenges of building a sufficient sample of independent replicates, i.e., herds of domestic animals, and the challenges posed by helping animal owners in real-time while conducting a robust experiment. We also discuss our approach to inference about treatment effects, in light of the scientific community's recent introspection over the statistical issues contributing to the reproducibility crisis (7, 8, 22-24).

Methods

Field methods and statistical analyses of treatment and order effects for both Masters studies are fully described in (25) and (26) respectively. The latter by SJH learned from the former by ARF and tried to replicate methods as closely as possible. If readers seek more detailed methods than those we focus on here, the preceding citations provide copious details.

Deterrents: fladry and lights

Foxlights® are battery or solar-powered deterrents that flash two colors of LED light at 360 degrees. According to the manufacturer, the lights can be seen at 1 km (https:// www.foxlights.com/usa.html accessed 24 September 2023). According to the manufacturer, the flashes of light are randomized, with the goal to prolong the period before predators habituate to the light (https://www.foxlights.com/instructions.html accessed 25 February 2024). These solar-charged lights can be mounted on stationary infrastructure or attached to mobile objects such as livestock (16). We affixed all lights to stationary objects in this study.

SJH attached the lights with cable ties (30 and 45 cm lengths) to available fencing (wood posts, T-posts, chicken wire, etc.) of the property on all sides of the study area. The manufacturer recommends placing the lights at a height visible to the predator species of interest, especially as the device intends to simulate a person moving around with a flashlight (<u>https://www.foxlights.com/instructions.html</u> accessed 25 February 2024). SJH deployed devices 0.9-1.8 m off the ground as an estimated median height for carnivores in the study area (13).

Deployment height also varied with site-specific conditions, though we attempted to be as consistent as possible. ARF installed one light roughly at the center of the fladry line to serve as a night-time deterrent. ARF installed lights above the top of fladry lines a height approximately equal to head height for coyotes (*Canis latrans*) and gray wolves (*C. lupus*). When a treatment area was in the control phase, ARF removed the light battery to serve as a placebo control (i.e., present but inactive).

We found constructing and deploying the fladry time-intensive. Wind, wetland landscapes, thick vegetation, and the tendency of cattle to chew on their surroundings made fladry deployment difficult at some of ARF's farms. While llamas did not chew on fladry, their curious behavior detached several flags from ropes. In addition, the forestry flagging tape used to construct fladry had the downfall of having low friction on the polywire, leading to the flags bunching. Traditional fladry uses a canvas-like material sewed to a rope spaced 35-50 cm apart. Recent research suggests the inter-flag spacing of fladry will influence how effective it is in deterring canids of different sizes (27). Fladry has historically been applied around the entire perimeter of livestock pasture (21). This was not feasible for all farms in our studies. Instead, we sought to disrupt wildlife corridors, namely deer trails, which mays serve as paths of least resistance for prey and predators alike (28, 29). The length of fladry lines varied between treatment areas on farms based on the length needed to cut off a corridor. Fladry lines averaged 36.6 m in a straight line before any change in direction. ARF could not feasibly encompass the hundreds of ha of pasture on six farms with fladry; SJH enclosed entire pastures on 4 farms and a partial pasture on one farm.

Our experiments evaluated if fladry lines and lights could reduce traffic of large wildlife and carnivore occurrences in and around livestock pastures. Wildlife corridors appear to be paths of low resistance for wild animals (29, 30)(31). We identified wildlife corridors based on aerial photos of waterways and habitats on farms and then confirmed by ground-based surveys that

wildlife had recently used the trails. Indirect signs of wildlife traffic included packed earth and flattened vegetation indicating trails and by distinct tracks. Trail camera photos of black bears (*Ursus americanus*), coyotes, foxes, free-ranging cats (*Felis cattus*), etc. confirmed the trails were being used during our field studies.

Field sites, landscapes

ARF's experiment in northern Wisconsin included six farms managing cattle or sheep, which bordered or were within the Mashkiiziibii Ma'iingan Relationship Plan buffer zone (32). Our study ran from June through early September, roughly the average grazing period in this area on continuously grazed farms- when snow cover has melted, the grass is growing, and farmers have their livestock out on pasture. SJH's experiment in western Colorado's Montrose and Ouray Counties included five farms with different livestock and took place between 2 June and 17 August 2022.

Deploying non-lethal deterrents materials was hard work that required bending, kneeling, and navigating uneven private property. Both researchers were under-equipped in the field and needed to make the most of their situations by developing intuitive field methods with their limited resources while still collecting reliable data. By contrast, some of the largest fladry studies (not RCTs) (Stone et al. 2017, Windell et al. 2022) used all terrain four-wheel vehicles, trailers, and specialized tools for deterrent deployment. We could not have completed this research without volunteer field assistants.

Hall and Fleming (14) showed the importance of ambient light in mediating any effect of Foxlights ®. The moon, stars, and nearby human settlements produced ambient light that varied by farm in our study. We do not have systematic measures of ambient light across farms, only quantitative notes. For example, the only farm in ARF's experiment with marked human-caused ambient light was JAV which is located next to a major highway. This ambient lighting in addition

to preexisting motion detection lights near buildings may have reduced the effectiveness of the experimental lights. Indeed, The only livestock losses in ARF's study were repeated losses of poultry on farm JAV. This treatment area was not included in the data analysis due to the light being repeatedly knocked down by barn animals. At JAV, lights failed to deter a red fox and a domestic cat repeatedly shown on camera entering JAV's poultry barn. All other farms in this study were located on guieter country roads without street lighting. On all other farms in this study, lights associated with human structures were further from treated areas. In SJH's experiment farm MAR resided immediately next to a busy road to the east, and ambient light from the house on the property shone on the closest alpaca enclosures. Neighbors' lights on the north side of the property sandwiched the alpaca paddock with ambient light, but the west side of the property contained no human-caused light. There was very little woody vegetation on the property. Farms FIR and BRI—while shaded by a few trees surrounding the perimeter of the experimental area—had no nearby neighbors, reducing human-caused ambient light. However, lights on the landowner's buildings produced ambient light. Lastly, the experimental enclosures at Farms COY and ERG were immediately adjacent to the landowner's houses with moderate amounts of shade trees. Not only was this a high human traffic area, but there was a small amount of pre-existing lighting from the livestock owner's residence.

Research design

We randomly set up treatment (fladry deployed and light devices operating) or placebo control (fladry rolled up on ropes or ropes without flags deployed and light devices present but turned off). We deployed trail cameras similarly across all farms near wildlife trails, near water sources, and in areas without thick vegetation where carnivores had recently been seen by farmers. We cleared vegetation around the fladry and cameras regularly.

Treatment bias occurs when the researcher does not standardize the amount of time or intensity between setting up and maintaining treatment and placebo conditions. We found this

challenging. Still, we worked to control treatment bias by allocating roughly the same amount of time to set up and maintain treatments and placebos on each farm. ARF visited farms equally by keeping a log of farm visits. Such a step will necessarily be constrained by travel time and physical capabilities. For example, farm JAV(also described above) experienced greater carnivore conflicts than any other farms, which necessitated that ARF spend more time at this farm. Rather than investing disproportionate time-altering setups at farm JAV, ARF invested extra time consulting and brainstorming for possible husbandry solutions with these farmers. JAV was therefore an outlier in carnivore visits and attacks on livestock but also on time spent on the property by the researchers. For this reason, we set it aside as an outlier.

Domestic animals

We worked with animals under our institutional IACUC with a waiver dated 14 April 2019 because we did not handle or capture any animals. We did not seek to control farmers' patterns in grazing their animals, and we assumed this produced random error unrelated to experimental treatment. Each farm varied in how frequently farmers visited pastures, paddocks, or the animals. Farmers also varied in how often or how far they moved livestock between paddocks (grazing units within the pasture, separated by temporary fencing). SJH studied llamas, alpacas, ducks, and chickens sometimes in the same enclosures. The landowner from Farm COY would let chickens roam free during the day and out of the experimental enclosure. Each night they would return to the enclosure. These and other differences between replicates might confound interpretation of a treatment effects, which justifies the use of crossover design. Because crossover design consists of within subjects analysis (treatment v control and phase 2 v phase 1 for treatment effects and order effects respectively), it exerts the most control of potentially confounding variables among commonly used study designs.

Owners

All farmers in our study went through our institutional IRB approved consent process (2019-0194 and 2021-0923). While none of the farmers disrupted the design or withdrew during either study, they were not available to help check on the fladry or monitor for carnivore presence despite ARF encouraging them to contribute qualitative observations. This is likely due to the socioeconomic situations these small farmers in rural Wisconsin found themselves in, based on numerous farmers self-reporting a lack of resources and capacity to add deterrents to their chores and expense lists. SJH struggled to locate interested participants. Initially, SJH consulted with several local government agencies and non-profit organizations to connect with livestock owners. The groups declined to assist SJH's search on the grounds of value-based disagreements about carnivores or non-lethal methods or privacy concerns—even though our Institutional Review Board protocols stated that participants remain confidential through the research process and beyond. The initial skeptical receptions required alternative means to locate farmer partners through private, personal networks instead. For example, after a puma killed an alpaca, the farm BRI property manager was given SJH's contact information by an existing participant. Farm BRI joined the study soon after.

While finding landowners to participate in the research was difficult, the relationships built with farmers were invaluable to our experiments and more broadly our learning. The landowners took time out of their busy schedules to consult with the researchers about the project and a number of the farmers seemed to derive mutual benefits in discussing the challenges and rewards of being a livestock farmer on the same landscape as carnivores. The landowners drew attention to system-wide problems, such as the lack of water availability during drought conditions in western Colorado. For ARF, building relationships with farmers was the foundation for studying carnivore coexistence and helped them to integrate teachings from Bad River tribal members. These interactions helped us to understand how intact Indigenous culture, language, and life-ways relate to the wider ecosystem. The farmers greatly impacted the lives of the

9 of 30

researchers with their kindness, hospitality, curiosity, and commitment to science and carnivore conservation.

Inference about treatment effects

We do not present new statistical analyses because the Masters theses (25, 26) did so and neither found strong support based on p-values. Nor do we believe that pooling the datasets would necessarily change that conclusion because some farms seems to tend towards attractive effects of lights and others towards deterrent effects in both studies.

But more importantly, we do not wish to obscure treatment effects with p-values. We discuss the several interpretations of a lack of treatment effects from indeterminate to no effect to inconsistent effect. Rather than emphasize statistical significance, we aim to discuss what we believe the patterns of carnivore approaches reveal and why other investigators may wish to replicate our methods.

Results and Discussion

Effective solutions to reduce flag bunching may involve heavier materials for flags like canvas or a stronger fastener between the flag and rope. However, these improvements may increase the time and costs required for deployment. With enough funding, partnering with professional manufacturers may increase the quality of the fladry and the speed at which it is prepared for the field. Government contracts could increase the scope of experiments to larger perimeters, and the quantity of projects across the USA. Thus far, USDA has had funding to construct durable and even electrified fladry. ARF tried to get USDA support for their study and SJH asked wildlife control agents to facilitate recruitment of farmers. Neither effort succeeded which we interpreted to be due to skepticism on the part of the government employees. Such skepticism may be based in value differences or idiosyncratic opinions. In our subfield the contrasts and similarities between perceived effectiveness of interventions for wildlife control

has not been studied until recently (33). Skepticism and different perceptions of effectiveness may reflect weak treatment effects or variable treatment effects across subjects.

Table 1. Carnivore visits to two sets of study farms divided by treatment or control and phase in the randomized, controlled trial with crossover design. Analyses explained in the footnote[†].

Study and farm	Camera Days for Treatment (T) and Placebo (P) sequence	Phase 1 Visits	Phase 2 Visits	Phase 1- Phase 2	Treatme nt- Placebo
ARF (25)					
Ady	68 (P=34, T=34)	3	1	2	2
Eut	70 (T=35, P=35)	0	0	0	0
Sor	70 (T=35, P=35)	0	1	-1	1
Tig	70 (T=35, P=35)	0	7	-7	7
Tik	67 (P=32, T=35)	0	0	0	0
(SJH (26)					
Bri	44 (P=22, T=22)	10	12	-2	2
Соу	62 (P=29, T=33)	32	26	6	-6
Erg	45 (T=22, P=23)	7	3	4	4
Fir	55 (P=26, T=29)	13	8	5	-5
Mar	57 (T=28, P=28)	0	1	-1	-1

[†] Experimental designs for ARF (25) and SJH (26) followed (13) and analyses followed (34). In brief, we assigned treatment or placebo randomly for Phase 1 then reversed condition in Phase
2. We relied on non-parametric tests of phase effects (Phase 2 - Phase 1) and repeated for treatment effects (Treatment - Placebo). The response variable was frequency of carnivore

visits (visits per camera-day). A visit was defined as an independent detection by camera or indirect sign (footprints, scat, landowner detailed observation) during a 12 h period (defined as day or night, 25, 26). Repeated detection within 12 h was scored as a single visit. We split data by day and night, by carnivore species, and all data pooled.

Table 1 presents the data from both studies on the frequency of visits by all species to the respective farms during the trials. We found neither deterrent nor attraction caused by treatments but instead heterogeneity among farms (25, 26). ARF detected only coyotes and deer. SJH detected more carnivore visits (got, puma, black bear, free-ranging cat) than in ARF's study, but less than anticipated from farmer self-reports. It is likely some carnivores were undetected during their visits or deterred by the experimental set up and monitoring regardless of treatment or placebo conditions. Seasonal or week to week changes in wildlife ranging patterns could also explain scarcity of observations. For example, wildlife may range from the highlands back to the river basin over the hot summer months. Therefore, carnivore visits during the early summer could have been suppressed, as there may have been less prey around the study areas (26).

Maintaining the potential effect of deterrents under field conditions

In both studies, wind and domestic animals reduced the potential deterrent effect of fladry as they altered the spacing between flags. Recent research shows attaching two ropes—one rope at each end of the flag—can reduce flag furling in the wind and wrapping upon itself, leaving it dysfunctional until researchers correct it (25, 27). The level of maintenance required for the fladry may be unappealing or inaccessible to a farmer, but it may also be a valuable way to increase human presence via maintenance checks. Human presence may act as a deterrent in itself (34). After detecting that cattle chewed on the fladry that was impossible to setup outside of their pasture and out of their reach, ARF tried treating the fladry with hot sauce to prevent the chewing but this proved ineffective, likely because the capsaicin quickly broke down when

exposed to the weather. Setting up the fladry outside of enclosures worked well for SJH, but the only solution to combat llamas detaching flags from the ropes was to proactively repair fladry lines at every opportunity.

We found it important to pair the day time deterrent of fladry with a night time deterrent of lights, since the timing of carnivore visits varied to include both day and night. The lights alone may have the potential to attract carnivores (See Introduction). ARF has also witnessed, via trail camera, one occurrence on farm JAV that appeared to show coyotes approaching a line of lights and fladry at night, from a distance. ARF interpreted this behavior as the lights potentially having the effect of initially attracting the coyotes, though they never entered the protected pasture based on continue trail camera monitoring. The potential for attracting animals to novel materials is an important risk factor to communicate with farmers before deploying lights. Fladry did not seem to cause the same attraction.

SJH learned solar-powered devices must receive full sunlight to operate effectively. If it is ideal to deploy a light device in a shaded area, replace it with a fully-charged solar device or use battery-powered devices instead of relying on a single device (Solar Foxlights® Instructions, 2023). Better yet, researchers should have a surplus of NLD materials to sufficiently repair or replace light devices, fladry, or trail cameras at a moment's notice. During his farm checks each week, SJH transported extra supplies.

Previous work in our lab revealed that farmers could turn on lights during the placebo control condition, undermining the crossover design (13, 16). Therefore, other approaches to control such as removing the device entirely or more frequent monitoring might be needed.

Domestic animals

In our sites, the major approaches to grazing livestock on pasture are called continuous and rotational grazing (Smith, 2007 https://hereford.org/static/files/02 07 RotationVsContinuous.pdf accessed 1 October 2023). Under both methods, farmers estimate how many head of cattle can be sustained per acre or hectare of pasture. In continuous grazing, the farmer does not control where or when the livestock are within the pasture system and allows the animals to direct their own grazing throughout the growing season. In rotational grazing, the farmer controls where and when the livestock are within smaller pastures by giving them access to paddocks or sections of the pasture in different periods, such as daily or weekly. The decision about where and when to move the livestock is based on the number of cattle relative to pasture quality and on allowing the cattle to graze the grass to a certain length. In humid habitats this rotation will allow the plants to grow back and be grazed again multiple times within one growing season (Smith, 2007 https://hereford.org/static/files/02 07 RotationVsContinuous.pdf accessed 1 October 2023). Because rotational grazing entails moving the livestock between paddocks, it also results in there being more frequent human presence around livestock. Heralding back to traditional pastoral agriculture, this might simulate the effect of having a human herder with the livestock and thus reduce carnivore presence or conflict. Rotational grazing may also help farmers notice any sign of carnivores approaching or interacting with livestock earlier than continuous grazing farmers who don't need to interact with livestock as regularly (34). While researchers shouldn't take lightly the work that goes into rotational grazing or in changing husbandry methods to rotate pastures, these interventions should be compared experimentally.

The only livestock losses in ARF's study were repeated losses of poultry on farm JAV. This treatment area was not included in the data analysis due to the light being repeatedly knocked down by domestic animals. Farm JAV's motion-detecting lights did not deter one or more foxes repeatedly visiting the poultry pens or a domestic cat repeatedly entering the poultry barn We attribute the disproportionate livestock loss on JAV to the large stream which is a part of a larger

river watershed running through the farm's pasture land. This served as a major corridor where most wildlife seemed to focus movement based on trail camera and indirect sign surveys.

During SJH's experiment, no domestic animals were lost during either phase or condition, nor during, before, or after a washout period. However, one landowner lost an animal to a puma prior to contacting SJH to participate in the experiment. If the animal was not lost, then this landowner likely would not have reached out to work with SJH. No animals were lost immediately following the conclusion of the experiment, either.

Wildlife

All farms were subject to unique environmental conditions. In ARF's study, all farms were within the Bad River Watershed and were surrounded by relatively similar wetland and mixed hardwood and pine forest habitat. We confirmed the presence of coyotes (eight total occurrences during the study period) and black bears (four total occurrences outside of the study period) within the research area based on trail camera data. Gray wolves were never detected on any farm and bobcats (Lynx rufus) were detected on two farms during daylight, but outside of the study period. A puma was reported by two different farm families during the study period, but never detected by ARF via trail camera or tracking. Tracking conditions at the time ARF investigated were very poor due to drought and clay soil leaving few tracks. In SJH's study, all farms were near the boundary of Montrose and Ouray counties. Visits from carnivores included one puma at night, seven black bears (1 day and 6 night visits) across three farms, and 44 visits from foxes (9 day visits, 35 night visits) across all five farms. SJH never recorded the occurrence of a coyote or bobcat, and gray wolves were not in this region of Colorado yet. Farms varied by habitat and topography (plateaus, lowlands, high tree density, at the base of a ridge line). Properties in closer proximity to suitable carnivore habitat may have a higher number of carnivore visits when compared to farms further away. Three of the five farms had direct access to water via creeks or rivers. These three properties had a higher occurrence of

carnivore visits captured on the trail cameras. Farms closer to water might have a higher rate of carnivore visits especially in dry habitats and seasons.

Owners

We recommend researchers build relationships with owners or managers over time ahead of intended research projects with livestock farmers to increase successful participant recruitment. The most effective means of recruitment was by showing up in person and having local connections. Most farmers expressed to ARF that they would enroll in the study to help ARF, as opposed to immediately seeing potential benefits for their farm. None of the participating farmers seemed to have the capacity or interest in personally helping monitor the deployed deterrents or to be involved in the review of the data. ARF assumed this mostly relates to the reality small livestock farmers face of being overburdened to make ends meet. This sentiment also reflects the role farmers have repeatedly told ARF that government and academic scientists should play, i.e., finding solutions for preventing livestock losses. For example, prior to this study, farmer JAV reported having no relationship to the Bad River Tribe. Out of all the farms, JAV is the only one ARF continued to work with after the study to continue to troubleshoot carnivore coexistence. Perhaps the farmer felt a good relationship was established with the Tribe.

SJH succeeded in recruiting private landowners for his experiment when his strategy shifted from working with local government agencies and NGOs to focusing on private meetings with each landowner. Local government officials and agriculture-based non-profit organizations did not help him to facilitate the landowner recruitment. Therefore, future research should take on a two-pronged approach of good faith communication with cognizant governments while at the same time, independently exploring private relationship-building with property owners. While the husbandry and backgrounds of farmers may vary substantially, trying to control for which types of farmers enroll in a study may not be feasible, based on our experience. It is important for some level of farmer agreement to accomplish carnivore coexistence research. If a farmer isn't convinced of any possible benefits they will not see it as worth their time, or any perceived risk to their livestock, to allow a researcher on their land and around their pastures. Additionally, conclusions and observations in research can be improved by a farmer's interest in the study and in informing the researcher of any carnivore sightings or encounters. We found it best to openly recruit farmers of any demographic (generational, first generation, organic, non-organic, etc.) and base enrollment on whether the farmer is interested rather than pushing to work with less interested farmers whose management balances the experimental design. In both studies, we could not afford to be choosy in recruiting participating farmers, and this could be connected to the outsider-insider problem.

Researchers may exhibit different characteristics than the communities in which they work, which can bring advantages and disadvantages (35). Among the disadvantages, a new person to the region requesting to speak with landowners who encountered problems with carnivores in the past) may trigger resistance or hostility. Resistance to research may reflect a local farmer's strategy of preventing access to insider knowledge. By contrast, farmers may perceive researchers as allies against outside groups with whom they have difficulties (35, 45, 46). Much has been written about participatory research, so we sum up by concluding that RCTs with crossover design on private farms benefit from individual recruitment of the farm owners, in our experiences and those of our colleagues (16). Successful interpersonal communication is essential to this work and it won't help a researcher to get off on an awkward start with a farmer or be in a position in which they feel added pressure to prove themselves to the farmer who less than willingly joined a study. If a researcher happens to recruit more interested farmers than the researcher actually has capacity to work with, there could be a good opportunity to qualitatively categorize farmers into husbandry and background types and then randomly select an equal sample from each category, but the researcher should not sacrifice being able to effectively work with recruited farmers for the sake of more robust experimental design. It seems

reasonable to assume that if some farmers enroll and have a positive experience working with the researcher, that additional and varied demographics of farmers will become interested and participate in future studies, which can also provide more insight to how carnivore coexistence may vary between farming demographic groups.

Many RCTs with crossover design fail to find consistent treatment effects (16). This raises the hypothesis that some livestock may experience deterrent effects and others attractive effects, and yet others no effect of treatments. Accordingly, below we specify individual features of the 11 subject farms to aid in future experimental design and interpretation.

Farms in ARF study

Farm TIG grazed 94 cattle rotationally on 134 acres and self-reported more frequent farmer visits to their cattle, perhaps in part due to the husbandry method of rotational grazing. This farmer had no previous relationship to the Tribe. In 2012, this farm experienced a wolf predation on a calf when a wolf entered the pasture through a drainage ditch. At that time, fladry was deployed for a week in the affected paddock by USDA. Outside this event, the farmer felt that deterrents were not necessary, because the pasture was protected by electrified high tensile fence. This farmer reported that he hadn't seen coyotes in the past two years on the farm, but our trail cameras revealed coyotes were present on an ATV trail adjacent to the pasture on seven separate occasions during the control period and none at all during the treatment period. That corridor running along farmland was regularly used by coyotes, despite the farmer not detecting coyote presence. It seemed that despite this corridor being in immediate proximity to livestock pasture, the coyotes using it were not bypassing the electric fence that separates the corridor from pasture. Additionally, our data suggest the deterrents seemed to keep the coyote(s) from using this stretch of the ATV trail during the treatment condition.

Farm JAV had no history of deterrent use in pastures and the farmer reported it was not feasible in terms of time or money to treat the entire pasture. During the study, this farm had 75 head of cattle grazing continuously on 97 ha. The pasture land was mostly fenced with non-electric high tensile wire and some barbed wire. Farm JAV has a western property border almost entirely overlapping with a growing wetland. The owners attributed the growing wetland to a wildlife conservation easement they reported existed on bordering land. This bordering wetland habitat rendered it difficult to fence off the farm's grazing land effectively from coyotes in particular. ARF worked with the owner of JAV to make a husbandry change with the goal of reducing calf vulnerability to predation by keeping the cow-calf pairs in a paddock surrounded by electrified fence and between the highway and human dwellings. Historically, the farmer allowed cows to birth their calves out on pasture. This resulted in coyotes coming in the pasture to eat the placenta and also resulted in occasional calf kills. The husbandry change of penning the cowcalf pairs was initially combined with a line of fladry and lights along the boundary with the open pasture. Trail cameras showed coyotes inspecting this line of deterrents, but from a distance and not approaching the paddock of cow-calf pairs. This method seemed to work from 2020 until spring of 2023 post-study, when ARF investigated a calf kill and concluded it was done by two coyotes based on forensic investigation and tracking (this was later confirmed by a USDA APHIS-WS investigation). ARF attributes the high amount of poultry loss experienced by this farm to a creek, which served as a wildlife corridor for carnivores including coyotes and bobcats. This is the only farm that lost livestock (poultry) during the study period (see Methods for description of barn lighting and fox forays).

Farmers who owned EUT and ADY reported a recent history of land use change from forested land to developed agricultural land. In order to reduce environmental degradation such as nutrient loading and erosion, agricultural government agencies like USDA NRCS and others promote vegetated riparian buffer zones. Future studies could explore how this stewardship method impacts wildlife movement along these corridors. For instance, does preserving intact vegetation and habitat along waterways, which serve as corridors, influence whether wild animals venture into pastureland and further prey on livestock once they encroach (36).

Farms EUT and TIK had the smallest differences in carnivore visits between treatment and control, because no carnivore visits were detected on these farms during the study period in treatment or control. On farm TIK, there was a coyote chasing a deer documented on trail camera, but this occurred five days before the deterrents were deployed and the study period begun. Similarly, on farm EUT coyotes were detected, but not until the months following the experiment. In both cases, carnivore use of the pasture land, at least at treatment areas, might have been less frequent during the summer compared to the spring and fall. This pattern would match what ARF has observed while living in their study area since December 2018- that deer tend to congregate in agricultural lands more in the fall and the spring.

Farm ADY saw the most carnivore presence during treatment period with one coyote being detected during the treatment period compared to three occurrences of coyote during the control period. Both the coyote visits during the treatment and control occurred during the day. There seemed to be a coyote family present, because one of the detections during the control period was a pair of pups. When a coyote appeared during the treatment period, the coyote was running parallel to the line of fladry as if it might have been corralled. This reflects how fladry was originally designed to control the movement of wolves in order to hunt them in Europe. Therefore, detecting the presence or visits of carnivores (Table 1) by itself may be an inadequate measure of risk of predation on domestic animals. Actual injuries or deaths of domestic animals may be the only useful measure of the effectiveness of deterrents.

Farms in SJH study

Because of its late entry into the study (see Methods), farm BRI was the only property SJH worked with that did not have a fladry line around the entire perimeter of the pasture. Farm BRI

lost an alpaca to a puma only days before contacting SJH. The puma climbed over an eight-foot fence to kill the alpaca in the enclosure that would be involved in the experiment. While there were plenty of human-made corridors on the property (even one directly east of the enclosure), the puma presumably followed the stream found on the west side of the property. Therefore, SJH deployed fladry only the that side of the enclosure. SJH distributed lights and trail cameras normally around the property as he did with the other farms. This farm was also the only subject farm with multiple livestock types in the same enclosure, which did not seem to change results. Nevertheless, farm BRI was excluded from the overall within-subjects analysis given its incomplete perimeter of fladry. Still, interesting observations surfaced. No pumas appeared on the trail cameras at Farm BRI. SJH captured many mule deer (O. hemionus) using a wildlife trail near the creek near the farm. It is possible the puma attacked the alpaca after following deer along this corridor. Furthermore, while no pumas appeared on the trail cameras, Farm BRI hosted a fair share of foxes which liked to use the access road to the east of the enclosure. SJH also observed one black bear at the transition zone between the wildlife trail and human-made corridor, indicating the bear could use both the natural and human-made trails near farm BRI. Lastly, the landowner notified SJH of a potential coyote den south of the enclosure. No coyotes appeared on the trail cameras, or were identified via indirect sign.

Farm ERG demonstrated the largest difference between the treatment and placebo control conditions: 5 more visits in the treatment condition than the placebo control condition (when not considering outdoor cats). The enclosed property was a garden and orchard, which house domestic ducks. The area sat on a slope leading up to a highland plateau, with an access road to the west and thick vegetation and wildlife trails to the east. Most of the vegetation was scrub oak. The treated areas were close to the landowner's residence. On the west side of the access road, a human-made channel diverged from a natural creek. Although the landowner claimed to hear coyotes howling close to their property during the experiment, the farmer reported they never approached the duck enclosure. Additionally, there was no indication via trail cameras nor

other identifiers that coyotes ever visited the property during the experiment. Additionally, the landowner witnessed many pumas between their house and the highland plateau over their residence at this property. At Farm ERG, all of the carnivore observations occurred on wildlife trails frequented by deer. Foxes were the only carnivores observed on this farm beside outdoor cats (7 times total). Foxes may not have been deterred from the property via the lights SJH deployed, as observed recently (13, 14). However, it is difficult to give any specific reasons for why there was a stark difference between the placebo control and experimental phases since there were very few observations of carnivores overall. Foxes visited the study area six times in the first phase (experimental condition) and only once during the second phase (placebo-control condition). No carnivores were observed using the human-made corridor to the west of the duck enclosure.

Farm MAR had the smallest difference between visits during the treatment and control conditions (one less visit in the treatment than in the placebo control condition). In all, Farm MAR recorded one red fox visit. This could be due to the geographic positioning of the property, as it is surrounded by cattle farms on three sides with a busy road nearby. There was little natural covering for carnivores throughout most of the property except to the west, where the landowner grew hay in the summertime. Neighbors preferred to protect their property with lethal deterrents, with unknown effect.

Inferring effects

An unproven method of wildlife control may have no effect, or a positive (attractant) effect or a negative (deterrent) effect. Alternately, we may have insufficient evidence to feel confidence in any of the three conclusions. Because of the low sample sizes, our statistical tests will have low power to discriminate between those alternatives (7). In our studies, the p-values do not suggest effective treatments —and even if they did, we are wary of false discoveries (false positive rates, FPR) (22, 23). We have mitigated FPR by using a randomized, crossover study design,

but FPR never equals zero (7). The only safe conclusion from the frequentist p-values we calculated is "indeterminate".

Generalizable science versus individual experiences of experimental treatments Yet, perhaps our farmers deserve more illumination than our scientific uncertainty leading to an indeterminate conclusion? Consider the treatment effects (Table 1). The early study (ARF) shows more of a suggested deterrent effect of treatments whereas the later study (SJH) does not. The statistical threshold for significance cannot be used to reject one farmer's satisfaction or dissatisfaction with lights and fladry. Statistical significance and perceived effectiveness operate in different realms of generality and specificity. Examining the effects of our experiments herd by herd as we have in these Results, we cannot reject the owner's claims for their own herd that a method deterred, attracted, or had no effect. Differences between treatment and placebo control were not always zero even if they averaged zero. Moreover, approaches by carnivores are not equivalent to attacks by carnivores as the example from farm TIG and its adjacent coyotes revealed. In short, we the investigators find no general treatment effect but the owners of those herds may honestly and reasonably disagree.

A difference in messaging between scientists seeking generality and individuals experiencing experimental effects is not a crisis for science. It does pose a challenge to public policy and individuals (domestic animal owners in our case). Clearly, we as scientists concerned with integrity must report indeterminate conclusions about treatment effects to the public. We must do so because we seek generalizable inferences that may inform policy and protect future owners and their domestic animals should they follow our recommendations. U.K. badger control studies exemplify the problem. These experimental evaluations of killing badgers to attempt to control the transmission of bovine tuberculosis (bTb) between cattle farms were funded by the U.K. government, designed by a panel of eminent scientists, and insofar as we can tell from peer-reviewed work, conducted expertly with rigor and care (37-42). Nevertheless,

the cattle industry representatives rejected the scientific conclusions that indiscriminate badger killing made the situation worse and targeted removal of infected badger groups did not improve the disease transmission rate generally. Indeed, the findings seem to suggest that targeted killing sometimes accelerated the spread of bTb and sometimes inhibited the spread of bTb. In the UK bTb case, the industry would be irresponsible in generalizing a different conclusion than the scientists. The same is not true for individual farmers.

If an owner in our study chooses to promote or demote one of our methods, should we contradict them with our general conclusion of indeterminate effect? Probably not. The crux of the issue is whether anyone is recommending the method in general or adhering closely to an individual-specific recommendation, e.g., 'method x worked on subjects y during the summer of z...' with appropriate specificity. That is not generalizable in a scientific sense but may be accurate and fair-minded nonetheless. In short, we are drawing a distinction between general conclusions that are actionable in a public policy intervention (funding, disseminating, promoting, etc.) versus participant testimonials. For example, in our field experiments, several land owners experienced apparent improvements in the safety of their domestic animals during the treatment condition. They might feel justified in promoting the treatments to their colleagues, friends, neighbors — but here we caution against generalizing. Likewise, some of our landowners experienced no benefits or even worsening of risk. They too might be justified in describing outcomes to others. As we search for generalizability, we can examine the conditions for our landowners who experienced either extreme of effects (attraction or deterrence of carnivores) and pose appropriate novel hypotheses. Those will await future experiments. In the meantime, word will get out and the consequences of non-generalizable experiences will be felt as we describe further below.

Trusted messenger theory in communication sciences (43, 44) informs us that testimonials from like-minded persons or people of similar background carry great weight with their appropriate

audiences. In such cases, an experimental participant who promotes or demotes a method based on whatever criterion is likely to influence other individuals who seek a solution for themselves. This is the modern dilemma of 'fake news' or irreproducible science. We cannot constrain our participants or anyone else from speaking about the methods we test experimentally. Perhaps we may try to share our view of our general conclusions with each participant in an effort to regulate their future communications. But when their audiences, including the media, pick up those communications we and our subjects may lose any control over the communication of specificity of individual experiences. The illusion of generalizable facts stemming from individualized and specific anecdotes worsens when audiences have short attention spans for details or cannot remember details outside their sphere of expertise. Then, generalizable science is likely to lose out in the current sociopolitical atmosphere that treats testimonials as generalizable facts.

Does this mean we should abandon the endeavor of experimental evaluations of treatment effects? No, not in our opinion. The discussion above points to the appropriate conduct of researchers. Remain cautious, repeat the most scientifically defensible conclusions as often as needed without abridging the caveats and uncertainties of one's results. Educate the reporters (and their editors) who cover science not to abridge details. Work towards public education about science. Hope for the best.

Specific recommendations on wildlife control methods we tested and their sustainability

Our work also speaks to three criteria for sustainability. Non-lethal methods that effectively protect domestic animals from wild predators can prevent the sorts of injuries that lead owners to seek lethal interventions. Therefore, non-lethal methods are more biologically sustainable for wild predator populations and potentially also save money for governments that are usually called upon to pay for lethal interventions (a second type of sustainability). Moreover the non-

lethal methods we studied (flagging and lights) are inexpensive and can be owned, purchased, and maintained by owners themselves. That demonstrates a third type of sustainability.

Acknowledgments

The Bad River Natural Resource Department and tribal members provided invaluable support to ARF. Summerlee Foundation and Animal Welfare Institute supported SJH. Derse Foundation and Therese Foundation supported AT. We thank all the farmers.

References

 Linhart SB. Managing coyote damage problems with non-lethal techniques: Recent advances in research. Proceedings of the Eastern Wildlife Damage Control Conference. 1981;1:105-18.

2. Mason JR, Shivik JA, Fall MW. Chemical repellents and other aversive strategies in predation management. Endangered Species Update. 2001;18:175-81.

3. Shivik JA, Treves A, Callahan M. Non-lethal techniques: Primary and secondary repellents for managing predation. Conservation Biology. 2003;17:1531-7.

Shivik JA. Tools for the edge: What's new for conserving carnivores Bioscience.
 2006;56(3):253-9.

Mukherjee S. The Emperor of All Maladies: A Biography of Cancer. Mew York: Scribner;
 2010.

6. Interactive Autism Network. Gold standard of evidence: The randomized controlled trial (RCT) 2017 [Available from: <u>https://iancommunity.org/cs/understanding_research/</u>

randomized_controlled_trials.

 Christie AP, Amano T, Martin PA, Shackelford GE, Simmons BI, Sutherlan WJ. Simple study designs in ecology produce inaccurate estimates of biodiversity responses. J Appl Ecol. 2019;56:2742–54.

8. Christie AP, David Abecasis, Mehdi Adjeroud, Alonso JC, Alvaro Anton, Barry P. Baldigo, et al. Quantifying and addressing the prevalence and bias of study designs in the environmental and social sciences. Nature Communications. 2020;11:6377.

9. Treves A, Krofel M, McManus J. Predator control should not be a shot in the dark. Front Ecol Environ. 2016;14:380-8.

 Treves A, Krofel M, Ohrens O, Van Eeden LM. Predator control needs a standard of unbiased randomized experiments with cross-over design. Frontiers in Ecology and Evolution.
 2019; 7 402-13.

11. Khorozyan I. Dealing with false positive risk as an indicator of misperceived effectiveness of conservation interventions. PLoS One. 2021;16(5):e0255784.

Ioannidis JP. Why most published research findings are false. PLOS Medicine.
 2005;2(8):e124.

13. Ohrens O, Bonacic C, Treves A. Non-lethal defense of livestock against predators: Flashing lights deter puma attacks in Chile. Front Ecol Environ. 2019;17(1):32-8.

14. Hall K, Fleming PA. In the spotlight: can lights be used to mitigate fox predation in a freerange piggery? Appl Anim Behav Sci. 2021;2:105420.

15. Naha D, Chaudhary P, Sonker G, Sathyakumar S. Effectiveness of non-lethal predator deterrents to reduce livestock losses to leopard attacks within a multiple-use landscape of the Himalayan region. PeerJ. 2020;8:e9544.

16. Treves A, Fergus AR, Hermanstorfer SJ, Louchouarn NX, Ohrens O, Pineda Guerrero AA. Gold-standard experiments to deter predators from attacking livestock. 2023 pre-print posted for pre-publication review.

17. Okarma H. Status and management of the wolf in Poland. Biol Conserv. 1993;66:153-8.

Musiani M, Visalberghi E. Effectiveness of fladry on wolves in captivity. Wildl Soc Bull.
 2000;29:91-8.

19. Davidson-Nelson SJ, Gehring TM. Testing fladry as a nonlethal management tool for wolves and coyotes in Michigan. Human–Wildlife Interactions. 2010;4(1):87-94.

20. Iliopoulos Y, Astaras C, Lazarou Y, Petridou M, Kazantzidis S, Waltert M. Tools for coexistence: Fladry corrals efficiently repel wild wolves (Canis lupus) from experimental baiting sites. Wildl Res. 2019;46(6):484–98.

21. Musiani M, Mamo C, Boitani L, Callaghan C, Gates CC, Mattei L, et al. Wolf Depredation Trends and the Use of Fladry Barriers to Protect Livestock in Western North America. Conserv Biol. 2003;17: 1538–1547.

22. Colquhoun D. An investigation of the false discovery rate and the misinterpretation of pvalues. Royal Society Open Science. 2014;1:140216.

Colquhoun D. The reproducibility of research and the misinterpretation of p-values.
 Royal Society Open Science. 2017;4:171085.

24. Benjamin D, Berger J, Johannesson M, Nosek B, Wagenmakers E, Berk R, et al. Redefine statistical significance. Nature Human Behaviour. 2018;2:6–10.

25. Fergus AR. Building carnivore coexistence on Anishinaabe land: gold standard nonlethal deterrent research and relationship building between livestock farmers and the Bad River Band of the Lake Superior Tribe of Chippewa Indians. Madison, WI: University of Wisconsin; 2020.

26. Hermanstorfer SJ. Western Colorado carnivore coexistence: gold-standard non-lethal deterrent experiments and human-carnivore coexistence in Montrose, Colorado: v; 2023.

27. Young JK, Draper J, Breck S. Mind the Gap: Experimental Tests to Improve Efficacy of Fladry for Nonlethal Management of Coyotes. Wildl Soc Bull. 2019:1-7.

28. Kittle AM, Anderson M, Avgar T, Baker JA, Brown GS, Hagens J, et al. Wolves adapt territory size, not pack size to local habitat quality. J Anim Ecol. 2015;84(5):1177–86.

29. Zellmer UJ, Goto BS. Urban wildlife corridors: Building bridges for wildlife and people. Frontiers in Sustainable Cities. 2022;4:954089.

30. Halfpenny JC, Sanders MR, McGrath KA. Human-lion interactions in Boulder County, Colorado: Past, present and future. In: Braun CE, editor. Mountain Lion-Human Interaction Symposium and Workshop: Colorado Division of Wildlife, Denver, CO.; 1991. p. 10-6.

31. Gallagher AJ, Creel S, Wilson RP, Cooke SJ. Energy landscapes and the landscape of fear. Trends in Ecology and Evolution. 2017;32.

32. Fergus A, Hill L. Mashkiiziibii Wildlife Program. Mashkiiziibii Ma'iingan (Gray Wolf) Relationship Plan (Edition 2. In: Department. MNR, editor. Odanah, WI: Bad River Band of Lake Superior Tribe of Chippewa Indians; 2019.

 Ohrens O, Santiago-Ávila FJ, Treves A. The twin challenges of preventing real and perceived threats to human interests. In: Frank B, Marchini S, Glikman J, editors. Human-Wildlife Interactions: Turning Conflict into Coexistence. Cambridge: Cambridge University Press; 2019. p. 242-64.

34. Díaz-Uriarte, R. (2002). Incorrect analysis of crossover trials in animal behaviour research. Animal Behavior 63: 815–22.

34. Jones, B. & Kenward, M.G. (1989). Design and analysis of cross-over trials. London, UK: Chapman and Hall.

35. Louchouarn NX, Treves A. Low-stress livestock handling protects cattle in a five-predator habitat. PeerJ. 2023;11:e14788.

36. Chavez AS, Gese EM. Landscape use and movements of wolves in relation to livestock in a wildland–agriculture matrix J Wildl Manage. 2006;70(4):1079-86.

37. Bielby J, Vial F, Woodroffe R, Donnelly CA. Localised badger culling increases risk of herd breakdown on nearby, not focal, land. PLoS One. 2016;11(10):e0164618.

38. Donnelly CA, Woodroffe R, Cox DR, Bourne J, Gettinby G, Le Fevre AM, et al. Impact of localized badger culling on TB incidence in British cattle. Nature. 2003;426:834-7.

Donnelly C, Woodroffe R, Cox DR, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, et al.
 Positive and negative effects of widespread badger culling on tuberculosis in cattle. Nature.
 2006;439:843-6.

40. Donnelly C, Wei G, Johnston W, Cox D, Woodroffe R, Bourne F, et al. Impacts of widespread badger culling on cattle tuberculosis: concluding analyses from a large-scale field trial. International Journal of Infectious Diseases. 2007;11:300-8.

41. Donnelly C, Woodroffe R. Reduce uncertainty in UK badger culling. Nature. 2012;485:582.

42. Vial F, Donnelly C. Localized reactive badger culling increases risk of bovine tuberculosis in nearby cattle herds. Biol Lett. 2012;8:50-3.

43. Dunwoody S. The challenge of trying to make a difference using media messages In: Moser SC, Dilling L, editors. Creating a climate for change. Cambridge: Cambridge University Press; 2007. p. 89-104.

44. Kinzig AP, Ehrlich PR, Alston LJ, Arrow K, Barrett S, Buchman TG, et al. Social Norms and Global Environmental Challenges: The Complex Interaction of Behaviors, Values, and Policy. Bioscience. 2013;63:164–75.

45. Cruikshank J. THE OUTSIDER: An Uneasy Role in Community Development. Canadian Social Work Review / Revue Canadienne de Service Social.1990; 7:245–59.

46. Merton RK. Insiders and Outsiders: A Chapter in the Sociology of Knowledge. American Journal of Sociology. 1972; 78: 9–47.