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Density of river otters (*Lontra canadensis*) in relation to energy development in the Green River Basin, Wyoming

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HIGHLIGHTS

- We studied North American otters, a sentinel species of aquatic ecosystems, in western Wyoming.
- Densities in the Green River were relatively high, but few individuals used the New Fork tributary.
- Habitat and prey availability were similar in all river reaches studied.
- Otter activity was potentially affected by elevated disturbance from the industrial gas fields.
- We detected an increase in conductivity likely associated with surface-water contamination.
- Continued monitoring of otter densities and surface-water quality in Wyoming are warranted.

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ABSTRACT

Exploration and extraction of oil and natural gas have increased in recent years and are expected to expand in the future. Reduction in water quality from energy extraction may negatively affect water supply for agriculture and urban use within catchments as well as down river. We used non-invasive genetic techniques and capture–recapture modeling to estimate the abundance and density of North American river otters (*Lontra canadensis*), a sentinel species of aquatic ecosystems, in Southwestern Wyoming. While densities in two of three river reaches were similar to those reported in other freshwater systems in the western US (1.45–2.39 km per otter), otters appeared to avoid areas near energy development. We found no strong difference in habitat variables, such as overstory cover, at the site or reach level. Also, fish abundance was similar among the three river reaches. Otter activity in our study area could have been affected by elevated levels of disturbance surrounding the industrial gas fields, and by potential surface water contamination as indicated by patterns in water conductivity. Continued monitoring of surface water quality in Southwestern Wyoming with the aid of continuously recording devices and sentinel species is warranted.

1. Introduction

In an effort to reduce dependence on imported fossil fuels, the United States (US) government has been facilitating increased exploration and extraction of domestic energy sources. Much of this exploration, focusing on natural gas, occurred in the Intermountain West since the mid 1980s. As of 2012, Wyoming ranks ninth in the total number of active wells in the US (US EIA, 2014a) and fourth in the total gas production (US EIA, 2014b). Much of this exploration occurs in Southwestern Wyoming around the Jonah Field and Pinedale Anticline. Exploration and extraction are predicted to further increase in this part of Wyoming as production estimates of natural gas exceed 852 billion m^3 (US EIA, 2014c). The Green and Little Snake Rivers, the headwaters of the Colorado River in Wyoming, provide essential water supplies for agriculture and urban use within the catchments as well as down river. Therefore, oil and gas developments may diminish water quality (Wilson and VanBriesen, 2012; Vidic et al., 2013) to a large region.

Hydraulic fracturing, an increasingly common drilling method for extraction of natural gas, relies on the injection of large quantities of water with hundreds of chemicals deep into the ground (Bowen et al.,

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2015). Nearly 75% of these chemicals are known to negatively affect multiple organ-systems in humans (Colborn et al., 2011). The injected fluids and associated formation waters from the drilling, fracturing and well finishing-processes are recovered as "produced waters". Produced waters are generally re-injected, stored in containment ponds or released into ground and surface waters after treatment. An acute toxicity test of the treated produced-waters, often using aquatic invertebrates (e.g., *Ceriodaphnia* or *Daphnia* spp.) or fathead minnows (*Pimephales promelas*), is required by the US EPA approximately every three months to a year (US EPA, 1987). Although strict state and federal regulations exist, evaluating water quality can be difficult because the identity of many compounds has not been disclosed. In addition, detecting these chemicals after they have been released into flowing water, when releases may be pulsed and at low concentrations, may be unattainable (Stuer-Lauridsen et al., 2000; Álvarez-Romero et al., 2013). Nonetheless, because oil and gas deposits are primarily associated with sediments of marine origin, produced waters usually contain salts in higher concentrations than found in freshwater streams. Thus, changes in conductivity, which reflects total dissolved solids and salinity, could indicate anthropogenic inputs of contaminants into surface waters (Bowen et al., 2015).

The Green River and its tributaries are one of the few watersheds in the western US where river otters (*Lontra canadensis*) persisted after European settlement (Melquist et al., 2003). River otters, top predators in many freshwater systems, are particularly sensitive to environmental degradation (Larivière and Walton, 1998; Bowyer et al., 2003), anthropogenically-derived diseases (Gaydos et al., 2007) as well as human disturbance (Guertin et al., 2012). Numerous studies demonstrated the negative effects of exposure to hydrocarbons and polychlorinated biphenyls (PCBs) on river otters (Ben-David et al., 2000; Bowyer et al., 2003; Guertin et al., 2010). Similarly, these mustelids exhibit high sensitivity to heavy metal pollution (Harding et al., 1998), which led to their designation as a sentinel species of freshwater and marine ecosystems (Bowyer et al., 2003).

To gauge the potential effects of disturbance and contamination associated with oil and gas development on river otters in Southwestern Wyoming, we estimated their densities using non-invasive (Pauli et al., 2010) genetic sampling (Hansen et al., 2008; Guertin et al., 2012; Mowry et al., 2011; Brzeski et al., 2013) along several reaches of river. We mapped the distribution of river otter latrines in relation to habitat availability and disturbance and evaluated water quality by deploying electrical conductivity loggers at strategic points throughout the watershed. We hypothesized that otter density and the distribution of their latrines will be negatively related to levels of disturbance and alteration of surface water quality.

2. Materials and methods

2.1. Study area

The Green River is the largest tributary of the Colorado River (Fig. 1). Its various tributaries drain the Wind River Mountain Range to the east and the Wyoming Range to the west. The region experiences long, cold winters (−18.0 to −2.7 °C) and short, hot summers (4.7 to 25.2 °C). Precipitation largely occurs in the form of snow and averages 164 mm annually (NOAA, 2004). The Green River and its tributaries exhibit hydrographs typical of western streams with large seasonal variances in discharge due to snowmelt. Streams in the region are bordered by a relatively narrow riparian zone of primarily willows (*Salix* spp.), alders (*Alnus tenuifolia*), and cottonwood trees (*Populus* spp.). The surrounding landscape is dominated by sagebrush (*Artemisia tridentata*) and herbaceous vegetation.

2.2. Sampling areas

To estimate river otter densities and distribution of latrines, we sampled three river reaches in 2010 including the New Fork River (NF) within the Pinedale Anticline natural gas field, the upper Green River (UGR) below the NF confluence and above the Fontenelle Reservoir, and the Green River within Seedskadee National Wildlife Refuge (SNWR) below the Fontenelle Dam (Fig. 1). In 2011 after exploring otter activity on all reaches and based on activity levels, we resurveyed the UGR and two reaches of the Green River through SNWR and did not re-survey the NF (Table 1).

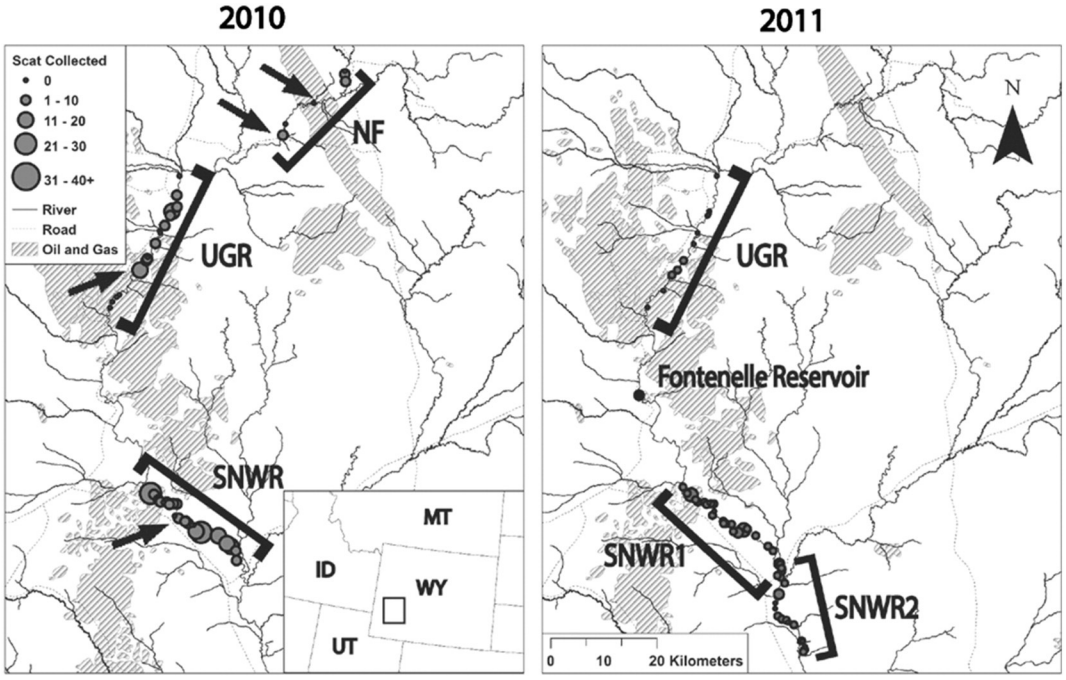

Fig. 1. Location of river otter latrine sites and number of feces counted at each site (fecal deposition rate) along surveyed river reaches in the Green River Basin, Wyoming in 2010 and 2011. Shaded areas represent the extent of oil and gas development and collection facilities, while arrows mark the location of deployment of conductivity loggers in 2012. Most active energy development is constrained to the northern areas surrounding the New Fork River.

Table 1
Total length (km), number of river otter latrines, latrine density (latrines per km), number of scat and hair samples collected, and fecal deposition rate (number of feces per site), and dates of primary occasions for four river reaches surveyed in 2010 and 2011 within the Green River Basin, Wyoming.

Year	River reach	Total length (km)	Number of latrines	Latrine density (latrines/km)	Number of scats	Fecal deposition rate (scats/latrine)	Number of hair samples	Dates of primary occasions
2010	NF	32.2	6	0.19	13	2.2	1	Jun 9–16, Jul 6–13, Aug 3–12
	UGR	35.4	17	0.48	60	3.5	1	May 31–Jun 8, Jun 27–Jul 4, Jul 25–Aug 2
	SNWR1	38.6	24	0.62	241	10.0	16	May 16–24, Jun 18–25, Jul 15–23
2011	UGR	35.4	12	0.34	17	1.4	0	Jun 12–19, Jul 20–27, Aug 6–13
	SNWR1	38.6	23	0.60	133	5.8	15	May 22–29, Jun 21–28, Jul 12–19
	SNWR2	38.9	21	0.54	128	6.1	9	Jun 1–9, Jun 30–Jul 7, Jul 28–Aug 4
Total		–	–	–	580	–	42	

2.3. Sample collection

In both 2010 and 2011, we surveyed three river reaches for otter latrine sites using rafts. Our sampling schedule followed the requirements of the robust-design capture–recapture model (Pollock, 1982). Each surveyed river reach was visited three times every summer (primary occasions; Amstrup et al., 2005). Within each primary occasion, latrine sites along each river reach were identified, marked with the aid of GPS technology, and then sampled on four consecutive secondary occasions, each lasting two days. Thus, each site was visited 12 times during a given year.

At each site, we only collected fresh otter feces (<24 h old). All fresh otter feces were preserved in sterile 50 ml vials with 100% ethanol and stored on ice during the float; samples were refrigerated for long-term storage. In addition, we placed 1–3 hair snares (modified commercial body-snares; DePue and Ben-David, 2007) at latrine sites with high activity (i.e., more than 5 relatively fresh feces per site). Hairs plucked from individual otters were collected in sterile 2 ml tubes filled with dry silica, and the snare was burned with a butane lighter to prevent cross contamination in subsequent collections.

2.4. DNA extraction and microsatellite genotyping

All fecal samples were sieved through fine-mesh stainless steel, autoclavable sieves to remove all hard parts of prey material such as fish scales, bones, and crayfish shell (Hansen et al., 2008). We extracted genomic DNA from feces using QIAamp DNA Stool Mini Kits (Qiagen, Valencia, CA, USA). Hair samples were extracted with tissue kits (Qiagen, Valencia, CA, USA). We amplified nine microsatellite loci using polymerase chain reaction (PCR). We used five markers (*Rio*-01, *Rio*-05, *Rio*-17, *Rio*-19, and *Rio*-20) developed for the river otter (Beheler et al., 2004, 2005) and four markers (*Lut*-701, *Lut*-733, *Lut*-801, *Lut*-829) for the Eurasian otter (*Lutra lutra*, Linnaeus 1758; Dallas and Piertney, 1998). PCR cocktails were mixed using sample product, Qiagen Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA), BSA, dNTPs, primer mix, and ddH20 for a final 10 μl reaction volume per well (Appendix A, Table A1). Positive (blood samples from river otters with known genotypes) and negative controls (PCR Blanks) were included with each PCR run to insure reliability and monitor for contamination (Hansen et al., 2008). DNA amplification was conducted in PTC-0200 DNA Engine Peltier Thermal Cyclers (MJ Research, Inc., Waltham, MA) with variable programs depending on the locus (Appendix A, Table A2). Amplified PCR products were resolved on a 3730 DNA Sequencer (ABI, Foster City, CA, USA) with formamide-LIZ ladder as an internal size standard in each lane at the Nevada Genomic Center, Reno, Nevada. Products were scored manually using the software Peak Scanner v1.0 (ABI, Foster City, CA, USA). In addition, we amplified the LutSRY locus for sex identification (Dallas et al., 2000).

All samples collected in 2010 were screened with the two most reliable markers (*Rio*-17 and *Lut*-733), and discarded if they failed to amplify after three runs of each (Paetkau, 2003). Genotypes were initially evaluated after two amplifications (Frantz et al., 2003) and amplified 3–4 additional times before their consensus genotype was determined. All samples with complete agreement in all loci were considered recaptures. We archived the DNA extracts from all samples collected in 2011 because of high genotyping costs.

2.5. Population analyses

We quantified genotyping error (allelic dropout [ADO] and false alleles [FA]) following procedures described in Prugh et al. (2005) and Guertin et al. (2010). We used the software Micro-Checker 2.23 (van Oosterhout et al., 2004) to screen for genotyping error and null alleles. Probability of misidentifying individuals (P_{ID}) based on the multilocus genotype was calculated using program Gimlet 1.3.2 (Valière, 2002). We estimated both unbiased and sibling P_{ID} (Waits and Paetkau, 2005). We tested for departures from Hardy–Weinberg equilibrium (HWE) using the program Arlequin 3.5 (Excoffier and Lischer, 2010). Population differentiation was assessed using program Structure 2.2 (Pritchard et al., 2000), with an admixture model with correlated allele frequencies. We estimated the number of putative subpopulations (K) by performing 10 independent runs of $K = 1$ to 3 with a burn-in period of 50,000 followed by 50,000 Markov Chain Monte Carlo (MCMC) repetitions. We also used *Jost D* (Jost, 2008) to evaluate whether river otters identified on the three river reaches in 2010 were genetically distinct.

After identifying recaptures, we constructed a capture history for each otter and estimated abundance ($\hat{N}$) using robust-design capture–mark–recapture (CMR) models in program Mark (Version 7.2; White and Burnham, 1999). We constructed a suite of a-priori models by varying survival, capture and recapture probabilities and assessed the fit to the data with Akaike Information Criteria (AIC) for small sample sizes. CMR models require that individuals are captured only once per occasion (Amstrup et al., 2005). Therefore, in cases where individuals were identified multiple times at different latrine sites, but during the same occasion (i.e., spatial recaptures), we considered them a single capture/recapture event. We constructed models based on the clustering assignment by program Structure (Guertin et al., 2012), as well as separately for each river reach.

To avoid discarding information derived from spatial recaptures we also used Capwire (Miller et al., 2005) to estimate population size. Capwire uses two capture models: the even capture probability model (ECM) assumes that every individual is equally likely to be captured in each trapping session, and the innate rate model (TIRM) assesses the occurrence of heterogeneity among individuals in capture probabilities. We used both model types in our analyses. River otter density was calculated by dividing the population estimates by river length. For a maximum density estimate we used only the sum of length of river reaches surveyed. For minimum density we calculated the total river length including reaches between those surveyed (Fig. 1).

2.6. Habitat analysis

At each latrine site we recorded the occurrence of dens, beaver sign, and ranked the anthropogenic disturbance from “low” to “high” based

on trails, roads, buildings, and drilling rigs. These ocular ranks (1–3) will be referred to as "observed disturbance."

We measured aspect perpendicular to the river and the distance from the location of feces to bankfull height. Total overstory cover, by species, was estimated using an ocular scale from 1 to 5 (converted to percent), following methods described by Bowyer et al. (1995) and DePue and Ben-David (2007). Similarly, we estimated total understory cover and categorized it into general types (i.e., brush, grass, etc.) using the same scale as overstory. We measured the slope of trail accessing the river and measured the depth of the river at 1 m from the water's edge. We estimated the width of the river in meters and, if visible, categorized the river substrate (e.g., mud and cobble). Herein, these data will be referred to as "observed habitat" variables.

In addition to site-specific habitat measurements, we estimated habitat availability within each river reach using four-band (red, green, blue and near-infrared) aerial imagery from the National Agricultural Imaging Program (NAIP) captured at a 1-m ground sample distance (USDA, 2013). Second, National Hydrological Database (NHD) high-resolution stream coverage was obtained from the US Geological Survey (USGS, 1999) to provide the base layer for analyses. Using ArcGIS 10.1 (ESRI Redlands, CA, USA) we masked the imagery to contain only a 2 km buffer around each river reach. We generated data for model fitting by hand-digitizing 50–60 polygons within each river reach for each of five categories: overstory (i.e., alder, cottonwoods and willows), tall green grass, bare ground (or ground sparsely covered with short brown grass), sagebrush, and water. Following methods outlined in Hayes et al. (2014), we used the *randomForest* package in Program R (Liaw and Wiener, 2002; R Development Core Team, 2013) to classify the vegetative cover in each reach. Random Forest is a bootstrapped classification and regression tree algorithm that creates a 'forest' of randomly generated classification trees (Breiman, 1996, 2001). During the generation of a single tree, 64% of the input data are used for model fit while the remaining 36% are retained to estimate the Out-of-Bag (OOB) classification error (Evans et al., 2011). We also conducted external-model-validation (True External Validation — TEV) by randomly selecting 70% of the available polygons within each vegetation class for model training, while the remaining 30% were used for validation. Finally, we used the Random Forest model to predict the most likely vegetation class for each pixel within the NAIP imagery. We then calculated the percent cover of each habitat type within each reach of river. Herein, these data will be referred to as "modeled habitat" variables.

2.7. Prey abundance

Capture–recapture data for salmonids (*Salmo trutta*, *Salvelinus fontinalis*, *Oncorhynchus* spp.), a major prey source for river otters in the Green River Basin, were obtained from A. Senecal and H. Sexauer (Wyoming Game and Fish Department; WGFD). Data were collected by the WGFD during summer 2011 in surveys conducted along approximately 5 km of river. Those surveyed reaches fell within those we sampled for river otters. Fish were captured with electrofishing equipment over a 3-day period. All captured fish were identified to species. Salmonids were marked with a passive integrated transponder (PIT; Biomark, Boise, ID, USA) for individual identification. Each marked fish was also weighed to the nearest 1 g. We transcribed the salmonid data into capture histories and estimated abundance with a closed-capture model with equal capture and recapture probabilities in program Mark. The resulting population estimates for each reach were converted to density by dividing $\hat{N}$ by river length (km). We calculated fish biomass per km and 95% confidence intervals by multiplying abundance by the average fish mass separately for each river reach. We generated these values using bootstrap procedures and the gamma distribution in program R (R Development Core Team, 2013) with 10,000 iterations.

2.8. Disturbance

We used a disturbance raster dataset, developed by Copeland et al. (2007), which describes levels of disturbance measured on a scale from 0 to 100, with 100 representing the highest disturbance possible, for each 30-meter pixel of our study area. This raster was derived from various anthropogenic disturbance factors including roads, power lines, buildings, and development (Copeland et al., 2007). We used ArcGIS to estimate the mean degree of disturbance around each individual latrine site as well as the surveyed river reaches. To measure the amount of disturbance surrounding latrine sites, we buffered each surveyed site by 200 m and calculated the mean 30-m pixel values within the buffer. Similarly, we buffered the NHD stream reaches by 200 m and obtained the mean disturbance value for those. These measurements will herein be referred to as "modeled disturbance."

In order to assess disturbance that might not be captured within the raster (Copeland et al., 2007), we used Google Earth (Version 5.1, accessed on 23 February 2014) and identified all active man-made structures around the river. We classified each structure as either industrial or residential. We exported the coordinates of these structures to ArcGIS and counted all points within respective buffers of 500 m, 1 km, and 2 km of each river reach.

2.9. Conductivity and salt load calculations

In June 2012, we deployed HOBO U-24 conductivity loggers (Onset Computer Corporation, Bourne, MA, USA) at four sites within the Green River Basin. We set two loggers in the NF, one above the Pinedale Anticline oil and gas field and another below the produced-water treatment plant (Fig. 1). A third logger was deployed in the UGR, and a fourth in SNWR. Loggers were sealed in PVC tubes drilled with 1-cm diameter holes to insure unimpeded water flow to the sensors. The PVC casings were then secured to cinderblocks and placed in the rivers deep enough to ensure they would remain submerged during periods of low discharge. We tied the cinderblocks to anchors hammered into the bank with plastic-coated stainless steel wiring. The conductivity loggers were programmed to record conductivity and temperature every 30 min. They were retrieved in December 2012, and the data were downloaded using HOBOware Lite Version 3.3.1.

Daily averages of conductivity from the HOBO U-24 recordings were temperature corrected using HOBOware Pro Conductivity add-on (Onset Computer Corporation, Bourne, MA; http://www.onsetcomp.com/products/software/add-ons-plug-ins). Because the recording times differed for the four loggers we calculated the daily averages of temperature-corrected conductivity values. Discharge, measured every 15 min, was obtained from USGS gauge stations 09205000 (NF),

Table 2
Abundance and density estimates (with 95% confidence intervals in parentheses) for river otters in the Green River Basin, Wyoming derived from non-invasive genetic analyses of samples collected in 2010. Densities are in kilometers per river otter.

Dataset	Robust design			Capwire		
	N	Maximum density	Minimum density	N	Maximum density	Minimum density
All	35 (28–47)	–	–	44 (38–49)	–	–
Excluding NF	31 (25–43)	2.39 (1.72–2.96)	3.65 (2.63–4.53)	51 (40–69)	1.45 (1.1–1.85)	2.21 (1.64–2.83)
UGR	–	–	–	17 (12–26)	–	2.08 (1.36–2.95)
SNWR	–	–	–	26 (22–32)	–	1.48 (1.21–1.75)

Table 3
Observed habitat variables (±95% confidence intervals — CI) measured at river otter latrine sites, estimated from disturbance rasters, and prey abundance along three river reaches in the Green River Basin, Wyoming. Numbers in bold represent significant differences between reaches.

Variable	Description	NF		UGR		SNWR	
		Estimate (6)	95% CI	Estimate (17)	95% CI	Estimate (24)	95% CI
Observed disturbance	Ranked 1–3	**1.7**	**1.6–2**	1.2	0.9–1.4	1.2	1–1.4
Model-derived disturbance	Reach level	24.4	21.4–27.3	28.2	25.0–31.3	**12.5**	**4.2–20.7**
	Latrine sites	30.0	28.5–31	28.7	28.0–29.2	**11.2**	**10.6–11.8**
Overstory	Willow (%)	23	11–35	27	17–37	22	12–33
	Cottonwood (%)	0	–	4	0–9	1	0–2
	Alder (%)	3	0–9	0	–	0	–
Understory	Brush (%)	30	21–39	34	26–42	43	33–54
	Grass (%)	70	61–77	62	51–73	51	40–51
	Bare ground (%)	0	–	4	0–10	6	0–6
River width	In meters (m)	32	27–38	40	34–47	**53**	**46–60**
River depth	At 1 m (in cm)	47	38–57	47	41–55	**31**	**25–37**
River substrate	Mud (%)	23	5–42	34	20–47	24	10–39
	Cobble (%)	77	58–77	61	46–75	72	56–87
	Large rocks (%)	0	–	6	0–17	4	0–12
Latrine area	In m^2	83	0–172	178	98–258	785	0–1610
Distance to bankfull height	In m	2	1–3	2	1–3	4	3–6
Bank slope	In degrees	32	27–38	33	29–37	37	31–43
Aspect	Mode	W	–	W	–	NW	–
Beaver sign	% of sites	17	–	65	–	17	–
Salmonid abundance	Per km of river	119	73–212	**266**	**221–327**	111	78–165
Salmonid mass	kg	0.93	0.76–1.11	0.82	0.74–0.90	**1.95**	**1.81–2.10**
Salmonid biomass	kg per km of river	111.2	45.4–177.0	227.2	175.9–278.6	216.4	131.1–301.8

09210500 (UGR), and 09211200 (SNWR), and converted from cfs to m^3/s. We calculated the daily average flow from the raw data.

The daily average specific conductance values were converted into salt equivalents (g/m^3) by multiplying conductance by 0.467 (2.14 μs = 1 mg NaCl/l), a value that was obtained through calibrating the HOBO U-24 loggers. Salt load was estimated by multiplying salt concentration and the daily averaged discharge of the river. We integrated the area under the curve to calculate the total amount of salt equivalents added for the period of July 12, 2012 to November 21, 2012 for each logger. To assess the salt load added above background to the NF, we subtracted our estimates derived from the logger stationed above the Pinedale Anticline oil and gas field from those of the logger positioned below the water treatment plant.

3. Results

3.1. Sample collection

During the 2010 field season we identified 47 active river otter latrine sites. The majority of sites occurred in SNWR and the least were detected along the NF (Table 1). Similarly, fecal deposition rate was lowest on the NF compared with the UGR and SNWR (Table 1). In 2011, the density of active latrines declined on the UGR but remained relatively high on the upper reach surveyed on SNWR. Latrine density was similar on the upper and lower reaches of the SNWR (Table 1). In 2010, for all three river reaches, we collected a total of 314 scats and 18 hair samples. The majority of samples were collected from the SNWR reach (Fig. 1). In 2011, we collected a total of 278 scats and 24 hair samples (Table 1).

3.2. Microsatellite genotyping and population genetics

Of the 314 feces collected in 2010, 160 (51%) and all 18 hair samples yielded river otter DNA. We were able to successfully genotype 97 fecal samples (31%) at a minimum of seven microsatellite loci. No hair samples amplified in sufficient loci to be included in subsequent analyses. Using fecal samples, we identified 38 unique individuals, with 11 river otters identified once and 27 identified 2–6 times. Of these, two river otters were identified from three sites along the NF, 12 individuals from 10 sites on the UGR, and 23 individuals from 16 sites in SNWR. One individual was genotyped at two sites in SNWR in late June and from two sites on the UGR in late July and early August. The sex assignment was 21 females and 17 males.

Because most samples did not contain anal jellies (89%), genotyping was accomplished with a minimum of 5 PCRs per locus. This yielded an overall multi-locus error rate of 0.022 with ADO ranging from 10 to 29% and FA from 2 to 4%. Theoretical $P_{ID\text{-}unbiased}$ was 2.34×10^{-08} and $P_{ID\text{-}sib}$ 1.02×10^{-03}. That equals a 1 in 42,826,552 chance that two unrelated river otters in the population share the same multi-locus genotype, and a 1 in 985 chance that two siblings in the population exhibit the same profile. Four of the nine loci (*Rio*-01, *Rio*-05, *Lut*-733, and *Lut*-801) exhibited evidence of a null allele. All microsatellite loci were polymorphic with a mean number of alleles per locus of 5.22 (range 3–9; Table A3). Observed heterozygosity was lower than expected (Table A3), as is common in small semi-isolated populations. Over all loci, the population was in HWE, although a few loci deviated from this pattern (Table A3). Both Structure and *Jost D* ($D_{est} = 0.003$) analyses indicated a single panmictic population.

3.3. Population size and density

The top robust-design model with constant survival ($\psi = 1.0 \pm 0.00$), and equal and constant capture and recapture probabilities ($p = c = 0.19 \pm 0.03$) accounted for 62% of model weight. AICc of the following model (variable capture and recapture probabilities) was

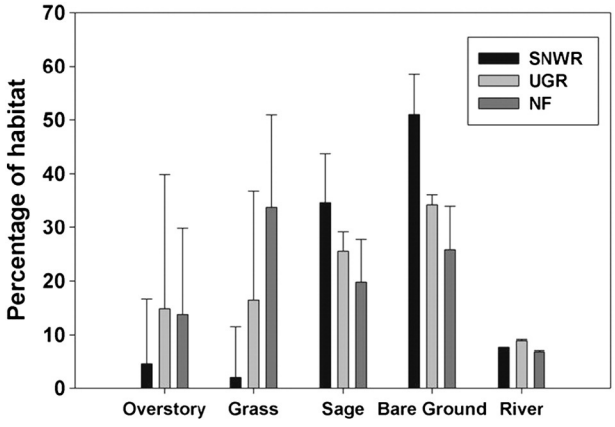

Fig. 2. Percent classification of habitat cover from NAIP imagery (National Agricultural Imaging Program) and Random Forest modeling for each river reach surveyed in the Green River Basin, Wyoming in 2010 and 2011. Error bars show Out of Bag (OOB) error for each classification.

Table 4
Total numbers of active man-made structures within varying buffer distances of each river reach in the Green River Basin, Wyoming, as counted from Google Earth images.

River reach	Structure type	Buffer distance from river		
		500 m	1 km	2 km
NF	Industrial	6	9	26
	Residential	10	12	12
UGR	Industrial	0	0	0
	Residential	6	6	6
SNWR	Industrial	0	1	1
	Residential	0	3	3

>3.0 units higher. The ECM was selected in most (3 of 4) Capwire analyses, suggesting little individual heterogeneity in capture.

While abundance estimates derived from the full dataset of capture histories with program Capwire were higher (44 river otters) than those obtained from the robust-design model (35 individuals), the 95% confidence intervals overlapped (Table 2). We re-calculated abundance estimates after excluding the two individuals identified from the NF to minimize potential bias in density calculations. The robust-design estimates were less sensitive to this exclusion than Capwire (Table 2). Because of the differences in disturbance regimes, and despite the strong evidence of a single genetic population, we also estimated the abundance of river otters separately for the UGR and SNWR reaches of river using Capwire. This analysis resulted in an estimate of 17 (12–26) individual river otters above the Fontenelle Reservoir (UGR), and 26 (22–32) below the dam (SNWR; Table 2), or a total of 43 individuals.

Both maximum and minimum density values resulting from abundance estimates of the robust-design model were lower than those derived from Capwire (Table 2). Because only one river otter crossed the area between the surveyed reaches, we also considered densities separately above and below the Fontenelle Reservoir. These estimates were 40% higher below the dam (Table 2).

3.4. Habitat, prey availability and disturbance

We found few differences among the numerous site-specific observed habitat variables across the four reaches of river surveyed in 2010 and 2011. Latrines on SNWR occurred where the river was wider and shallower than on other reaches, likely because this was a prevalent feature of this reach. The overstory was dominated by willow at all sites, but SNWR and UGR sites also had a low percentage of cottonwood, while NF had a small percentage of alder (Table 3). Also, the NF sites were exposed to higher levels of observed disturbance (Table 3).

The near infrared spectrum was most important for classification of modeled habitat types for all survey reaches based on mean decrease in accuracy (Fig. B1). Average OOB error for all habitat classes varied among the reaches between 7.7% and 10.2%, with the SNWR river reach exhibiting the least, and the NF reach the highest values. The highest confusion in all reaches was between overstory and grass, and between sagebrush and bare ground (Table B1). Errors using the TEV method were generally higher, but showed similar trends (Table B1). These error rates are higher than other studies in this region (Hayes et al., 2014), but adequate for quantification of habitat features important for river otters (i.e., overstory cover; Crait and Ben-David, 2007; DePue and Ben-David, 2010).

River reaches analyzed using the classified vegetation Random Forest modeling demonstrated some differences in habitat within a 500 m buffer of the river (Fig. 2). SNWR had the greatest percent of bare ground and sagebrush cover compared with the UGR and the NF. UGR and NF reaches had higher percent cover of overstory and grass (Fig. 2).

Density estimates of salmonids were similar in SNWR and NF, but greater in UGR (Table 3). Similarly, fish biomass was higher in UGR compared with the NF, while values were intermediate for SNWR (Table 3). This was likely because fish in SNWR were in general larger, and the UGR is generally wider than the NF and deeper than SNWR below the Fontenelle Dam. Such a large and deep river likely provides more habitat for these fish. In addition to salmonids, the WGFD survey recorded several non-salmonids in all three river sections including: non-native burbot (*Lota lota*), non-native white sucker (*Catostomus commersonii*), as well as native mountain whitefish (*Prosopium williamsoni*), mottled sculpin (*Cottus bairdii*), speckled dace (*Rhinichthys osculus*), redside shiner (*Richardsonius balteatus*), Utah chub (*Gila atraria*), mountain sucker (*Catostomus platyrhynchus*), flannelmouth sucker (*Catostomus latipinnis*), and sucker hybrids. High abundance of white suckers was noted especially in the NF section.

Modeled disturbance values measured at the reach level were substantially lower in SNWR than in UGR or NF. This trend was maintained in the

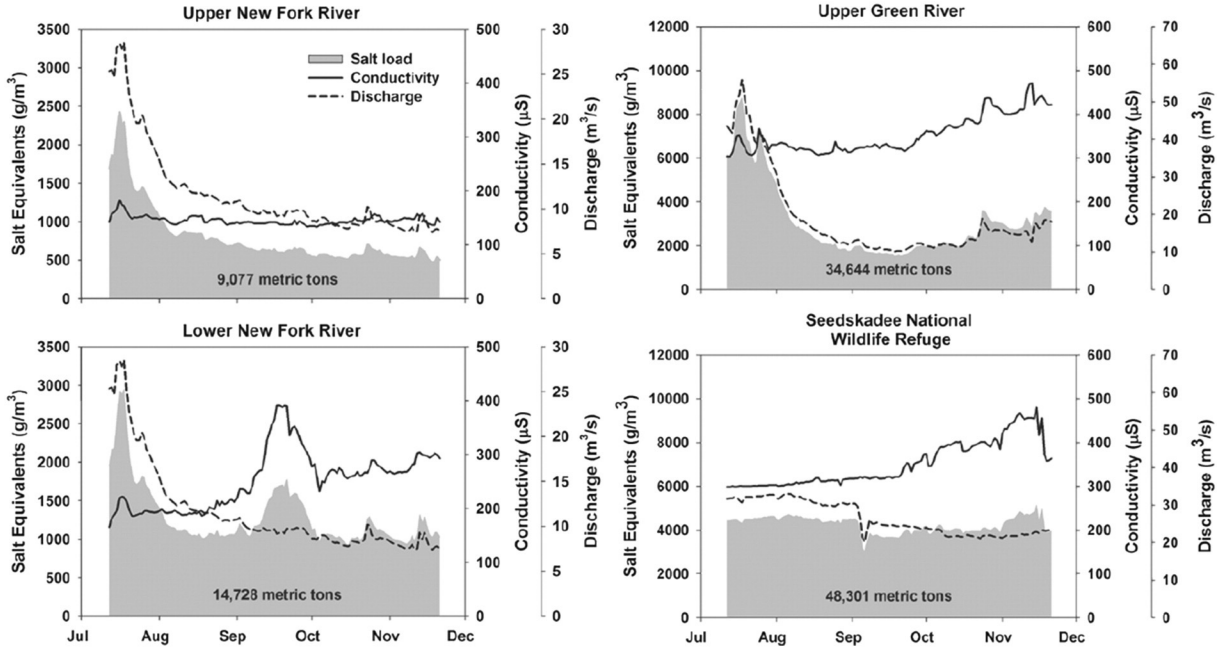

Fig. 3. Daily averages of specific conductance, discharge, and total load of salt equivalents at four sites in the Green River Basin, Wyoming, July 12 through November 21, 2012. The upper New Fork logger was located above the Pinedale Anticline oil and gas field while the lower was deployed below the wastewater treatment plant (see Fig. 1).

analyses of buffers around specific latrine sites (Table 3). The NF reach harbored the greatest number of residential and industrial structures throughout all respective buffers (Table 4), potentially indicating higher levels of noise and light pollution that can be associated with man-made structures.

3.5. Conductivity analysis

The patterns of specific conductance and load of salt equivalents differed significantly at the four stations sampled and could not be solely explained by discharge (Fig. 3). Temperature corrected conductance as measured in the SNWR, UGR and the upper logger of the NF largely matched the hydrograph. In SNWR there were few fluctuations in discharge and conductance because of steady water release from the Fontenelle Dam. For the UGR and the upper NF loggers, the high discharge associated with snow melt was closely tracked by conductance (Fig. 3). The lower NF logger recorded a spurious, 4-week long, increase in conductance in September, a period with minimal flow. This total increase in conductance over the period from July 12, 2012 to November 22, 2012 (134 days) in the lower NF logger was equivalent to 5651 metric tons of salt over the background recorded by the upper NF logger (Fig. 3). With no major tributaries flowing into the NF reach between these two loggers, and no recorded increase in discharge over this time, this value represents an increase in salt equivalents greater than expected by a factor of 1.62.

4. Discussion

Our results illustrate that river otter use of reaches in the Green River Basin, Wyoming, varied and was most likely influenced by anthropogenic disturbance and potentially pollution associated with oil and gas development. Prey availability and habitat characteristics were similar in the NF, UGR and SNWR reaches of river suggesting that these factors were less influential on river otter distribution. The highest density of river otters occurred at the least disturbed reach (SNWR), while we only identified two individuals on the NF from an estimated population of 35–45 animals. Concurrently, we observed negative correspondence between latrine density and disturbance, both at the scale of whole river reaches and at individual latrine sites. The observed increase in conductivity during September 2012 may be indicative of contamination from oil and gas development, and suggest that river otter avoidance of this reach may have been affected in part by unaccounted contamination events over time.

Our results of river otter abundance and densities fall within the range of similar studies (Guertin et al., 2012; Brzeski et al., 2013; Johnson et al., 2013). Additional support for the reliability of these estimates stems from our use of well-established non-invasive genetic methods, and the concordance with levels of error and probability of identity reported in such studies (Hansen et al., 2008; Guertin et al., 2012; Mowry et al., 2011). However, the lack of continuity between the reaches we surveyed complicated density calculations in this river system. The robust-design capture–recapture sampling scheme (Pollock, 1982; Amstrup et al., 2005), which was less sensitive to the exclusion of the two NF individuals and yielded estimates we consider more reliable, prevented the monitoring of the entire length of the rivers. Given that river otters in our study area belong to the same genetic and likely demographic population (i.e., we recorded movements between the UGR and SNWR reaches by one river otter), obtaining estimates for the full length of the Green and New Fork Rivers (including the Fontenelle Reservoir) would have been preferable.

Our reach-specific Capwire-derived density estimates matched our indirect measures of river otter activity. Latrine density and fecal deposition were higher in SNWR in 2010 and 2011, corresponding with 40% higher densities than the UGR. Annual differences in fecal deposition in UGR and SNWR were likely a function of higher flood levels in 2011. In that year flood-level flows persisted through July, inundating many latrines and likely washing away feces.

Differential use of river reaches may be related to resource availability. It is possible that river otters are attracted to SNWR because, at least in summer, it may provide higher prey diversity and availability than UGR and NF. The data we obtained from WGFD demonstrated no difference in salmonid densities or biomass between NF and SNWR, the reaches with the most disparate river otter densities. These prey data were collected in one year (2011) and represent a partial assessment of food availability because for most species no abundance data were collected. However, other fish species such as suckers (*Catostomus* spp.) and the large non-native burbot (*L. lota*), which are commonly consumed by river otters in this system (Gardunio et al., 2011; BLG, *personal observation*), were recorded in large numbers in all reaches, including the NF. Indeed, all the river reaches we surveyed have been renowned sport-fishing destinations for many years (Pinedale Outdoors, 2012), indicating sustained prey populations.

In contrast with the other river reaches, SNWR is characterized by high occurrence of *Orconectes* spp., a large invasive crayfish (Hubert, 2010). These crayfish occur frequently in river otter scats in this location (Hansen, 2004; BLG, *personal observation*). However, the availability of this prey below Fontenelle Dam could not solely explain the low use of the NF by river otters. Because these crustaceans are active only during the summer months (Hubert, 2010), they are available to river otters only seasonally. River otters exhibit high fidelity to latrine sites (Bowyer et al., 2003; DePue and Ben-David, 2010), which are used over decades. When river otters are present, such long-term use is usually easy to detect. Thus, a seasonal feeding migration to SNWR, while possible as river otters can travel the intervening distance and readily cross potential barriers such as the Fontenelle Dam and Reservoir, would not have resulted in the near-absence of river otter sign from the NF.

In addition, differential use of reaches by river otters could not be explained by habitat composition. First, our observed site-specific habitat measurements have shown that when establishing a latrine in all river reaches, river otters selected for similar features, namely high overstory and grass cover, moderately sloping banks, and relatively deep pools. These features have been previously described as suitable for river otters in western streams and lakes (Crait and Ben-David, 2007; DePue and Ben-David, 2010). As indicated by our habitat modeling, the NF had the highest availability of overstory and grass cover at the landscape level compared with the other two reaches. In contrast, SNWR, the reach with the highest density of river otters, had the least ideal vegetative cover (bare ground and sagebrush). As such, it appears that habitat composition had little influence on the observed distribution of river otters in this system.

In arid landscapes, river and Eurasian otters may seasonally abandon river reaches during periods with reduced discharge (Prenda et al., 2001). Indeed, river otters rarely use streams with flows less than 0.3 m^3/s (Boyd, 2006). The USGS gauging stations located on the NF, UGR and SNWR indicated that ice-free flows never fell below this threshold. Thus, it is unlikely that the differential use of these reaches by river otters resulted from differences in the river hydrographs.

Other studies have shown that human presence and associated disturbance, even at low levels, may deter river and Eurasian otters from occupying or colonizing various waterways (Prenda et al., 2001; Gaydos et al., 2007; Guertin et al., 2012; Romanowski et al., 2012). For example, Kalz et al. (2006) found limited dispersal of Eurasian otters across a highway that bisected a nature reserve in Germany. Also, Guertin et al. (2012) documented asymmetrical emigration of river otters around the industrial harbors of Victoria, British Columbia, with individuals born in relatively pristine locations avoiding the heavy boat traffic. Our analyses of observed and modeled disturbance, as well as quantification of man-made structures, demonstrate that along the NF river otters are exposed to higher levels of industrial disturbance from oil and gas development, as well as non-industrial activities associated with human habitation. The effects of noise from heavy machinery, illumination, seismic activity, traffic and high human presence may deter river otters from foraging and scent marking along the banks of

the NF. Studies have shown that other species such as mule deer (*Odocoileus hemionus*) avoid areas surrounding energy extraction well pads, especially those with high activity and traffic (Sawyer et al., 2009), and that sage-grouse leks (*Centrocercus urophasianus*) are negatively affected by intermittent anthropogenic noise associated with natural gas extraction (Blickley and Patricelli, 2010; Blickley et al., 2012).

Nonetheless, disturbance alone may be insufficient to explain the low levels of river otter activity along NF, as these mustelids are known to habituate to long-term human disturbance. For example, although river otters born outside the industrial harbors of Victoria, British Columbia rarely immigrated to this highly disturbed environment, Guertin et al. (2012) recorded typical levels of survival, successful reproduction, and high emigration among resident individuals. Similarly, Shenoy et al. (2006) found that smooth-coated otters (*Lutrogale perspicillata*) in India avoid anthropogenic activity and disturbance, though avoidance was largely temporal rather than spatial, suggesting partial acclimation. Whether the time-span of increased oil and gas development in Southwestern Wyoming is too short to allow habituation by river otters is unknown.

In addition to the effects of industrial disturbance, surface water pollution may determine use of the NF by river otters. We have evidence of an unexplained discharge of solutes (i.e., salt-equivalents) into the NF downriver from the Pinedale Anticline oil and gas field, and below a regional wastewater treatment facility, in September of 2012. Hydraulic fracturing waste-fluids and other production waters are highly saline (from bromide, chloride, barium, arsenic, naturally occurring radioactive material, and more), ranging from 5000 mg/l to greater than 200,000 mg/l (Warner et al., 2013). Although our monitoring of river otters in the Green River Basin preceded the timing of this episode, if similar events occurred in previous years, such discharge could explain the low density of latrines and low fecal deposition we observed. For example, DePue (2009) documented limited dispersal of river otters across a 40 km contaminated reach of the Gunnison River, Colorado. The paucity of old latrine sites along the NF suggests that river otters, a sentinel species for aquatic contamination (Bowyer et al., 2003), have been avoiding this reach for some time.

Unfortunately, given the scope of this study we could not identify specific solutes that could account for the increase in conductivity. Nonetheless, our findings illustrate the need for additional intensive sampling and an in-depth investigation into the potential effects of oil and gas development on surface waters in Wyoming and elsewhere. Thus, continued monitoring of rivers with the aid of continuously recording devices (Stuer-Lauridsen, 2005) and sentinel species, such as river otters, is warranted.

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Appendix A. Genetic analyses and results

We extracted genomic DNA from feces using QIAamp DNA Stool Mini Kits (Qiagen, Valencia, CA, USA). Hair samples were extracted with tissue kits (Qiagen, Valencia, CA, USA). We amplified nine microsatellite loci using polymerase chain reaction (PCR). We used five markers (*Rio*-01, *Rio*-05, *Rio*-17, *Rio*-19, and *Rio*-20) developed for the river otter (Beheler et al., 2004, 2005) and four markers (*Lut*-701, *Lut*-733, *Lut*-801, *Lut*-829) for the Eurasian otter (*L. lutra*, Linnaeus 1758; Dallas and Piertney, 1998). PCR cocktails were mixed using sample product, Qiagen Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA), BSA, dNTPs, primer mix, and ddH20 for a final 10 μl reaction volume per well (Table A1). Positive (blood samples from river otters with known genotypes) and negative controls (PCR Blanks) were included with each PCR run to insure reliability and monitor for contamination (Hansen et al., 2008). DNA amplification was conducted in PTC-0200 DNA Engine Peltier Thermal Cyclers (MJ Research, Inc., Waltham, MA) with variable programs depending on the loci (Table A2). Amplified PCR products were resolved on a 3730 DNA Sequencer (ABI, Foster City, CA, USA) with formamide-LIZ ladder as an internal size standard in each lane at the Nevada Genomic Center. Products were scored manually using the software Peak Scanner v1.0 (ABI, Foster City, CA, USA). In addition, we amplified the LutSRY locus for sex identification (Dallas et al., 2000).

We quantified genotyping error (allelic dropout [ADO] and false alleles [FA]) following procedures described in Prugh et al. (2005) and Guertin et al. (2010). We used the software Micro-Checker 2.23 (van Oosterhout et al., 2004) to screen for genotyping error and null alleles. Probability of misidentifying individuals (P_{ID}) based on the multi-locus genotype was calculated using program Gimlet 1.3.2 (Valière, 2002). We estimated both unbiased and sibling P_{ID} (Waits and Paetkau, 2005). We tested for departures from Hardy–Weinberg Equilibrium (HWE) using the program Arlequin 3.5 (Excoffier and Lischer, 2010). Population differentiation was assessed using program Structure 2.2 (Pritchard et al., 2000), with an admixture model with correlated allele frequencies. We estimated the number of putative subpopulations (K) by performing 10 independent runs of $K = 1$ to 3 with a burn-in period of 50,000 followed by 50,000 Markov Chain Monte Carlo (MCMC) repetitions. We also used *Jost D* (Jost, 2008) to evaluate whether otters identified on the three river sections in 2010 were genetically distinct.

Genotyping, accomplished with a minimum of 5 PCRs per locus, yielded an overall multi-locus error rate of 0.022 with ADO ranging from 10 to 29% and FA from 2 to 4%. Theoretical PID-unbiased was 2.34×10^{-08} and PID-sib 1.02×10^{-03}. That equals a 1 in 42,826,552 chance that two unrelated otters in the population share the same multi-locus genotype, and a 1 in 985 chance that two siblings in the population exhibit the same profile. Four of the nine loci (Rio-01, Rio-05, Lut-733, and Lut-801) exhibited evidence of a null allele. All microsatellite loci were polymorphic with a mean number of alleles per locus of 5.22 (range 3–9; Table A3). Observed heterozygosity was lower than expected (Table A3), as is common in small semi-isolated populations. Over all loci, the population was in HWE, although a few loci deviated from this pattern (Table A3). Both Structure and *Jost D* ($D_{est} = 0.003$) analyses indicated a single panmictic population.

Table A1
PCR reaction mixtures for nine microsatellite loci amplified in scat and hair samples collected from river otter latrine sites in the Green River Basin, Wyoming, in summer 2010.

PCR reaction mixture	Amount (μl)
Sample	1
ddH_2O	2.6
dNTP mix (2 mM)	1
Qiagen Multiple PCR Master Mix	5
Forward labeled primer	0.2
Reverse primer	0.2
Total volume (μl)	10

Table A2
Thermal cycler programs for nine microsatellite loci amplified in scat and hair samples collected from river otter latrine sites in the Green River Basin, Wyoming, in summer 2010.

PCR Rxn step	*Lut*-701	*Lut*-733	*Lut*-801	*Lut*-829	*Rio*-01	*Rio*-05	*Rio*-17	*Rio*-19	*Rio*-20
1. Activation	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00	95° for 15:00
2. Denaturation	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30	94° for 0:30
3. Annealing	60° for 1:30	48° for 1:30	55° for 1:30	60° for 1:30	48° for 1:30	60° for 1:30	60° for 1:30	60° for 1:30	60° for 1:30
4. Extension	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00	72° for 1:00
5. Cycles	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35	Goto 2, ×35
6. Final extension	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00	60° for 30:00
7. End of cycling	4° forever	4° forever	4° forever	4° forever	4° forever	4° forever	4° forever	4° forever	4° forever

Table A3
Number of alleles amplified (mean ± SE), expected (H_E) and observed heterozygosity (H_O), deviation from Hardy–Weinberg Equilibrium (HWE, *p*-value) from 97 river otter fecal samples collected in the Green River Basin, Wyoming in 2010.

Loci	Number of alleles	Range	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)	HWE (*p*)
Lut-701	5	194–210	0.62	0.58	0.378
Lut-733	5	172–184	0.65	0.79	0.076
Lut-801	4	226–238	0.54	0.34	0.343
Lut-829	6	234–252	0.78	0.63	0.036
Rio-01	6	273–292	0.79	0.66	0.228
Rio-05	9	327–351	0.71	0.32	0.001
Rio-17	3	171–177	0.56	0.45	0.469
Rio-19	5	276–292	0.53	0.40	0.024
Rio-20	4	244–262	0.62	0.58	0.347
Overall	5.22 (±0.57)		0.65 (±0.03)	0.53 (±0.05)	0.211

Appendix B. Modeling habitat availability with remote sensing data

We estimated habitat availability within each river section using four-band (red, green, blue and near-infrared) aerial imagery from the National Agricultural Imaging Program (NAIP) captured at a 1-m ground sample distance (U.S. DA, 2013). Secondly, National Hydrological Database (NHD) high-resolution stream coverage was obtained from the United States Geological Survey (U. S. GS, 1999) to provide the base layer for analyses. Using ArcGIS 10.1 (ESRI Redlands, CA) we masked the imagery to contain only a 2 km buffer around each river section. Following methods outlined in Hayes et al. (2014), we used the *randomForest* package in Program R (Liaw and Wiener, 2002; R Development Core Team, 2013) to classify the vegetative cover (overstory [i.e., cottonwoods and willows], tall green grass, bare ground [or ground sparsely covered with short brown grass], sagebrush, and water) in each section. Random Forest is a bootstrapped classification and regression tree (CART) algorithm that creates a 'forest' of randomly generated classification trees (Breiman, 1996, 2001). During the generation of a single tree, 64% of the input data are used for model fit while the remaining 36% are retained to estimate the Out-of-Bag (OOB) classification error (Evans et al., 2011). This process is repeated for a predetermined number of individual trees and the votes across all trees are compiled to complete the ensemble model.

We generated data for model fitting by hand-digitizing 50–60 polygons within each river section. Prior to fitting a single Random Forest model, 70% of the available polygons, within each vegetation class, were randomly selected for model training, while the remaining 30% were used for external model validation. A total of 750 pixels within each vegetation class were randomly selected. The number of randomly sampled pixels within a single area of interest was determined by the proportion of the current polygon's area divided by the total area of all polygons within the current vegetation class. This same process was followed for the validation polygons, but the number of validation pixels was limited to 225 for each vegetation class due to the reduced number of polygons from which to sample. We then extracted the four reflectance bands for training and validation points. Using the training dataset, we fit a Random Forest model using 501 individual trees to create the ensemble. For each branch within an individual tree, three random bands were chosen from the possible four and the best discriminating variable was chosen. The model was then used to predict the most likely vegetation class for each validation point. We created a variable importance plot to assess the relative importance of each band within the NAIP imagery used to classify the habitat types. We assessed model goodness of fit using two different approaches. First, we obtained the OOB classification error within the Random Forest model. Secondly, we created a confusion matrix of correct classification for each vegetation class within the validation dataset. Finally, we used the Random Forest model to predict the most likely vegetation class for each pixel within the NAIP imagery.

The near infra-red spectrum was most important for classification of habitat types for all survey sections based on mean decrease in accuracy (MDA; Fig. B1). Average OOB error for all habitat classes varied among the sections between 7.7% and 10.2%, with the SNWR river section exhibiting the least, and the NF section the highest values. The highest confusion in all sections was between overstory and grass, and between sagebrush and bare ground (Table B1). Errors using the true-error-validation (TEV) method were generally higher, but showed similar trends (Table B1). These error rates are higher than other studies in this region (Hayes et al., 2014), but adequate for quantification of habitat features important for river otters (i.e., overstory cover).

Table B1
Correct classification (diagonal in bold) and average Out-of-Bag (OOB) error (top) and true external validation (bottom) of the Random Forest classification model of NAIP imagery for river sections in the Green River Basin, Wyoming.

	Classified					
Observed	Overstory	Grass	Bare ground	Sage	River	Overall
Overstory	**0.823**	0.168	0.008	0.000	0.001	0.177
Grass	0.254	**0.843**	0.002	0.000	0.000	0.157
Bare ground	0.006	0.003	**0.931**	0.060	0.000	0.069
Sage	0.000	0.000	0.058	**0.942**	0.000	0.058
River	0.000	0.000	0.000	0.001	**0.998**	0.002
Overstory	**0.780**	0.219	0.002	0.000	0.000	0.220
Grass	0.249	**0.751**	0.000	0.000	0.000	0.249
Bare ground	0.001	0.005	**0.916**	0.077	0.000	0.084
Sage	0.000	0.000	0.096	**0.904**	0.000	0.096
River	0.002	0.003	0.000	0.000	**0.995**	0.005

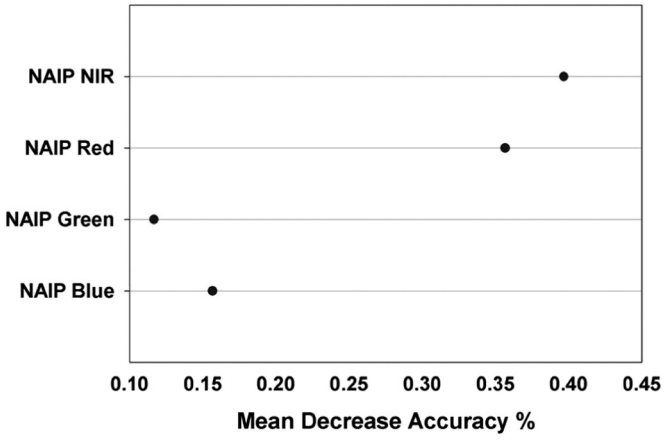

Fig. B1. Variable importance plot for the Random Forest classification model for three river sections in the Green River Basin, Wyoming.

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