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Sub-lethal effects of energy development on a migratory mammal—The enigma of North American pronghorn

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GRAPHICAL ABSTRACT



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ABSTRACT

To meet societal demands for energy, ~50,000 gas sites are developed annually in North America, among which many are in western less-developed and wildlife rich areas. To evaluate effects of increasing energy infrastructure requires sufficiently robust study designs, an onerous issue given the vastness of scale, limited funds, and an abject dearth of baseline data. Here we address these issues, first by discussion of the type of approaches needed to develop proper inference about potential effects of energy footprints, and subsequently through an empirical approach by examining the biological performances of more than 370 GPS radio-collared adult female pronghorn (*Antilocapra americana*). A rigorous attempt to examine if industrial development has any impact on pronghorn is based on three assumptions: (i) late-winter body mass reflects a period of inadequate food availability because winter habitat is altered; (ii) variation between population segments reflects spatial differences in food availability, increased energetic costs, or varying survival rates between gas field and non-developed sites; and (iii) reproductive correlates including

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physiological and immunological markers and adult survival are lower at sites varying in habitat quality. Our study area situated in one of the world's premier wildlife regions – the southern Greater Yellowstone Ecosystem – harbors approximately 100,000 wintering ungulates, some of North America's longest migrations, and two of the continent's largest gas fields. We compared the response of five variables between wild pronghorn in control (not disturbed) and experimental (developed gas fields with well pads, roads, and traffic) sites—pregnancy, chronic stress, immune function, body mass, and adult survival. Despite shifts in animal movements, which included avoidance of energy infrastructure where development is occurring at the highest densities inside two of the largest natural gas fields in North America (Pinedale Anticline Project Area [PAPA] and Jonah fields) and other behavioral or ecological observations of sub-lethal effects, we failed to reject the null hypothesis that development is unrelated to parity in pronghorn biological responses. Studies intent on producing knowledge to assess whether energy development is inimical or not to ungulates will increasingly require appropriate time scales and understanding whether populations are below an expected food ceiling. Further, as with pronghorn in our study region, knowing if individuals are at the limits of their biological range (e.g. altitude) where stressful winter conditions may mask impacts of development is important.

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1. Introduction

As the human footprint continues to expand globally, governments will be the final arbiters of strategies implemented on behalf of people and the planet's biodiversity. This is especially true of relatively intact geographies such as the neotropics, Arctic, and savannas where hydrocarbon development presses into wildlife-rich areas (Copeland et al., 2009; Berger and Beckmann, 2010; Naugle, 2010). The establishment of current and future conservation practices is partially dependent on credible science which, in turn requires appropriate study designs to detect if change occurs, and if so the magnitude of change from habitat alteration and other disturbance on population performances. Ideally, such designs would include baseline data on species abundance and distribution, biological attributes, abiotic factors (e.g. temperature and precipitation) and demographic trends (Beckmann et al., 2011). Such information is however rarely available (Northrup and Wittemyer, 2013; Lynch et al., 2015).

The most detailed studies of mammals in petroleum-rich areas have concentrated on four species in North America—caribou (*Rangifer tarandus*), elk (*Cervus elpahus*), mule deer (*Odocoileus hemionus*), and pronghorn (*Antilocapra americana*). While abundant GPS data are available (Hebblewhite and Haydon, 2010; Hebblewhite, 2011) much remains unknown about biological impacts of development at the individual or population level (Sawyer et al., 2009a,b; Lendrum et al., 2012, 2013). Most studies have addressed questions about habitat fragmentation and road avoidance (Beckmann et al., 2011, 2012; Seidler et al., 2015) with responses broadly classified as sub-lethal which may include behavior and ecological components such as movement, density alterations, and habitat shifts (Bayne and Dale, 2011). Since most work has been in areas where baseline data lack, projects have generally been observational and correlative, although at times Before–After–Control–Impact (BACI) approaches have been deployed. Less frequent are studies with replication or comparative design (i.e. control vs. treatment groups). For instance, 66% of 38 studies of the above four ungulates had only a weak observational approach or failed to have control study regions (Hebblewhite, 2011). Such limitations are serious because the power of inference becomes more restricted when null models cannot be tested by randomization of ecological data.

Here, we capitalize on a study design using control and experimental treatments to report the extent to which expanding industrial footprints affect life history parameters, health, and survival in adult female pronghorn. We focus on females because females are the critical element for population growth in all sexually reproducing species. The primary purpose of the study was to examine potential demographic differences between animals wintering in proximity to gas field development and wintering in undeveloped areas. Our goals were to examine pronghorn response (body mass, stress, pregnancy rates, immune-responses (health), and survival) to gas field development. To do this we compare these five parameters between experimental (individuals that winter inside natural gas field boundaries) and control (individuals that winter outside of natural gas field boundaries) subpopulations to understand how varying and increasing densities and scale of development and infrastructure impact pronghorn on their crucial winter range. We pose as a hypothesis that rapid alteration of habitat by energy development negatively affects reproductive correlates of pronghorn. If true, then pronghorn in such areas will be in poorer body condition than at control sites and might similarly be characterized by depressed immune-responses, lower pregnancy rates, higher levels of stress, and poorer survival.

Our study region is within several of North America's largest natural gas fields, both situated within the southern tier of the Greater Yellowstone Ecosystem (Fig. 1; Berger, 2003, Sawyer et al., 2009a, Beckmann et al., 2012, and Seidler et al., 2015). Two large mammals – pronghorn and mule deer – from this site have some of the longest reported migratory movements of any New World terrestrial mammals between Canada and Argentina; each species has also been the subject of regional or

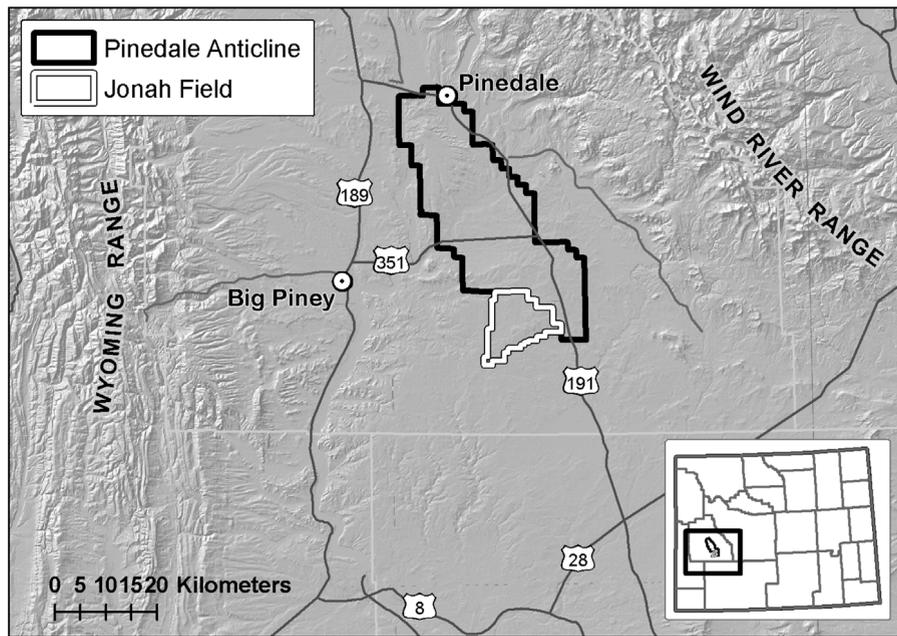


Fig. 1. Location of the Upper Green River Basin (UGRB) in western Wyoming, USA. The Pinedale Anticline Project Area (PAPA; northern outline) and Jonah (southern outline) gas fields are highlighted.

national legislation (Berger, 2003; Sawyer et al., 2009a,b; Sawyer and Nielson, 2011). Concomitant with these conservation efforts, gas field expansion with new roads and infill including areas of Wyoming, Colorado, Montana and across all of North America have been increasing, with approximately 50,000 new wells per year for almost two decades (Allred et al., 2015). This trend is likely to continue given that global demand for energy resources is projected to increase by nearly 40% in the coming two decades (Northrup and Wittemyer, 2013). Intensive gas field development has resulted in a 43% loss of mule deer over the past decade (Sawyer and Nielson, 2011) in our study area, though some of this may have been caused by a habitat shift rather than a demographic crash. For pronghorn, abandonment of crucial winter range occurs (Beckmann et al., 2012).

1.1. Assumptions and approach

A rigorous attempt to examine if industrial development has any impact on pronghorn – just as for the other above three North American ungulates – is based on three assumptions that we address: (i) late-winter body mass reflects a period of inadequate food availability because winter habitat is altered; (ii) variation between population segments reflect spatial differences in food availability, increased energetic costs, or varying survival rates between gas field and non-developed sites; and (iii) reproductive correlates including physiological and immunological markers and adult survival are lower at sites varying in habitat quality.

Although prior studies have been designed to assess effects of disturbance including habitat loss associated with energy development, rarely are assumptions explicitly offered yet they are critical because they underlie two fundamentally different – though at times related – processes, (a) habitat loss and (b) behaviorally-mediated disturbance. If habitat availability is associated with population density, then with other factors equal, it seems reasonable to expect food availability to affect an individual's body condition (Bowyer et al., 2005). On the other hand, numerous factors affect density and distribution including food quality (as opposed to quantity; Van Horne, 1983), social factors, and legacy effects (Berger, 1986; Berger et al., 2015), so a broader or comparative approach may be needed to evaluate the role, if any, of habitat loss through energy's footprint. If populations are below food carrying capacity, perhaps effects at individual or population levels will not be detectable (Beckmann et al., 2012).

Behaviorally-mediated disturbance with associated energetic costs will occur, independent of habitat loss, if individuals respond to a suite of factors associated with gas field development. Such effects might come in the form of increased vigilance, avoidance of roads, gas pads, and/or compressor stations or might entail habituation. Irrespective, sub-lethal effects might compromise immune-function, probability of pregnancy, and reduce body mass. Here, we examine more fully these assumptions by using a comparative framework and empirical data gathered across five years on female pronghorn in the southern Greater Yellowstone Ecosystem (GYE). This comparative study approach involving our evaluation of correlates of fitness (e.g. body mass, pregnancy rates, stress) and survival coupled with our previous work on pronghorn behavior (e.g. Beckmann et al., 2012, and Seidler et al., 2015), allows us to examine the above three assumptions to evaluate impacts of industrial development.

2. Materials and methods

2.1. Study site and pronghorn sampling overview

The Upper Green River Basin (UGRB) of western Wyoming encompasses part of the southern GYE (Fig. 1) and contains an estimated 30–50 trillion cubic feet of natural gas. Due to topography and climate the region receives strong winds which creates areas of typically lighter snow cover and relatively better low elevation (~2200 m) winter habitat for pronghorn and mule deer (Sawyer et al., 2006; Beckmann et al., 2012).

Across five successive years we captured 388 female pronghorn at least 1.5 years of age or older, using a net-gun from a helicopter in February 2005 ($n = 50$), January and December 2006 ($n = 100$), February 2007 ($n = 100$), January 2008 ($n = 68$), and February 2009 ($n = 70$; see Beckmann et al., 2011, 2012 for details on animal capture and handling). We classified animals as either control (over-wintering locations outside natural gas field development boundaries) or experimental (over-wintering locations inside natural gas field development boundaries) based on site of winter capture and subsequent GPS collar locations (Fig. 2). This classification was assessed *a priori* at time of capture, but was supported by analysis of >500,000 GPS collar locations from 2005–2009 from these pronghorn, which showed movements consistent with 100% winter site fidelity at the scale of natural gas field boundaries (Beckmann et al., 2011, 2012). Sample sizes for comparisons (e.g., stress hormones, and pregnancy) reported here vary because we did not collect data on all measures every year nor were we successful in collecting all parameters from all individuals. Body mass measures occurred during handling of females for collar deployment. All handling was in accordance with Institutional Animal care protocols established by the Wyoming Game and Fish Department and the American Society of Mammalogists (Animal Care and Use Committee, 1998).

2.2. Processing and extraction of corticosteroids and progesterone from fecal samples

We evaluated the possibility of stress and pregnancy by reliance on fecal metabolites, a commonly-used approach for *in situ* wildlife (Berger et al., 1999; Creel et al., 2002). The secretion of glucocorticosteroid (GC) is a useful marker of stress as it is a product of the adrenal cortex, and chronic stress is known to affect condition, immunity, and reproduction (Sapolsky, 1992). We used corticosterone, a type of GC, levels to assess potential variation in chronic stress among pronghorn in different wintering areas, an assay that has proved useful to distinguish between stress-related responses of elephants in areas with different intensities of poaching (Foley et al., 2001). Baseline measures for non-stressed pronghorn were derived from fecal metabolites using the above procedures on two adult pronghorn females at the Pocatello Zoo, Idaho (similar longitude and latitude) in 2006, and seven wintering adult females in 2007 at the National Bison Range in Montana; this latter site is ~1100 m lower and more mild than our UGRB winter area situated at ~2200 m.

A handful of fecal pellets (6–15 pellets) were collected from each captured animal and shipped to Smithsonian's National Zoo Endocrinology Laboratory at the Conservation Biology Institute (Front Royal, VA). The frozen samples were subsequently dried using a lyophilizer. Corticosteroids were processed and extracted following protocols of Young et al. (2004), while progesterone extraction used protocols from Graham et al. (2001).

Briefly, samples were manually crushed and put into labeled 12×75 polypropylene storage tubes. Then 0.05 g of feces were placed in numbered 16×125 mm glass tubes. We then added 5 ml of 90% ETOH to each tube and they were vortexed. Samples were boiled in a pre-heated water bath (96°) for 30 min and centrifuged at 2500 rpm for 20 min. The supernatant was poured off into a second set of identically labeled 16×125 mm tubes. Another 5 ml of the 90% ETOH was added to the original tubes containing the fecal pellet and then vortexed for 30 s. We then centrifuged the original tubes at 2500 rpm for 15 min then poured off the supernatant into the second set of labeled tubes that contained the first supernatant. Samples were then dried under forced air under a hood. Finally, samples were re-suspended in 1 ml of Enzymeimmunoassay (EIA) dilution buffer. Progesterone samples were diluted to 1:150 (for some peaks further diluted to 1:300) and run on a progesterone EIA (CL425) assay. Fecal samples were subjected to a double antibody 125 I Radioimmunoassay (RIA) (MP Biomedicals, Inc. which was formerly ICN Biomedicals) at a dilution of 1:10. We evaluated potential variation in pregnancy rates by contrasting fecal progesterone levels/individual ($\mu\text{g/g}$ dry weight) between control and experimental sites. Corticosteroids were processed in a similar manner, but followed protocols of Young et al. (2004). Unless otherwise noted statistical analyses for mass, corticosterone, and progesterone are means \pm SE.

2.3. Tests for disease exposure and ecotoxicology

In 2005, 21 pronghorn designated as experimental animals and 32 designated as control animals were tested for a variety of health indicators. Costs prohibited collection and analysis of health indicators across all years, but in 2010, 30 additional pronghorn were also tested for the same health indicators in a subsequent study in conjunction with the National Park Service (Cain, Dewey, Seidler and Beckmann, pers. comm.). Blood samples were analyzed for disease-specific antibodies, biochemical nutritional markers, and ecotoxicological compounds. Sera were screened for the following pathogens: epizootic hemorrhagic disease virus, bluetongue virus, bovine respiratory syncytial virus, infectious bovine rhinotracheitis, *Brucella spp.*, and *Mycobacterium avium paratuberculosis* (Johne's Disease) (Williams and Barker, 2001). We measured levels of iron, magnesium, potassium, zinc, calcium, vitamin E, and sodium in the blood similar to Dunbar et al.

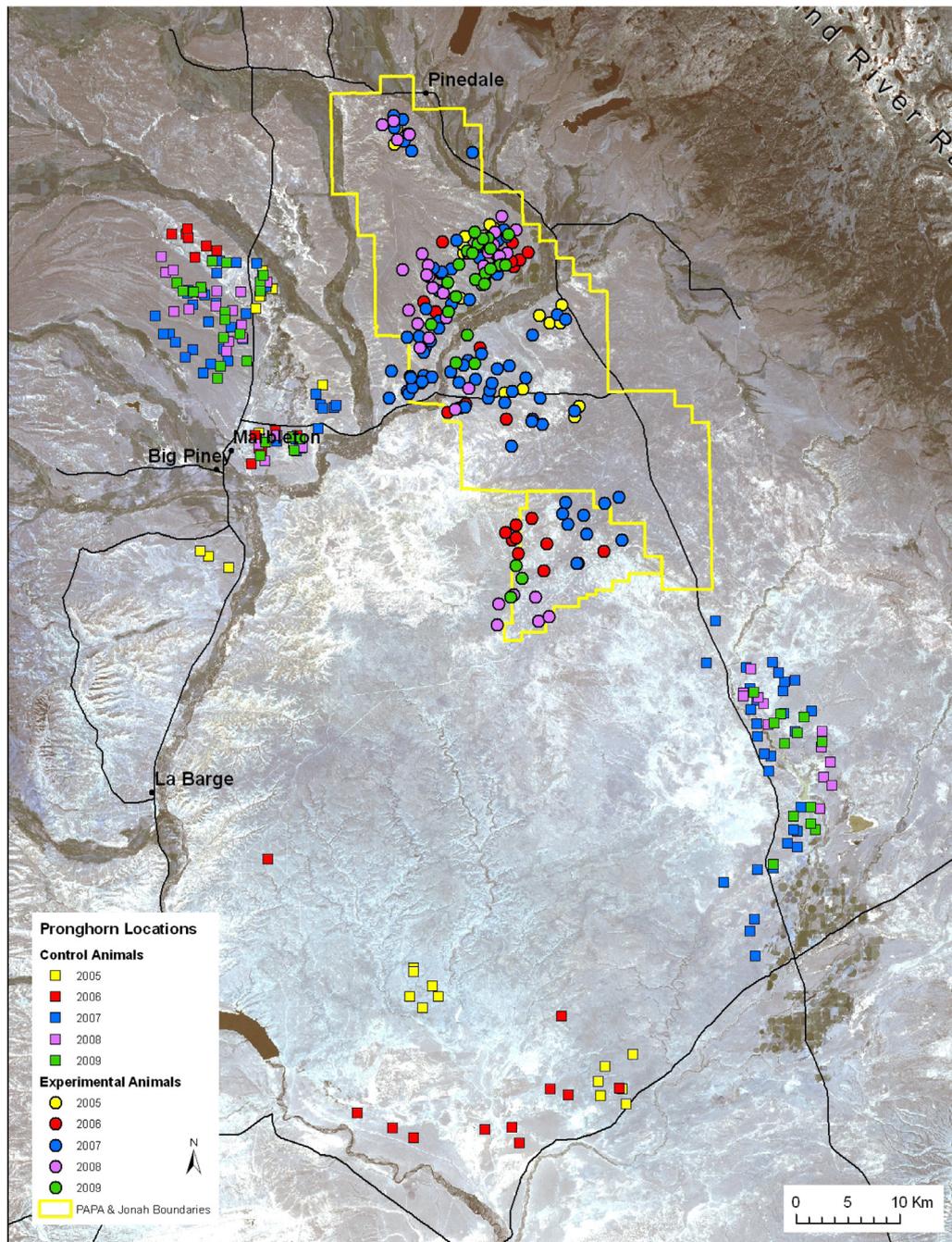


Fig. 2. Locations of 388 adult, female pronghorn captured in 2005, 2006, 2007, 2008, and 2009 indicating classification as experimental or control based on proximity of capture location to gas fields.

(1999). We also screened for compounds with ecotoxicological effects (cobalt, arsenic, cadmium, lead, thallium selenium, PCBs, and organochlorines) (see Raisbeck et al., 1996).

2.4. Survival of control and experimental animals

We conducted a power analysis at the beginning of the study to determine the likelihood of detecting a statistically significant difference in survival rates of control and experimental pronghorn in each year. In order to have a 95% chance of detecting a 10% difference in survival rates at a significance level of 0.05, we would have needed to monitor 726 animals annually. Due to constraints of capturing and collaring large numbers of pronghorn, we chose an annual sample size of 150

animals. This sample size allowed us a 95% chance of detecting a 25% difference in survival rates at the 0.05 significance level, or an 85% chance of detecting a 20% difference in survival rates, or a 70% chance of detecting a 15% difference in survival rates.

We estimated survival rates of radio-collared pronghorn from 2005 through 2009 using a known fate model in Program MARK (White and Burnham, 1999). The analysis was based on monthly encounter histories where encounters represented either initial captures or relocations by radio-telemetry during subsequent months. We evaluated 25 models to assess the effects of site (control or experimental), year (2005, 2006, 2007, 2008, 2009), month, season, and body mass on pronghorn survival. Seasons were classified based on similarities in monthly survival rates as winter (January–March), hunting (September and October; which coincides with fall migration), migration (April–May), summer (June–August), and post-hunt (November and December). We also tested trend models to assess evidence of increasing or decreasing linear trends in pronghorn survival that might be associated with habitat loss over time (see Beckmann et al., 2012), or with changes in hunting pressure if hunters are shifting their activities to avoid developed areas inside gas field boundaries. Mortality during the hunting season could not be attributed to direct (hunter killed) or indirect causes. We included a single covariate for body mass at the time of capture as a surrogate for condition. The most global model included parameters for body mass, month, and site, with an interaction term that allowed survival patterns to differ at control and experimental sites over time. We used Akaike's Information Criterion adjusted for small sample sizes (AIC_c) and Akaike weights to assess model fit (Burnham and Anderson, 2002). For comparative purposes, all survival rates are reported as annualized measures. Annual survival estimates and standard errors were calculated from model-averaged monthly survival estimates following Burnham et al. (1987).

2.5. Climate parameters to examine winter severity

As winter weather, particularly low temperatures, snowfall and snow depth significantly impact pronghorn populations in the UGRB (Beckmann et al., 2011, 2012), we examined four measures of climate for our study area using National Oceanic and Atmospheric Administration (NOAA) data from climate stations located near Pinedale, Wyoming and Big Piney, Wyoming. We compared monthly mean values using *t*-tests during winter (December–February) for: (1) number of days with minimum temperature less than or equal to -17.8 C (0.0 F); (2) average monthly number of days with greater than or equal to 1.27 cm (0.5 inches) of snow (measure/threshold determined by NOAA weather station data reporting); (3) average monthly maximum snow depth (cm); and (4) average monthly total snow fall (cm) for 1960–2015 to 2005–2009 as an index of winter severity during our study.

3. Results

3.1. Body mass

Across all years, analysis of variance (ANOVA) showed no significant differences in mass between control and experimental animals ($F_{1, 316} = 0.586$, $P = 0.445$). Differences across years ($F_{4, 316} = 575.37$, $P = 0.001$; Fig. 3) are reflective of annual winter conditions. There was no interaction effect between treatment (experimental or control) and year ($F_{4, 316} = 0.690$, $P = 0.599$).

3.2. Corticosteroids and progesterone

For corticosterone, the overall ANOVA was significant ($F_{10, 327} = 11.02$, $P = 0.001$), but differences between control and experimental animals were not detectable ($P > 0.56$); inter-annual variation occurred across all years (Tukey's pairwise comparisons; $P < 0.05$; Fig. 4). There was no interaction effect between treatment (experimental or control) and year ($P > 0.50$). Across all years, UGRB animals had elevated corticosterone levels above control animals from both the Pocatello Zoo in Idaho and the National Bison Range in Montana likely reflecting more challenging winter conditions in the UGRB (see Beckmann et al., 2011; Fig. 4).

With respect to fecal progesterone, mean levels failed to differ between control and experimental animals across our five sampling years ($F_{1, 195} = 0.296$, $P = 0.587$), but varied by year ($F_{3, 195} = 18.401$, $P = 0.001$; Fig. 5). There was no interaction effect between treatment (experimental or control) and year ($F_{3, 195} = 2.186$, $P = 0.091$).

3.3. Disease exposure and ecotoxicology

Irrespective of treatment (experimental vs. control), antibodies for all eight screened diseases were below detectable levels for all animals in both 2005 and 2010 (Cain, Dewey, Seidler and Beckmann, pers. comm. for 2010 data). Similarly, for selected minerals and vitamins in blood sera no differences were detected except that in 2005 experimental animals had significantly lower levels of sodium ($X_{\text{control}} = 3783$ ppm [$n = 27$], $X_{\text{experimental}} = 3683$ ppm [$n = 20$], $p = 0.004$). Finally, appreciable levels of polychlorinated biphenyls (PCBs) or other organochlorines were not detected in 2005 or 2010.

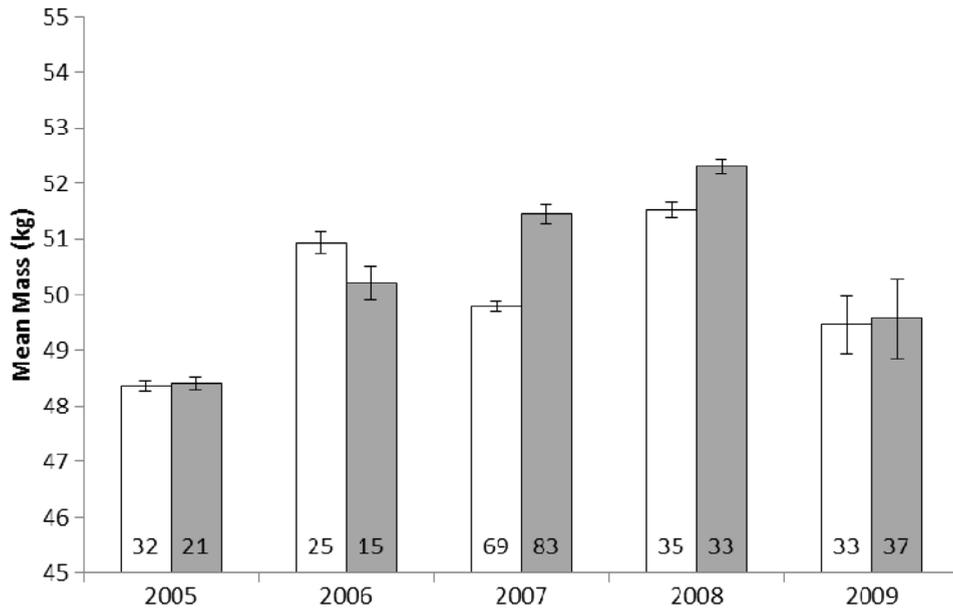


Fig. 3. Mean body mass (kg) of control (white) and experimental (grey) female pronghorn in the UGRB from 2005 to 2009. All captures were done in winter (December–February). Error bars represent \pm SE and sample sizes are shown in each box. Mean body mass was not significantly different between control and experimental animals ($F_{1, 316} = 0.586$, $P = 0.445$), but was significantly different among years ($F_{4, 316} = 575.37$, $P = 0.001$).

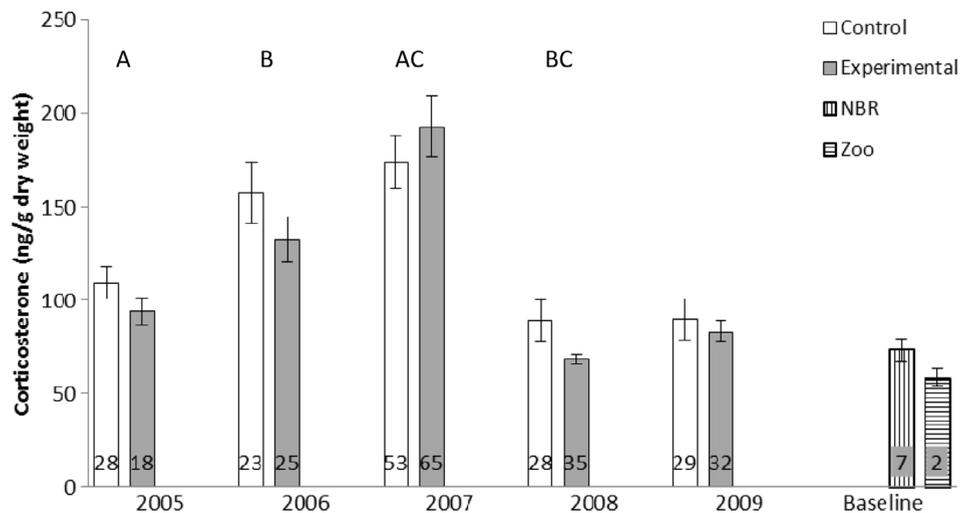


Fig. 4. Mean fecal corticosterone (ng/g dry weight) levels of adult, female pronghorn from control (white), experimental (grey), National Bison Range, MT (vertical bars), and Pocatello Zoo, ID (horizontal bars). Error bars represent \pm SE and sample sizes are shown in each box. Mean corticosterone levels were different between years ($P < 0.05$), but not for category of animals ($P > 0.56$). Letters (A, B, and C) denote years that were significantly different (Tukey's pairwise comparison).

3.4. Survival of control and experimental animals

We included 371 marked individuals in the survival analysis. Site was included as a variable in all models because the primary purpose of the study was to examine potential demographic differences between animals wintering in proximity to gas field development (experimental, $N = 177$) and wintering in undeveloped areas (control, $N = 194$). On the basis of minimum AIC_c , the survival model that best fit our data suggests that survival was constant among years but differed between control and experimental animals, and between winter months (January–March) and the hunting season (September–October) relative to other times of year (Table 1). This model had 36% of the Akaike weight, but performed just slightly better than a model that suggests survival was also positively related to body mass ($\Delta AIC_c = 0.94$; Akaike weight = 23%). The third-ranked model suggests that there has been a decreasing linear trend in pronghorn survival during the hunting season in the UGRB since 2005 ($\Delta AIC_c = 0.94$; Akaike weight = 11%; Table 1).

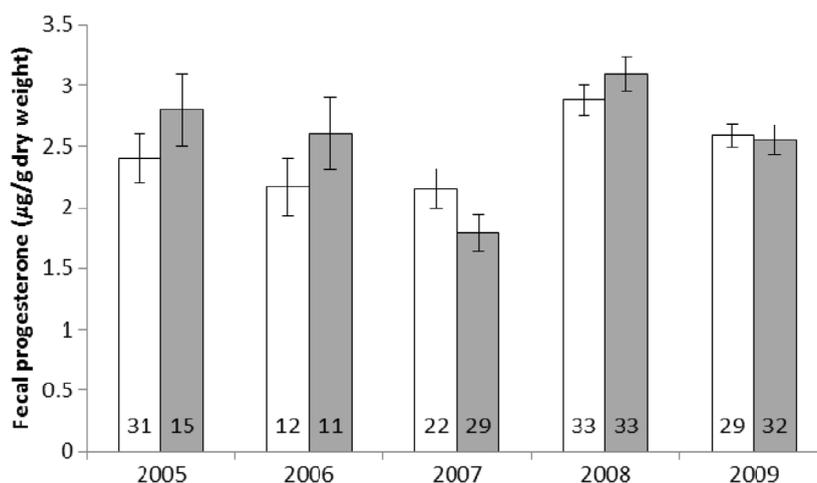


Fig. 5. Mean fecal progesterone levels ($\mu\text{g/g}$ dry weight) of control (white) and experimental (grey) adult, female pronghorn. Error bars represent \pm SE and sample sizes are shown in each box. Mean fecal progesterone levels were not significantly different between control and experimental animals ($F_{1, 195} = 0.296, P = 0.587$), but were different among years ($F_{3, 195} = 18.401, P = 0.001$).

Table 1

Model selection results for survival of female pronghorn in the Upper Green River Basin, Wyoming USA from 2005–2009.

Model	K	AICc	Delta AICc	Akaike weight	Model likelihood	Deviance
S(site + winter + hunting)	4	878.871	0.000	0.363	1.000	870.863
S(site + winter + hunting + mass)	5	879.815	0.944	0.227	0.624	869.803
S(site + winter + trend in hunting)	4	881.316	2.445	0.107	0.295	873.308
S(site + winter + trend in hunting + mass)	5	882.222	3.351	0.068	0.187	872.209
S(site + season)	6	882.624	3.753	0.056	0.153	870.607
S(site * trend in hunting + winter)	5	882.911	4.040	0.048	0.133	872.899
S(site * winter + site * hunting)	5	883.396	4.524	0.038	0.104	873.383
S(site + season + mass)	7	883.573	4.702	0.035	0.095	869.550
S(site * trend in hunting + winter + mass)	6	883.826	4.955	0.031	0.084	871.809
S(site * winter + hunting + mass)	6	884.386	5.515	0.023	0.064	872.369
S(site * season)	10	889.822	10.951	0.002	0.004	869.777
S(site * season + mass)	11	890.782	11.911	0.001	0.003	868.728
S(site)	2	891.653	12.781	0.001	0.002	887.650
S(site + mass)	3	892.654	13.783	0.000	0.001	886.649
S(site + month)	13	892.865	13.994	0.000	0.001	866.789
S(site * linear trend)	4	893.191	14.319	0.000	0.001	885.182
S(site + linear trend)	3	893.282	14.411	0.000	0.001	887.277
S(site * linear trend + mass)	5	894.273	15.402	0.000	0.000	884.261
S(site + linear trend + mass)	4	894.322	15.451	0.000	0.000	886.314
S(site + year)	6	896.667	17.796	0.000	0.000	884.650
S(site * month)	22	897.177	18.305	0.000	0.000	852.967
S(site + year + mass)	7	897.448	18.577	0.000	0.000	883.425
S(site * month + mass)	23	898.109	19.238	0.000	0.000	851.880
S(site * year)	10	901.510	22.638	0.000	0.000	881.464
S(site * year + mass)	11	902.391	23.520	0.000	0.000	880.337

While model-rankings suggest that there were no differences in survival among years, model-averaged survival estimates (Burnham and Anderson, 2002) indicate that survival was slightly lower at the control ($\hat{S} = 0.803 \pm 0.036$) and experimental ($\hat{S} = 0.812 \pm 0.035$) sites in 2007 compared to other years (Fig. 6(A)). Based on estimates from the top-ranked model, survival was significantly higher at the control ($\hat{S} = 0.892 \pm 0.033$) and experimental ($\hat{S} = 0.899 \pm 0.031$) sites during winter, and significantly lower at the control ($\hat{S} = 0.650 \pm 0.059$) and experimental ($\hat{S} = 0.668 \pm 0.057$) sites during the hunting season, compared to other times of year (Fig. 6(B)). The top-ranked trend model suggests that there has been an increase in hunting-related mortality since 2005, with survival rates during the hunting season (September–October) declining from 77% to 58% at the control site, and from 78% to 68% at the experimental site. However, confidence intervals for the trend overlapped markedly for all years.

3.5. Climate parameters to examine winter severity

Mean values for: (1) number of days with minimum temperature less than or equal to -17.8 C (0.0 F); (2) average monthly number of days with greater than or equal to 1.27 cm (0.5 inches) of snow; (3) average monthly maximum snow depth (cm);

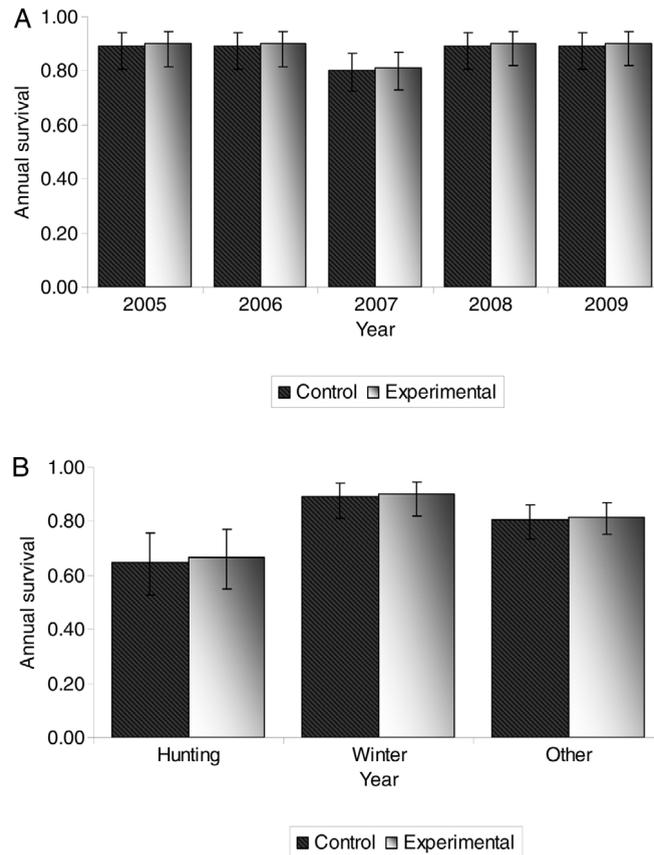


Fig. 6. (A) Annual survival rates estimated by Program MARK for control ($n = 177$) and experimental ($n = 194$) animals in the Upper Green River Basin, Wyoming USA 2005–2009. (B) Annual survival rates estimated by Program MARK for control ($n = 177$) and experimental ($n = 194$) animals based on seasonal survival rates during the hunting season (September–October), winter (January–March), and all other months in the Upper Green River Basin, 2005–2009. These rates reflect the annual survival rates that the pronghorn population would have experienced assuming the monthly survival rate during each season was in effect all year.

Table 2

Mean (\pm SE) values for measures of winter severity in the Upper Green River Basin, Wyoming USA study area. Data for the four measures of climate for our study area were collected from National Oceanic and Atmospheric Administration (NOAA) climate stations located near Pinedale, Wyoming and Big Piney, Wyoming. Long-term (1960–2010) means for winter months (Dec–Feb) were compared to study period (2005–2009) means using two-tailed t -tests.

Parameter	1960–2010	2005–2009	t	P
Avg. number of days below -17.8 C (0 F)	15.01 ± 13.49	14.83 ± 7.08	2.01	0.92
Avg. monthly number of days with >1.27 cm precip	0.07 ± 0.02	0.03 ± 0.005	1.67	0.56
Avg. monthly max snow depth (cm)	240.26 ± 16.93	273.47 ± 42.10	2.1	0.57
Avg. monthly total snow fall (cm)	237.06 ± 16.34	338.57 ± 51.77	2.57	0.15

and (4) average monthly total snow fall (cm) did not significantly differ ($P \geq 0.15$ in any case) during our study period (2005–2009) compared to the long-term means (1960–2015) for the region (Table 2).

4. Discussion and conclusion

4.1. Tests of assumptions in relation to energy development

Winter body mass reductions reflect food availability and alteration of important winter habitat.—While it is well documented that temperate and northern ungulates reduce metabolic rates and lose body weight during winter when food availability decreases, the important issue is whether loss of mass is exacerbated either by direct habitat loss or indirectly as a consequence of sub-lethal effects of other disturbance. That we failed to detect differences between adult females from control and experimental sites (Fig. 3) is indicative that mass is neither mediated by habitat reductions nor disturbance. Despite the fact that pronghorn of the UGRB winter at extreme high elevation relative to all other populations in North America, it may be that the comparatively long and cold winters at this locale mask effects of physical habitat loss. In other

words, increased rates of body mass loss during winter due to harsh winter conditions found at extreme high elevations may conceal any additional loss of mass that would occur due to habitat loss per se, thus masking the effect of habitat loss. It is also possible that because the UGRB animals are not at a putative food ceiling due to human harvest (on average more than 2450 pronghorn/year are removed from the six hunt units in our 4000 km² study site; Beckmann et al., 2012), any expectation of food limitation is illusory.

Habitat quality varies between population segments.—If habitat quality varies between control (e.g. non-developed) and experimental (e.g. gas field infrastructure) sites and if it governs bio-performances, then differences in measurable traits must exist. The problem with the assumption, as stated, is it is insufficiently explicit for testing. Incumbent within the context of hydro-carbon development is that habitats lose suitability or are somehow compromised by the energy footprint whether by fragmentation, loss of plant cover, general productivity, or human presence. The conundrum however is not that changes occur but the meaning of habitat. Habitats have variously been defined (Mitchell and Hebblewhite, 2012)—critical or non-critical, suitable or marginal, density-regulators or something else (Van Horne, 1983; Mayor et al., 2009). It is obvious that sites with and without gas wells, roads, and traffic differ. The critical assumption in need of testing is whether physical alteration of habitat and associated effects are differentially affecting traits of wildlife, which we assess below.

Developed sites reduce habitat quality and lower biological performance.—Our results on pregnancy, stress, and ecotoxicology and pathogen exposure across five years obviate discernible effects of habitat alteration and associated disturbances. Our sample of 371 marked female pronghorn included in our survival analyses likewise enables opportunities to develop broader inferences to understand inter-annual variation in adult survival which, like the above metrics, revealed no overall difference between sites.

The within year sample sizes limited us to detecting a 15% difference in survival among groups (experimental versus control) at a significance level of 0.05 with a probability of 70%. Hence it is possible that we missed differences between treatment groups, but the weight of evidence suggests that no difference in survival occurred between control and experimental groups. We detected a change in adult female survival during the hunting season and this trend seemed to be more influential in control sites away from gas field development. That survival decreased during the hunting season across the years of our study is indicative that either hunters target areas away from gas fields or that survival rates are lower in general in this population segment in fall regardless of direct hunting effects. Overall, our results indicate that we cannot reject the null hypothesis that development is unrelated to parity in pronghorn biological responses. However, given our previous results that industrial development and associated infrastructure, including well pads and roads, has altered pronghorn habitat selection patterns and migration (Beckmann et al., 2012; Seidler et al., 2015), it may be that a lag effect due to incremental changes in population dynamics and health will require longer monitoring efforts.

4.2. Scientific rigor

These findings beg a broader question—does the failure to reject the null mean that the energy footprint in the UGRB has little impact on pronghorn performance? An answer is steeped in a wider issue, scientific rigor, which in turn necessitates knowing if the assumptions are reasonable, if the strength of attempts to discredit competing hypotheses were vigorous, and whether the study design was adequate.

With respect to assumptions, for reasons previously described, we believe they were sufficient to address the possibility of inimical effects of energy infrastructure. Was our attempt sufficiently strong to discount alternative hypotheses? Although there are some obvious weaknesses in our approach—small annual sample sizes despite the fact that our sample sizes are large relatively to other similar studies, our lack of willingness to harvest animals for the purposes of examining liver, kidney, and other organs where eco-toxicological investigation queries might have been more revealing, or replications across additional study areas—we believe we have broken new ground by focusing on biological traits in addition to our prior work on behavioral aspects of avoiding infrastructure (Beckmann et al., 2011, 2012; Seidler et al., 2015). As for study design, a BACI procedure would have been additionally beneficial but given a lack of baseline values prior to energy development we relied on a control sub-population. On the other hand, studies of five years may be inadequate to detect prominent deleterious effects, overall gas field disturbances may be trivial, or in the absence of more extreme winters, weather in conjunction with the human energy footprint is trivial (see Christie et al., 2015).

That we failed to detect differences between control and experimental segments might result from comparatively mild winters in 2005–2009 relative to the long term average. However, given that mean values for: (1) number of days with minimum temperature less than or equal to -17.8 C (0.0 F); (2) average monthly number of days with greater than or equal to 1.27 cm (0.5 inches) of snow; (3) average monthly maximum snow depth (cm); and (4) average monthly total snow fall (cm) did not significantly differ during our study period (2005–2009) compared to the long-term means (1960–2015) for the region, suggests that the lack of significance in our measures of pronghorn health between control and experimental groups was likely not due to the five years of our study being outliers in terms of mild or harsh winters.

It is also likely that the reduction of populations below a food threshold due to significant fall harvest (see above; Beckmann et al., 2012) has resulted in adequate nutrition independent of the loss of usable habitat within gas field sites, which at the time of our study had been small (<3% in the Pinedale Anticline Project Area (PAPA) and <14.3% in the Jonah from 2000 to 2009) (Beckmann et al., 2012; Seidler et al., 2015). The lack of detected disease in these pronghorn populations may further bolster this argument if populations are below density-dependent disease thresholds (e.g. Langwig et al., 2012) which are also difficult to measure in free-ranging wildlife (Lloyd-Smith et al., 2005).

4.3. The context of energy development

While natural gas extraction in the US. is a clean-air alternative and may be a critical component in changing the United States' focus from foreign to domestic petroleum resources, harvest of natural gas must be balanced within the laws and best management practices associated with mandates of public land management. To protect native habitats and ecosystem processes such as long-distance migration of wildlife, while harvesting natural resources, careful planning must occur (Berger and Cain, 2014; Seidler et al., 2015).

Maintaining intact-systems in the face of large scale energy development is accomplished through properly designed wildlife monitoring research protocols, on-site mitigation, and adaptive management where detrimental development practices are either altered or suspended when the effects of natural resource extraction cause wildlife populations to decline from baseline levels, either demographically or from a health perspective (Beckmann et al., 2011). Baseline data are ideally collected before natural resource extraction begins and before decisions are made regarding how, when, where, and for what duration disturbance will proceed. If, for some reason, baseline data are not collected, then our understanding of the effects on wildlife will be compromised. In the case of the UGRB, unfortunately few pre-drilling data existed and baseline health data were unavailable until initiation of our project in 2005. However, Sawyer and Lindzey (2000) did examine movements of radio collared pronghorn between 1998–2000 at a relatively early period when gas field development had commenced (official field development in the PAPA was approved in the 2000 Record of Decision and the Jonah gas field was first drilled in the mid 70's; BLM, 2000, and Lanning, 2012).

At a time when the world's energy demands are growing, uncertainty remains about the effects of energy development on wildlife and strategies to minimize consequent impacts. In many cases, efforts to minimize potential harmful effects on wildlife are hampered by a lack of information on past trends in ungulate abundance, associated and independent effects of weather (Christie et al., 2015), and site-specific responses to the development and production of energy resources. As the construction of facilities and infrastructure to harvest these natural gas resources continues, it has become clear that the absence of biological data on wildlife, particularly baselines, is an impediment to prudent land use planning. Our efforts here have established recommended approaches to addressing questions regarding impacts of natural resource extraction and at the same time critical baseline data on pronghorn health and population performance that will be indispensable to continue assessing the impacts of the gas fields of the southern Greater Yellowstone Ecosystem, as they are further developed over the next half century. At a broader scale, effects of natural resource extraction span all continents and ecosystems and vary from deserts to tropical forests and polar regions (Contreras-Hermosilla, 1997; Peres and Lake, 2003; Joly et al., 2006). The approach we outline and employ here, in conjunction with our inferences on the impact of industrial development can serve as a model for addressing similar issues for other ungulate species globally.

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