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Assessment of environmental DNA for detecting and monitoring translocated North American beaver

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Abstract

There is growing interest in working with beavers (*Castor canadensis* and *Castor fiber*) to restore and maintain ecosystem function, improve hydrologic conditions and build climate resiliency in freshwater ecosystems. Beaver translocation into historically occupied but degraded systems has been increasingly applied as a restoration practice over the last two decades. Knowledge of beaver distributions on the landscape is critical to understanding where and when beaver translocations may be effective. However, current understanding of beaver occupancy and translocation success is limited by uncertainty, subjectivity and inefficiency associated with available monitoring methods. We evaluated the efficacy and spatial inference associated with environmental DNA (eDNA) techniques for detecting beaver presence in natural wetland and stream systems in the Cascade mountains of Washington State. We conducted eDNA sampling paired with radio-tracking of translocated beavers at four relocation sites from October 2020 through October 2022 to elucidate spatial patterns of site use, eDNA detection probability and eDNA quantity. We found that eDNA techniques detected beaver rapidly over long distances – up to 2.9 km from known locations within the first week after release – and reliably detected beavers when they were upstream, with positive detections in 92.4% of downstream eDNA samples collected 1–3 months after release. We also found that eDNA quantity decreased with increasing distance from beaver and increased with the amount of upstream beaver activity. Our study suggests that eDNA is a sensitive tool for monitoring translocated beaver and can provide spatial information on beaver location and site use within a stream system. Hence, eDNA methods could be a valuable tool for rapid inventory and assessment of beaver occupancy and our findings highlight important implications for using eDNA to monitor other semi-aquatic mammal species that share similar life histories.

Introduction

Freshwater ecosystems are home to disproportionately high levels of biodiversity and provide critical ecosystem services to natural systems and humans alike. However, land use practices have degraded and simplified freshwater ecosystems and consistently driven loss of biodiversity and ecosystem function (Young *et al*., 2016; Reid *et al*., 2019; Albert *et al*., 2021). Climate change is projected to exacerbate or accelerate these trends (Dudgeon *et al*., 2006; Erwin, 2009; Reid *et al*., 2019; Albert *et al*., 2021). The aquatic engineering activities of beavers (*Castor spp*.) are increasingly recognized for their ability to restore and maintain freshwater ecosystem function and build climate resiliency (Hood & Larson, 2015; Law *et al*., 2017; Fairfax & Small, 2018; Willby *et al*., 2018; Jordan & Fairfax, 2022). Consequently, interest in working with beavers to restore freshwater ecosystems is a burgeoning climate-change focused restoration strategy (Willby *et al*., 2018; Law *et al*., 2019; Jordan & Fairfax, 2022).

As a component of beaver-related restoration, beaver translocation has gained popularity over the last two decades (Pilliod *et al*., 2018). Translocation projects place beavers into degraded systems where their engineering may improve hydrologic function, increase freshwater system resilience and create habitat for many species (Jordan & Fairfax, 2022; Pollock *et al*., 2023). Translocation is predicated on the absence of resident beaver to increase chances of establishment and limit disruptions to resident populations; therefore, knowledge of beaver distributions is important for understanding where

translocation may be effective. However, current standardized methods for assessing beaver occupancy and establishment success are limited (Pilliod *et al*., 2018; Pollock *et al*., 2023; see Campbell-Palmer *et al*., 2021 for standardized sign surveys) and may not be applicable or efficient in hard to access areas (Graham *et al*., 2022). Additionally, the American beaver (*Castor canadensis*) is invasive in parts of northern Europe and southern South America, and efficient monitoring methods could aid in detecting the spread of these non-native populations and prove useful for evaluating outcomes of eradication projects (Parker *et al*., 2012; Huertas Herrera *et al*., 2020). Thus, to better understand beaver distributions on the landscape, we need flexible, efficient and sensitive tools to detect and monitor beaver occupancy across a variety of ecosystems.

Aquatic environmental DNA (eDNA) may provide sensitive and flexible tools for assessing beaver distributions and presence within specific sites. Environmental DNA techniques collect genetic material from environmental samples and can provide robust estimates of presence–absence while reducing field time and uncertainty of detection status. Current methods for establishing beaver presence (aerial surveys and visual searches for signs of activity; Hay, 1958; Robel & Fox, 1993; Campbell-Palmer *et al*., 2021: Pollock *et al*., 2023) have high upfront costs and low detection rates, and can be subjective, especially when beavers do not create dams (Petro, Stevenson, & Taylor, 2020). Alternative methods for monitoring beaver movement, such as radio-tracking, have limited long-term success and are not feasible at landscape scales (McKinstry & Anderson, 2002; Petro, Taylor, & Sanchez, 2015; Doden *et al*., 2022). Environmental DNA techniques have been used to detect semi-aquatic mammals including beaver (*Castor spp*. Harper *et al*., 2019b; Sales *et al*., 2020: Smith & Goldberg, 2022: Ushio *et al*., 2017). However, few studies have estimated detection probabilities or spatial inference of detections for beaver, though one study demonstrated ~300 m transport of detectable levels of beaver DNA (Broadhurst *et al*., 2021). Furthermore, spatially and temporally explicit studies of species-specific eDNA dynamics for semi-aquatic mammals are limited (Lugg *et al*., 2018; Croose *et al*., 2023; Mangan *et al*., 2023; Shiozuka *et al*., 2023). Detailed information about dynamics of eDNA detections in relation to animal movement and site use may provide important information that facilitates the use of these techniques to monitor beaver and other semi-aquatic mammal species.

Evaluation of detection probabilities and spatial inference for eDNA can be challenging because it relies on detailed data about species presence, location and activity in conjunction with eDNA sample collection. Beaver translocations provide unique *in vivo* study systems where absence of beaver DNA can be confirmed prior to release and beaver movement can be carefully monitored. This creates a unique opportunity to spatially and temporally attribute patterns of eDNA detection to animal locations and habitat use in the new environment.

The aim of this study was to evaluate the efficacy and spatial inference associated with species-specific eDNA techniques for detecting and monitoring beaver presence in natural wetland and stream systems of Washington State. We conducted eDNA sampling paired with radio-tracking translocated beavers at four sites to elucidate spatial patterns of site use and eDNA detections. There were two main objectives of this study: First, we sought to establish whether species-specific eDNA sampling is a viable and reliable technique for monitoring beaver by comparing positive detections to known beaver presence at release sites; second, we sought to establish spatial relationships between beaver activity, eDNA detections and eDNA quantity at multiple spatial and temporal scales.

Materials and methods

We collected all data between October 2020 and October 2022 from four low order stream systems in the Washington Cascade Range (Fig. 1). Sites consisted of open-canopy riparian corridors or wetland meadows connected to low-order streams, ranging in elevation from 800 to 1100 m. All sites had comparable hydrology, consisting of 2nd to 3rd order streams, catchment area ranging from 9 to 27 km^2 and average stream velocity < 0.5 m/s (Appendix S1a). We selected study sites based on the Washington Department of Fish and Wildlife (WDFW) beaver relocation habitat suitability protocol (WDFW 2019). Active beavers were absent and natural colonization was unlikely to occur during the study period. We confirmed beaver absence by collecting eDNA samples that spanned ≥2 km of stream at release sites, no more than 2 weeks prior to release.

Beaver translocation and tracking

All beavers were captured as part of ongoing translocation projects under permits from WDFW (permit details in Appendix S1b). We fitted beavers with tail-mounted radio-transmitters (Advanced Telemetry Systems, Model ATS MH3500; Arjo *et al*., 2008; Windels & Belant, 2016; Appendix S1 Fig. S1). We released two to three tagged beavers at each site: one site in fall 2020 and three during the summer/fall of 2021. We relocated beaver using homing techniques with ≤10 m accuracy (White & Garrott, 1990; Neill & Jansen, 2014), except for some locations that we estimated from single azimuths collected perpendicular to stream systems, primarily at night (Doden *et al*., 2022). We located each beaver every hour throughout the first night, every day during the first week and once monthly after release. We expected beaver movement to be greatest in time periods closer to their initial release and to decrease over time; thus, we decreased tracking intensity over time. During nighttime tracking, we limited movements and remained >30 m away to reduce impacts on beaver activity. At all sites, we conducted additional telemetry surveys following the first winter (7–14 months) after release.

Environmental DNA sample collection

We collected eDNA samples at fixed locations across release sites at intervals matching different periods of radio-tracking

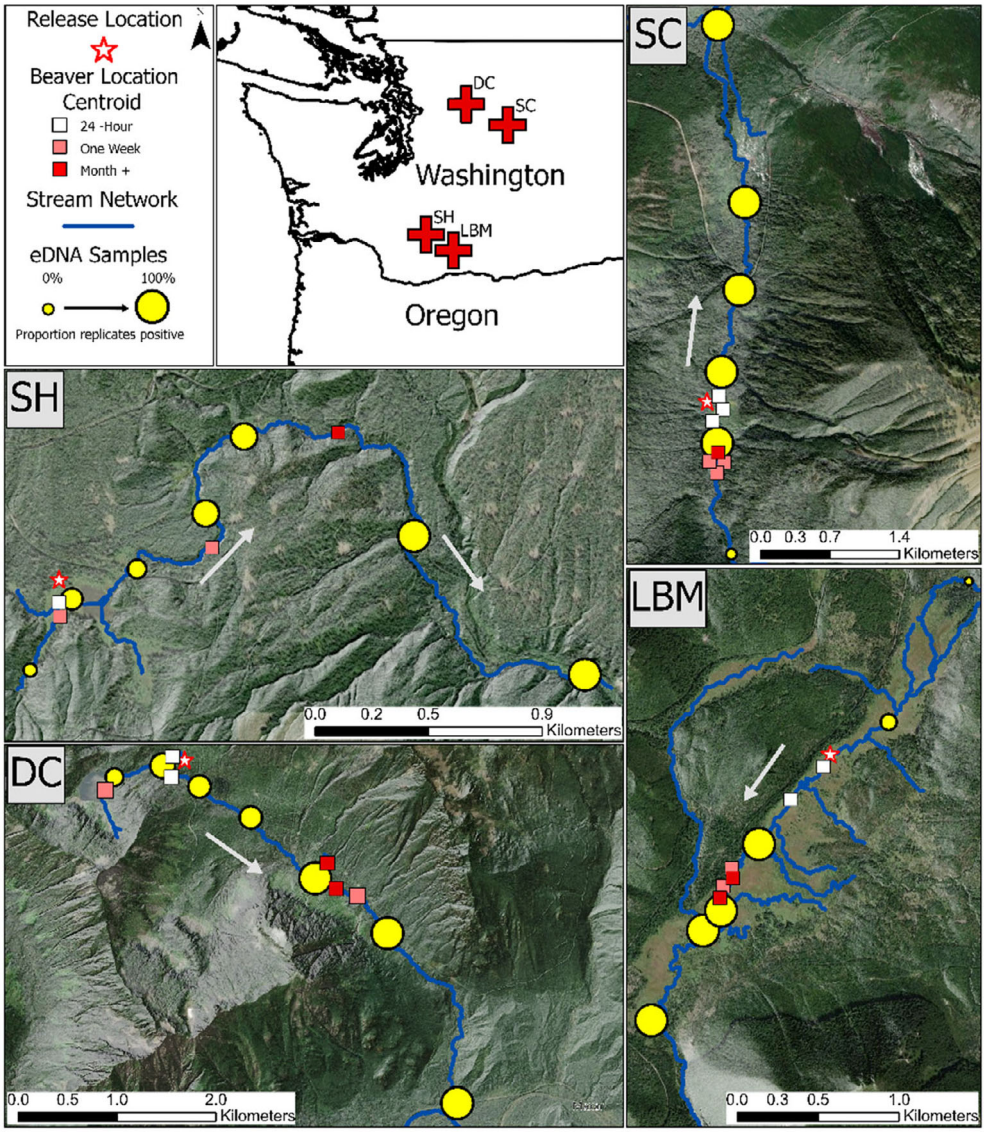

Figure 1 Study site locations, beaver locations and eDNA detections for beaver translocations in Washington State, USA, 2020–2022. Red stars on maps indicate initial release locations for beavers, squares indicate centroid locations for individual beavers for each tracking interval (24 h, first week and 1 month plus), yellow circles indicate eDNA sample locations, and circle size indicates the proportion of positive eDNA sample replicates. Blue lines are stream networks generated using DEMs, and gray arrows indicate the direction of flow in each system. Site abbreviations are as follows: SC = Snowy Creek, SH = South Helens, DC = Deer Creek and LBM = Lone Butte Meadows.

intensity. Full site eDNA sampling occurred prior to beaver release, 24 h after release (at 3 of 4 sites with sampling locations based on beaver's overnight movements), 1 week post-release, 1 month post-release and every month after that, until site access was no longer possible due to winter weather. Additionally, we collected eDNA samples during site visits conducted after the first winter since beaver release. Full site sampling designs spanned >1 km of habitat and included a minimum of five sampling locations, including samples at the upstream end of suitable beaver habitat, approximately 2 km downstream of release locations but upstream of major confluences and additional points in the main channel upstream of confluences with smaller tributaries (Fig. 1; more details in Appendix S1c). When beavers moved beyond fixed sampling designs, we opportunistically added sampling locations based on movements over time.

At each sampling location, we collected two eDNA replicates at the same location (thalweg of stream) in succession using a Smith-Root backpack eDNA sampler (Smith-Root Inc., Vancouver, WA, USA) and 5 μm polyethersulfone filters in sterile self-contained filter packs (Smith-Root Inc., Vancouver, WA, USA, Thomas *et al.*, 2018; Appendix S1 Fig. S2). We directly filtered 2 L of surface water and immediately preserved filters in the field using silica bead

desiccant (2–5 mm beads, Honeywell Fluka, Charlotte, NC, USA, Yamanaka *et al.*, 2016; Thomas *et al.*, 2018; Thomas *et al.*, 2019; details in Appendix S1d). Prior to each sampling occasion, we collected field negative samples by filtering 1 L of distilled water from decontaminated containers. To minimize the risk of contamination, we implemented best practice precautions in the field (Goldberg *et al.*, 2016; details in Appendix S1d). Additionally, we measured environmental parameters (pH and water temperature) that may influence eDNA detection rates (Strickler, Fremier, & Goldberg, 2015; Tsuji *et al.*, 2017) immediately following sample collection.

Environmental DNA extraction and quantitative PCR analysis

We extracted DNA using the QIAshredder/Qiagen DNeasy Blood and Tissue DNA extraction method in a limited-access clean room within 6 months of sample collection (Qiagen, Germantown, MD, USA; Goldberg *et al.*, 2011). We used a species-specific probe-based qPCR assay for American beaver described in Smith & Goldberg (2022). Each reaction plate included a standard curve consisting of a serial dilution of DNA (10^{-3} – 10^{-6} X) extracted from a beaver tissue sample or a gBlock standard (IDT, Coralville, IA, USA). The limit of detection (LOD) and the limit of quantification (LOQ) for this assay are ≤5 copies and 6 DNA copies per reaction, respectively (Klymus *et al.*, 2020; details in Appendix S1e). We analyzed each sample in triplicate and considered it negative if no reactions amplified and positive if all technical replicates amplified. We retested samples with mixed results in triplicate and considered the sample positive if at least one replicate amplified on the second round of qPCR. We considered a sample inhibited if the internal control Cq value was >3 higher than those in the standard curve, and we cleaned and reran these samples (4/249 eDNA samples were inhibited; details in Appendix S1e). We conducted all DNA extraction and qPCR using best practices for preventing and detecting contamination and included field collected equipment negatives, DNA extraction negatives and PCR negatives (Goldberg *et al.*, 2016).

Spatial predictors and movement analysis

We used ArcGIS Pro 2.9.1 (ESRI, Redlands, CA, USA) to create detailed linear systems of the aquatic habitat at each study site (details in Appendix S1f) and combined these with beaver locations and eDNA sampling locations to characterize spatial relationships overtime using the *riverdist* package (v0.15.5; Tyers, 2022) in R (v4.1.2; R Core Team, 2022). For beaver movement, we used the release location as the origin point and calculated movement distances with upstream locations assigned positive values and downstream locations assigned negative values. To develop spatial predictor variables for eDNA models, we quantified distances between each eDNA sampling location and each beaver location for each radio-tracking day prior to eDNA sample collection. For these calculations, we used eDNA sampling locations as the origin.

Beaver activity and eDNA analyses

We evaluated relationships between beaver activity and eDNA detection (using generalized linear mixed models; GLMMs) and eDNA quantity (using linear mixed models; LMMs). All models were fit using random intercepts for each site (4 levels) to account for variation between release sites. We inspected residual and Q-Q plots for violations of model assumptions and tested hypotheses using likelihood ratio tests (for GLMMs) and conditional F-tests with Satterthwaite estimations for denominator degrees of freedom (for LMMs) with a cutoff value of $\alpha = 0.05$. Predictor variable correlation coefficients were < 0.6 in all models. Water temperature and pH were not included in any models as they were not informative (details in Appendix S1g).

We used data collected during the first week (short-term data) at each site to evaluate the spatial relationship between beaver activity and eDNA detection rates and quantity. To assess eDNA detection rates, we fit binomial generalized linear mixed models using the *lme4* package in R (v1.1–28; Bates *et al.*, 2015) with the number of positive detections in two eDNA sample replicates as the response variable. Predictor variables included the average distance between an eDNA sampling location and beaver locations, the number of days since a beaver was upstream of the sampling location and the total biomass (kg) of beavers released. To assess beaver site use influence on eDNA quantity, we fit linear mixed models using the *lme4* package in R (v1.1–28; Bates *et al.*, 2015). We averaged DNA quantity across replicate samples collected at the same location and used the $\log_{10}$ transformed number of DNA copies per liter plus 1 (to avoid 0 s) as a response variable. For DNA quantity analyses, we used a subset of the total data that only included eDNA samples collected downstream of beaver locations (DNA quantity calculation details in Appendix S1h). Predictor variables for this model included the average distance from upstream beaver locations, the total biomass (kg) of beavers released and the amount of upstream beaver activity during the first week after release. We calculated upstream activity as the number of beaver locations that occurred upstream of an eDNA sampling location and divided by the total radio-tracking days during that week.

We used data collected 1 month or longer after beavers were released (long-term data) to further evaluate spatial relationships between beaver activity and eDNA detection rates and quantity over longer time periods. Model structures and assessment were largely the same as the short-term analyses with the following exceptions. For long-term eDNA detection rates, we used a single binary fixed predictor variable – whether beaver location was upstream or downstream from an eDNA sampling location at the time of sample collection – because the long-term data did not capture spatially explicit movement. For long-term analyses of eDNA quantity, we used the minimum upstream distance to a beaver and upstream beaver biomass (kg) at the time of eDNA sample collection as predictor variables and included sampling occasion as a random effect (3 levels) to account for non-independence of samples collected within the same survey. For long-term detection and quantity

analyses, we also used site-specific sampling location as an additional random effect (30 and 17 levels, respectively) to account for non-independence of samples collected at the same location at different times.

Results

Beaver movement patterns

We collected 183 beaver locations from 10 beavers released at four sites (Table 1). Nine beavers remained within ~1500 m of release locations during the first week. Seven beavers remained within ~2500 m of release locations for the total radio-tracking period (25 to 83 days depending on the site and length of transmitter retention). Dam building occurred at one site (SC) during the study period, but beavers exhibited foraging and food caching behaviors and used bank dens at all sites. Two beaver transmitter signals were lost during the first 30 days, five transmitters became detached within the 83-day initial tracking period, and no transmitters remained active after the first winter. Average transmitter retention was 37.78 ± 2.96 (standard error) days (Table 2). There was no evidence of predation, and no beaver carcasses were recovered from release sites. A detailed site-by-site summary of telemetry results is available in Appendix S2.

Beaver activity and short-term eDNA dynamics

All sampling locations were negative for beaver DNA prior to release, and we detected beaver at all sites post-release. Of 202 eDNA samples collected after beaver release, 155 yielded detections. No DNA was amplified in field blanks, DNA extraction blanks or PCR negatives.

Twenty-four hours after release, we detected beavers at all three sampled sites and 87.5% (7/8) of sampling locations. Detection distances ranged from 60 to 2440 m downstream of beaver activity. Two of three sites were lotic habitats where beaver eDNA was detected in all samples and replicates, SC and DC. The third site, SH, featured a lentic, impounded wetland system where beaver activity was concentrated (see Appendix S2 Section S2b). Beaver eDNA was detected at the inflow of this pond and the adjacent connecting flow channel (within 60 meters of activity) but only one

Table 1 Summary of beaver radio-tracking data and eDNA samples collected from each of four release sites in the Washington Cascade Range, USA, 2020–2022

Radio-tracking summary					eDNA samples			
Site[a]	Number of beaver released	Number of beaver locations	Release date	Last location date	24 h	1 week	1–3 months	7–14 months[b]
LBM	2	23	2020-10-20	2020-12-29		12	18	2
SH	3	30	2021-07-19	2021-10-19	6	12	50	6
SC	3	87	2021-08-12	2021-11-01	4	10	36	6
DC	2	36	2021-10-08	2021-11-02	6	14	14	6

[a] Site name abbreviation: SH = South Helens, SC = Snowy Creek, DC = Deer Creek and LBM = Lone Butte Meadows.
[b] No active transmitters remained at the site when eDNA samples were collected.

Table 2 Summary of individual beaver life stages, release sites, period of radio tracking in days, average movement distance in meters and transmitter fate from four release sites in the Washington Cascade Range, USA, 2020–2021

Beaver ID	Life stage	Release site[a]	Tracking days[b]	24-h distance[c]	7-day distance	30-day distance	Transmitter fate[d]
21SH-U1	Kit	SH	13	N/A	50	N/A	DT
21SH-M1	Adult	SH	2	N/A	N/A	N/A	SL
21SH-F1	Yearling	SH	56	10.8	−902.5	−1334.3	DT
21SC-M1	Adult	SC	55	−42.6	366.6	359.5	DT
21SC-U1	Kit	SC	49	5.4	289.8	339.2	DT
21SC-F1	Adult	SC	83	14.7	383.6	365.2	ACT
DC-F1	Adult	DC	25	−82.6	−2531.9	−2504.3	ACT
DC-M1	Adult	DC	25	20.4	−629.5	−914.3	ACT
LBM-F1	Yearling	LBM	32	N/A	−1014	−1063	DT
LBM-M1	Yearling	LBM	18	N/A	−1202	N/A	SL

[a] Release site name abbreviation: SH = South Helens, SC = Snowy Creek, DC = Deer Creek and LBM = Lone Butte Meadows.
[b] Tracking days end with the last positive location for each individual.
[c] Distances are mean distance moved within the first 24 h, first week and first month of radio-tracking in meters. Negative values indicate downstream movements from the release site.
[d] Transmitter fate codes: SL = signal lost, DT = dropped transmitter and ACT = active at last visit to the release site before winter. For beavers that still had active transmitters at the beginning of winter, retention time was from release until the last location.

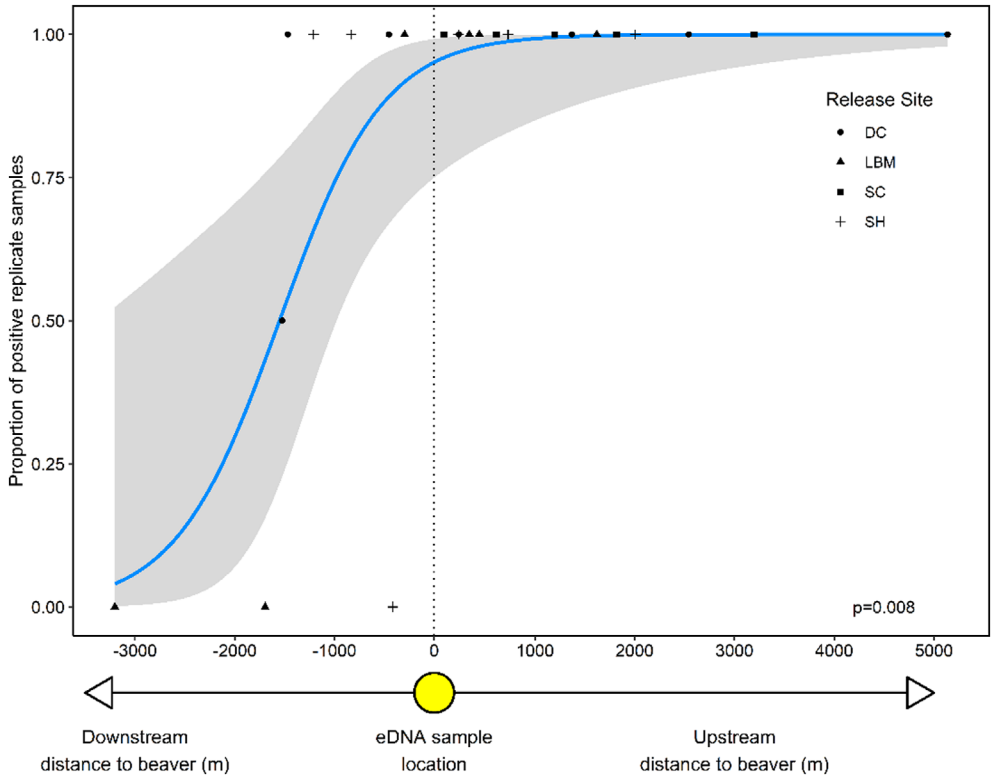

Figure 2 Relationship between beaver locations and eDNA detection 1 week after translocation. The estimated proportion of positive eDNA sample replicates explained by the average upstream or downstream distance between beaver telemetry locations and eDNA sampling locations during the first week after initial release across all four release sites. Negative numbers indicate beaver locations downstream of eDNA sampling points, and positive numbers indicate beaver locations upstream. Gray shading signifies Wald confidence intervals, and points represent raw data from eDNA samples.

of two replicate samples was positive in both cases. We did not detect beaver at the main outflow of the system, ~212 m from areas of overnight activity.

For short-term (1 week after release) eDNA samples, detection probability increased as the mean distance to beavers transitioned from negative (beaver locations downstream of eDNA sampling points) to positive (beaver locations upstream) ($P = 0.008$, $\chi^2 = 6.96$, $df = 1$; Fig. 2). There was no evidence that the time since a beaver was upstream of the sampling location or the biomass of beavers released at the site influenced eDNA detections ($P > 0.15$ in both cases). Short-term eDNA quantity increased with upstream activity ($P = 0.0055$, $F = 11.86$, $df = 1, 11$), with a 475.35% (95% profile confidence interval 131.56–1399.39%) increase in DNA copies per liter for each additional upstream location per tracking day (Fig. 3). There was no evidence that the average distance to upstream beavers nor the biomass of beavers influenced the quantity of DNA copies collected in eDNA samples ($p \geq 0.1$) in the short term.

Beaver activity and long-term eDNA dynamics

After the first week, we continued to detect beaver DNA at all sites in all sampling occasions. For samples collected 1–3 months after release (long-term), eDNA detection probability was >90% when a beaver was upstream (61/66 samples, model-predicted probability 0.99 with CI 0.775–1.0) and < 50% when beavers were not upstream (12/30 samples, model-predicted 0.22 with CI 0.024–0.765) (Fig. 4. $P < 0.001$, $\chi^2 = 15.299$, $df = 1$). The five negative samples collected downstream of beaver locations came from the lentic site, downstream of a single active kit in the wetland complex, but upstream of an active subadult female. Long-term eDNA quantity decreased with increasing distance between upstream beavers and eDNA sampling locations ($P = 0.01$, $F = 8.21$, $df = 1, 16.4$), with a 64.88% (95% profile confidence interval 25.89–83.00%) decrease in DNA copies captured in a liter of water for each additional 1000-m of stream distance (Fig. 5a). Additionally, eDNA quantity increased with increasing upstream beaver biomass in the long term ($P = 0.049$, $F = 9.23$, $df = 1, 3.30$), with a 213.18% (95% profile confidence interval 42.46–499.72%) increase in DNA copies captured in a liter of water for each additional 10 kg of upstream beaver biomass (Fig. 5b).

Beaver eDNA persistence

At four eDNA sampling locations, beavers were initially active upstream, moved downstream and did not appear to

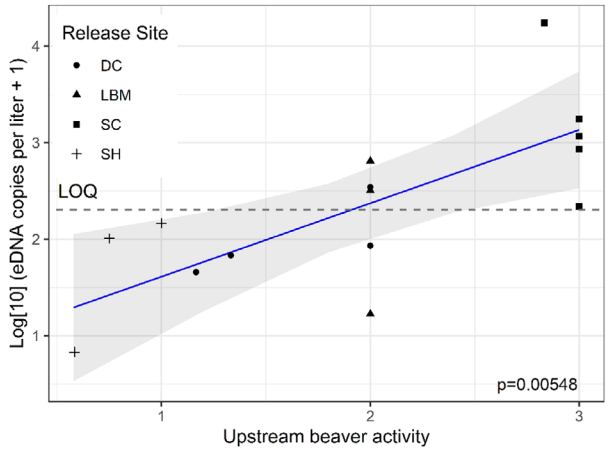

Figure 3 Relationship between beaver locations and eDNA quantity 1 week after translocation. Model-derived estimates for replicate averaged $\log_{10}$(eDNA copy number per liter +1) in eDNA samples for short-term models show DNA quantity was strongly impacted by the amount of upstream beaver activity during the first week, as measured by the number of upstream beaver locations normalized by the number of tracking days at each site. Blue lines show model estimates, gray shaded areas are 95% confidence intervals calculated with Kenward–Roger approximations, dashed lines indicate the limit of quantification for the assay used, and different point symbols are the raw data differentiated by each site.

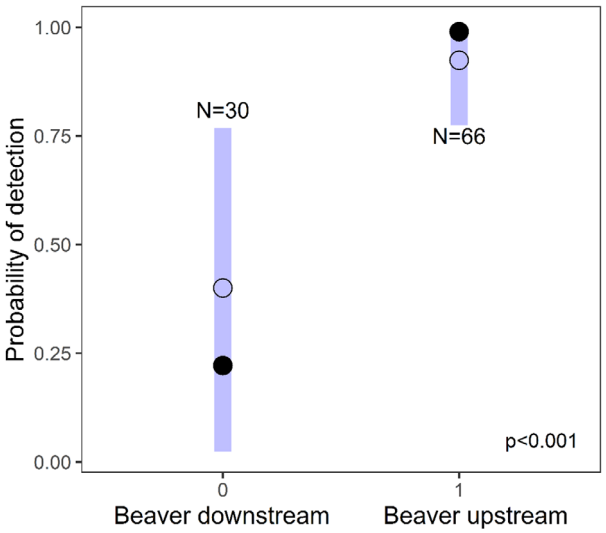

Figure 4 Relationship between relative beaver locations and eDNA detection 1–3 months after translocation. Model-derived estimates for the probability of detecting translocated beavers in eDNA samples collected 1–3 months after release, dependent on beaver location at the time of sample collection. Solid points represent estimated marginal means, open points are raw detection percentages, and shaded areas represent 95% confidence intervals.

return upstream during our study period. We collected samples at three of these locations 4 days after beavers had moved downstream and all eDNA replicates were positive. We collected additional samples at three locations (two the same, one new) 25–29 days after beavers left and detected beaver at two locations, though only 33% (2/6) of field replicates were positive and no location had >1 positive replicate.

We collected eDNA samples at all sites 7–14 months after release (5–11 months after last confirmed locations) when no transmitters remained active (either missing or emitting dropped signals). We continued to detect beaver at three of four sites in 55% (11/20) of all samples collected during this timeframe, though only one site had clear signs of beaver activity through winter (chewed sticks at heights requiring snowpack). Samples collected at the site with overwinter activity were all positive (6/6 sample replicates) with a mean eDNA quantity of 126.33 copies per liter SE 93.86. At the site where beavers constructed a dam and then apparently abandoned the site, samples collected within the dam complex and 1000 m downstream had low detection rates (1/4 sample replicates) with a mean eDNA quantity of 25.07 copies per liter SE 12.52.

Discussion

Robust methods that accurately detect beaver occupancy are critical for understanding contemporary beaver distributions and assessing outcomes of beaver translocations. We tested eDNA methods for detecting and monitoring beaver presence in low-order streams of the Washington Cascade Range, a focal region for beaver translocations. We detected beaver within 24 h of release and at distances up to 2930 m downstream of nearest known beaver locations. Detection probabilities were high (~99%) when eDNA samples were collected downstream of beaver activity at all time periods. In our study, eDNA sampling rapidly and consistently detected translocated beaver in montane stream systems at a variety of spatial and temporal scales.

Assessing eDNA transport distance from source organism locations is critical for understanding spatial inference from eDNA detections. Transport distances were consistently long in our system; 93.75% of samples collected >1 km downstream of beaver locations were positive. While eDNA detection over distances ≥7 km has been demonstrated (Deiner & Altermatt, 2014; Wood *et al.*, 2021), most studies (experimental and natural streams) have estimated transport distances of ≤1 km (Wilcox *et al.*, 2016; Shogren *et al.*, 2017; Fremier *et al.*, 2019), and a recent metanalysis suggests 2 km as a maximum eDNA transport distance in smaller lotic systems (Jo & Yamanaka, 2022). We hypothesize that beavers deposit relatively large amounts of DNA into the streams they inhabit which allows for detection over relatively long downstream distances because detectable levels of DNA can persist. Beavers deposit large volumes of fecal matter in aquatic habitats and actively engage with aquatic substrates through dam building, canal digging, food caching and other activities. Given the potential for beaver eDNA detections >2 km downstream of beaver locations, this tool may not be suitable for describing patterns of beaver occupancy and movement at finer spatial scales.

At the ~3 km stream reach scale, eDNA may reliably confirm presence of upstream beaver. We found positive

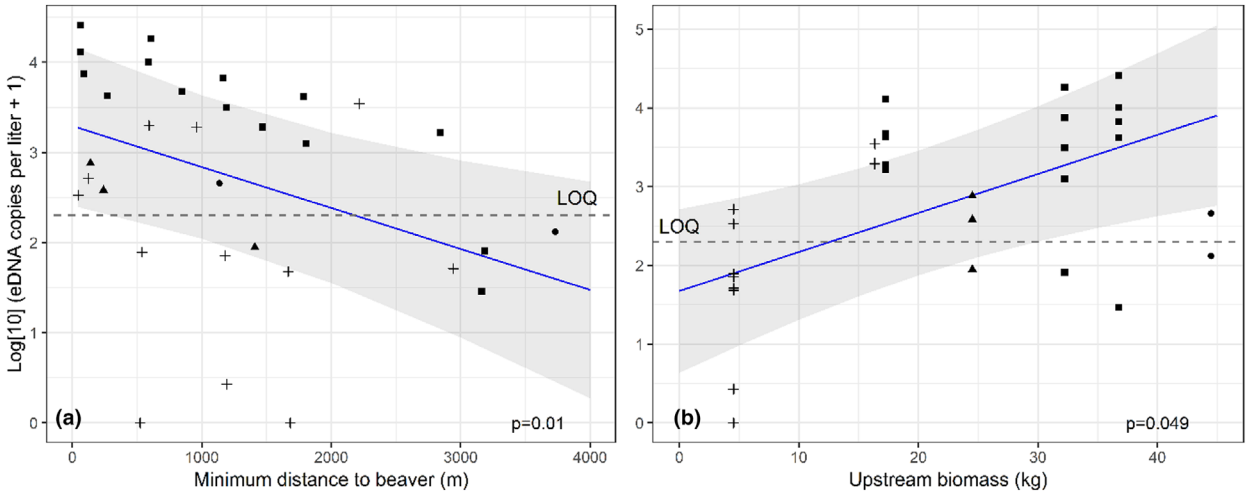

Figure 5 Relationship between beaver locations and eDNA quantity 1–3 months after translocation. Model-derived estimates for $\log_{10}$(eDNA copy number per liter +1) in eDNA samples for the long-term period show DNA quantity was strongly impacted by (a) the minimum upstream distance to beaver location at the time of sample collection and moderately impacted by (b) the total beaver biomass upstream at the time of sample collection. Blue lines show model estimates, gray shaded areas are 95% confidence intervals calculated with Kenward–Roger approximations, dashed lines indicate the limit of quantification for the assay used, and different point symbols are the raw data differentiated by each site.

detections in >90% of eDNA sample replicates collected downstream of beaver, regardless of the number of upstream individuals or distance. Detections dropped to ≤40% of sample replicates when beavers were downstream. Detections of downstream beavers may have occurred because of persistence of residual eDNA or could result from our relatively coarse long-term radio-tracking intervals failing to fully capture individual beaver movement.

Relationships between DNA quantity and locations of target organisms may increase spatial inference of eDNA detections (Shogren *et al.*, 2017; Wood *et al.*, 2020). Our quantitative PCR results demonstrate clear spatial relationships between DNA quantity and beaver locations 1–3 months after release. DNA quantity decreased with increasing distance between sampling locations and upstream beaver locations, with >50% eDNA loss for every 1000 m. Similar distance-eDNA quantity decreases have been demonstrated in other lotic systems where organism locations were known – highest quantities of DNA are generally captured ≤1000 m from target organisms (Robinson *et al.*, 2019: Wood *et al.*, 2020, 2021; alternatively, see Jane *et al.*, 2015). Similar to these studies, our DNA quantity estimates were variable across sites, samples and sampling occasions, but varied predictably with increasing stream distance from beavers, indicating DNA quantity may provide information about beaver position within a stream reach if multiple eDNA samples are collected along the stream.

Spatial patterns in eDNA detection and quantity have been used to provide insight into site use patterns in other systems (Eichmiller, Bajer, & Sorensen, 2014; Buxton *et al.*, 2017; Tillotson *et al.*, 2018; Robinson *et al.*, 2019). In our study, patterns of decreasing DNA quantity with increasing distance from beaver were most robust where beavers established dams, primarily SC (Fig. 5a.). Distance-decay patterns were not as obvious where beavers moved around more, and no site engineering occurred. This suggests resident beaver may produce a decaying pattern in DNA quantity with increasing distance from their territory, but transient beaver may not. Given the high detection rates of upstream animals and the distance-decay patterns in DNA quantity, eDNA samples collected using an interval design along stream reaches may provide relatively precise (~1 km) estimates of established beaver presence while balancing potential for detection of beaver from non-target areas due to long transport. Further research is needed to refine such a design, but interval sampling for eDNA is likely to increase the spatial inference of detections and has the potential to provide information about patterns of beaver activity in a watershed.

While we consistently detected beaver DNA over long distances in lotic systems, detection patterns in our sole lentic wetland site (SH) highlight the need to apply system-specific sampling designs. Consistent eDNA detection within lentic systems can be difficult due to patchy distributions of organisms, increased rates of eDNA degradation and limited DNA dispersion (Eichmiller *et al.*, 2014; Goldberg, Strickler, & Fremier, 2018; see review Harper *et al.*, 2019a). We did not detect beaver at distances >60 m from confirmed activity in a large lentic wetland system. This may indicate limited eDNA dispersion through the system, as beavers were observed using the entire release pond but were not detected in adjacent connecting channels. Furthermore, the SH site consistently had lower DNA quantities than other release sites, which may be partially explained by DNA depositing in this lentic wetland system instead of being transported downstream. While our lentic data are limited, it does support eDNA sample collection at closer spatial intervals

within sites characterized by slow flowing water and highlights the need to carefully design eDNA studies that include complex lentic–lotic habitats.

Our data indicate that beaver DNA remained detectable after beavers appear to have vacated a site. Beaver DNA was detected at some sites at least 25 days after beavers appeared to leave and we continued to detect beaver DNA 5–11 months after cessation of activity sign and transmitter activity at sites. Other reports of eDNA persistence times in aquatic systems vary widely and are drawn primarily from lentic systems or mesocosm studies, but generally range from 8 to 21 days after species removal (Dejean *et al*., 2011: Goldberg *et al*., 2013: Barnes *et al*., 2014; but see Balasingham, Walter, & Heath, 2017 for lotic systems). Beaver use sites intensively, depositing saliva while building lodges and dams and defecating in the water (in contrast to otters, which are difficult to detect with eDNA; Sales *et al*., 2020: Broadhurst *et al*., 2021), and detection patterns in our data suggest that beaver DNA persistence times may be longer than those for other species. We did see indications of reduced eDNA (fewer positive detections and lower quantity, Appendix S1i) at sites we presume were abandoned; however, given our uncertainty of true site abandonment, further research is needed to understand how long beaver DNA remains detectable after animals have left. The greatest monitoring utility of eDNA derives from the assumption that detections reflect contemporary presence of target organisms; thus, our data highlight the need for taxon-specific assessments of eDNA residence times for effective applications of environmental DNA.

The performance of VHF transmitters in our study was shorter (37.78 ± 2.96 days [s.e.]) than in a previous study in the Oregon Coast Range (60 ± 14 days [s.e.]; Petro *et al*., 2015) and 70% of individuals had unknown fates caused by transmitter loss and long-distance emigration, similar to other recent studies of translocated beaver (73%; Doden, 2021). After the first winter, no transmitters remained active and no obvious sign of beaver activity was observed, leaving occupancy status uncertain and subjective. Thus, our tracking data add additional evidence highlighting challenges in using VHF radio-telemetry to monitor beavers over long time periods and support the development of additional tools for monitoring and tracking beaver occupancy through time.

Management implications

The eDNA methods used in this study can detect beaver rapidly over long distances and consistently when they are upstream of sampling locations. Thus, eDNA may be a sensitive, efficient and minimally invasive way to monitor beaver occupancy. Additional spatial information may be obtained from DNA quantities, especially when samples are collected in interval designs along streams and through time. These methods may provide a useful tool for understanding beaver distributions, especially in remote areas or where land access is an issue. Furthermore, given the sensitivity of these methods, the absence of eDNA detections likely provides a relatively robust indication that there are no beavers in close proximity upstream of the sampling location. The eDNA methods detailed in this study may be useful for many applications, including the selection of translocation sites and understanding how beaver populations are distributed on the landscape, especially in areas where they are invasive. The assay used in this study has not been validated against tissue samples for *C. fiber* but *in silico* validation using the Primer-BLAST algorithm (Ye *et al*., 2012; Appendix S1j) indicates that it may distinguish the two species. Further *in vitro* and *in situ* validation is recommended (Goldberg *et al*., 2016) prior to using this assay in areas of where *C. canadensis* and *C. fiber* co-occur, such as parts of northern Europe. However, the spatiotemporal patterns of eDNA detections in this study should apply well to either species. Finally, to our knowledge, our study provides the most detailed comparison of semi-aquatic mammal site use and eDNA detection dynamics to date. Our findings highlight important details about eDNA transport distances and potential eDNA persistence that may have implications for using eDNA for other semi-aquatic mammals that share similar life histories as beaver.

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Author contributions

JASB, *CSG, JPS and SG* conceived idea and designed methodology; *JASB* collected and analyzed the data; *ACD* analyzed the eDNA samples; and *JASB* led writing of the paper. All authors contributed critically to drafts and gave final approval for publication.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1.

Figure S1. VHF radio transmitter attached to a beaver during translocation.

Figure S2. eDNA sample collection using the Smith Root eDNA Backpack Sampler.

Appendix S2. Beaver translocation radio-tracking and eDNA study site descriptions.

Figure S1. Beaver translocation and eDNA sampling at Lone Butte Meadows. Data were gathered in fall and winter of 2020. This system flows to the south west. (a) Data from the first week of this release. (b) Data from the entire first month of this release. Beaver locations that overlap are summed for viewing.

Figure S2. Beaver translocation and eDNA sampling at the South Helens site. Data were gathered in summer and fall of 2021. This system flows to the northeast. (a) A zoomed in section of the release site and within site eDNA sampling locations. (b) Data covering the entire South Helens stream system inclusive of all beaver locations and across-system eDNA sampling locations. Beaver locations that overlap are summed for viewing.

Figure S3. Beaver translocation and eDNA sampling at the Snowy Creek site. Data were gathered in summer and fall of 2021. This system flows to the north. Beaver locations that overlap are summed for viewing.

Figure S4. Beaver translocation and eDNA sampling at the Deer Creek site. Data were gathered in summer and fall of 2021. This system flows to the east southeast. Beaver locations that overlap are summed for viewing.