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Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods

Jared Hobbs[1] | Ian T. Adams[2,3] | Jessica M. Round[4] | Caren S. Goldberg[5] | Michael J. Allison[4] | Lauren C. Bergman[4] | Ali Mirabzadeh[6] | Heather Allen[6] | Caren C. Helbing[4]

[1]Hemmera Envirochem Inc., Victoria, BC, Canada

[2]Larix Ecological Consulting, Cranbrook, BC, Canada

[3]VAST Resource Solutions, Cranbrook, BC, Canada

[4]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada

[5]School of the Environment, Washington State University, Pullman, WA, USA

[6]Bureau Veritas Laboratories, Guelph, ON, Canada

Correspondence
Caren C. Helbing, Department of Biochemistry and Microbiology, University of Victoria, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada.
Email chelbing@uvic.ca

Present address
Jared Hobbs, J Hobbs Ecological Consulting Ltd, Pender Island, BC, Canada

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Abstract

The Rocky Mountain tailed frog, *Ascaphus montanus*, is a species at-risk in Canada. Based upon time- and area-constrained physical search surveys completed between 1996 and 2004, its Canadian distribution was defined as occurring in 19 tributaries and reaches within the Yahk and west side Flathead River Basins of British Columbia. We undertook a five-year (2014–2018 inclusive) environmental DNA (eDNA) survey to reassess the distribution of Rocky Mountain tailed frog, focusing on tributaries proximal to known extant occurrence records. Seventeen days of field sampling were performed over the five-year period. Targeted qPCR-based eDNA approaches proved more effective than conventional physical search methods for detecting tailed frogs due to relatively rapid field collection, low cost of filter materials, elimination of observer bias, and higher detection probabilities compared to conventional time-constrained survey methods. One hundred and forty sites were examined (138 for eDNA plus two visual only). Thirty-two of the 138 sites (23%) tested positive for Rocky Mountain tailed frog DNA, including from the four extant populations sampled, whereas visual observations occurred at only seven of the sites (5%) during the survey. During the study, we evaluated two tailed frog tests and the mitigation of false negatives through testing for qPCR inhibition and sample degradation, and we demonstrate their utility in evaluating eDNA data quality. These results expand the extant range of Rocky Mountain tailed frog in the Flathead, Wigwam, and Yahk watersheds and add two new watersheds (Moyie and Tepee) by identifying five newly recorded occupied drainages in Canada: Elder Creek, Upper Wigwam River, Tepee Creek, Gilnockie Creek, and Elmer Creek. These data are important to refine and augment wildlife habitat conservation areas for Rocky Mountain tailed frog.

KEYWORDS

Ascaphus montanus, endangered amphibian species, environmental DNA, nonlethal sampling, population distribution, robust methods development, Rocky Mountain tailed frog, time-constrained sampling method

1 | INTRODUCTION

Tailed frogs are a distinct and ancient lineage of frogs associated with mid- to high-elevation mountain streams (Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 2013). There are two tailed frog species in British Columbia (BC), Canada: the coastal tailed frog (*Ascaphus truei*) and the Rocky Mountain tailed frog (*Ascaphus montanus*).

The coastal tailed frog has a wide distribution west of the Coast Mountain Ranges extending north almost to the Alaskan Panhandle. Recent work using environmental DNA (eDNA) survey techniques has expanded the known distribution range in the southern Coastal Mountains (Hobbs, Round, Allison, & Helbing, 2019). Prior to 2014, the Rocky Mountain tailed frog was broadly accepted to be in two geographically disparate populations (Flathead and Yahk) in BC (COSEWIC, 2013; Dupuis & Friele, 2006a; Green, Weir, Casper, & Lannoo, 2013; Matsuda, Green, & Gregory, 2006). These populations were discovered as a result of an extensive inventory effort, which occurred in the late 1990s and early 2000s using time- and area-constrained physical search survey methods (Dupuis & Bunnell, 1997; Dupuis & Friele, 2004a, 2004b, 2006b; Dupuis & Wilson, 1999).

The Rocky Mountain tailed frog (Figure 1) is listed as at-risk in Canada due to a restricted range, low number of known occurrence records, low population size, geographic isolation, and ongoing threats from stream sedimentation due to resource development activities (including road building and logging) and wildfires (BC Ministry of Environment, 2014; COSEWIC, 2013). Both watersheds with known Rocky Mountain tailed frog populations have been extensively altered by previous and ongoing forestry practices and, to a lesser extent, by recent fire disturbance (Dupuis & Friele, 2005). As a result, the Rocky Mountain tailed frog is provincially blue-listed by the BC Conservation Data Centre (2018) and is listed on the Category of "Species at Risk" under BC's *Forests and Range Practices Act* (FRPA). The species is also designated as Threatened by both COSEWIC (2013) and Schedule 1 of the federal *Species at Risk Act* (SARA). Natural processes in interior stream systems (e.g., debris torrents and sediment floods) may also contribute to local extinctions (Lamberti, Gregory, Ashkenas, Wildman, & Moore, 1991).

As Rocky Mountain tailed frogs are listed as Species at Risk in BC, Wildlife Habitat Areas (WHAs) have been established along streams with confirmed tailed frog occurrence records. WHAs are area-based legal designations intended to provide species-specific management to conserve habitat values at known extant or previously occupied sites (BC Ministry of Environment, 2014). Within designated WHAs, general wildlife measures are mandated to promote conservation of biodiversity values. For tailed frogs, these measures typically designate no-timber-harvesting zones of 30 m on both sides of the streams and an additional 20 m zone of managed forest, where harvest is permitted, adjacent to the 30 m forest reserve zone.

Based upon previously collected data from time- and area-constrained physical search surveys (Dupuis & Bunnell, 1997; Dupuis & Friele, 2004a, 2004b, 2006b; Dupuis & Wilson, 1999), nineteen WHAs were legally designated at all known Rocky Mountain tailed frog occurrence records in the Kootenay Region (ten in the Flathead, nine in the Yahk). Each WHA is centered on known tailed frog core breeding reaches observed prior to 2005. A federal recovery strategy identified Critical Habitat under Canada's federal *Species at Risk Act* in a more precautionary fashion than the provincial WHAs (Environment Canada, 2015); Critical Habitat under federal definition also includes headwater streams and (where appropriate) adjacent streams within previously known drainages to reflect the uncertainty regarding the precise distribution of Rocky Mountain tailed frogs in each drainage.

Additional time-constrained visual detections collected by the Montana Department of Fish, Wildlife, and Parks during electro-fishing studies conducted between 2008 and 2012 documented Rocky Mountain tailed frog in Canadian reaches of the Flathead River, indicating that this tailed frog may be more widely distributed in BC than previously thought (Hobbs, Vincer, Adams, & Goldberg, 2015). We conducted field surveys paired with recently-developed eDNA methods for Rocky Mountain tailed frog detection (Pilliod, Goldberg, Arkle, & Waits, 2013; Veldhoen et al., 2016) to better understand the range of this at-risk species in BC.

Environmental DNA is any trace material that includes DNA released by an organism into the environment (Herder et al., 2014; Murray & Flores, 2013). The reliable detection of aquatic vertebrate species, including tailed frog, using eDNA methods, has been confirmed prior to the present study (Hobbs et al., 2019; Rees et al., 2014). Ex situ testing for the presence of a species' DNA using quantitative polymerase chain reaction (qPCR) methods requires development and validation of species-specific primers and probes that target a small section of the target species' mitochondrial DNA (Goldberg et al., 2016).

The present study sought to more accurately characterize the known distribution of the Rocky Mountain tailed frog by taking advantage of noninvasive, cost- and effort-effective eDNA methods to survey potential inhabited streams throughout southeastern BC. Any newly identified inhabited stream reaches were recommended for additional designation as WHAs and for future mapping of Critical Habitat to further conserve Rocky Mountain tailed frog habitat in Canada.

2 | MATERIALS AND METHODS

2.1 | Study area and sample site selection

The study area encompassed the known and potential range of Rocky Mountain tailed frog in BC, including streams in the Flathead, Wigwam, Yahk, Kootenay, Tepee, and Moyie watersheds. Sample sites were selected based on the consideration of several criteria: results from previous time-constrained searches and habitat suitability surveys, tailed frog observations from Lincoln County, Montana (Montana Natural Heritage Program, unpublished data) and Boundary County, Idaho (Idaho Natural Heritage Program,

FIGURE 1 (a) Rocky Mountain tailed frog eggs are adhered to the underside of large rocks in stream pools. (b-c) Their tadpoles have an adhesive oral disk, or mouth, to attach to rocks in stream habitats. (d) The 'tail' is visible on the adult male (white arrow) (Photo credit: J Hobbs)

unpublished data), and consideration of tailed frog ecology, DNA transport, and habitat suitability in tandem with open-source satellite imagery to determine accessible stream reaches. COSEWIC (2013) and BC Ministry of Environment (2014) both mentioned that Rocky Mountain tailed frog had been reported in Elder Creek but the occurrence was considered "unconfirmed" by both reports. This was initially reported to one of the present study coauthors by Montana Fish, Wildlife & Parks staff in 2013. The Flathead River watershed (Figure 2) is a large, broad valley in the extreme southeast corner of BC with no permanent human population. Forested habitats are characterized as a dry, cold Montane Spruce variant according to BC's Biogeoclimatic Ecosystem Classification (BEC) system (Meidinger & Pojar, 1991). Above 1,400–1,500 m elevation, this area transitions into the Kootenay dry, cold Engelmann Spruce Subalpine Fir variant (See Dupuis and Friele (2005) for a more extensive description of the Flathead study area). The Wigwam River (Figure 2) flows north from its headwaters in Montana and includes Weasel Creek as a headwater tributary (originating from its headwaters in Montana). Ram (Bighorn) and Lodgepole creeks contribute large inputs before the Wigwam River drains into the Elk River south of Elko, BC. The Elk River drains into the Kootenay River watershed (Figure 2) at Lake Koocanusa above Libby Dam. The headwaters of Wigwam River in Kootenai National Forest were affected by a large wildfire in 2005.

The Yahk (including Gilnockie Creek, a Yahk River tributary), Tepee and Moyie (including Elmer Creek, a Moyie River tributary) River watersheds (Figure 3) are in the McGillivray Ranges of the Columbia Mountains south of Cranbrook, BC. The McGillivray Range is typified by relatively lower mountain peaks with little to no alpine habitat. These drainages are more ecologically diverse than the Border ranges. Both the Yahk and Moyie rivers drain into the Kootenay River below Libby Dam and Kootenai Falls in northwest Montana. Tepee Creek drains into the Kootenay River watershed at Lake Koocanusa above Libby Dam. Forests in these three watersheds are characterized as having a diverse mix of conifers, and the area has an extensive history of forestry as well as fire, insect, and windthrow disturbances.

2.2 | Field sample collection and filtration

Duplicate 1 L water samples were collected at each site between July 2014 and September 2018. Sites near a stream confluence were sampled upstream of the confluence to eliminate ambiguity regarding eDNA source. Polypropylene Nalgene sample bottles were prepared by rinsing them in 50% (v/v) fresh bleach solution (Javex 12 Bleach by Clorox—10.3% sodium hypochlorite by weight) and labeled with the site name and sample replicate identifier, Universal Transverse Mercator (UTM) coordinate, date, collection time, and name of the collector. The field sampling crew wore clean nitrile gloves to triple rinse the sample bottles with stream water to

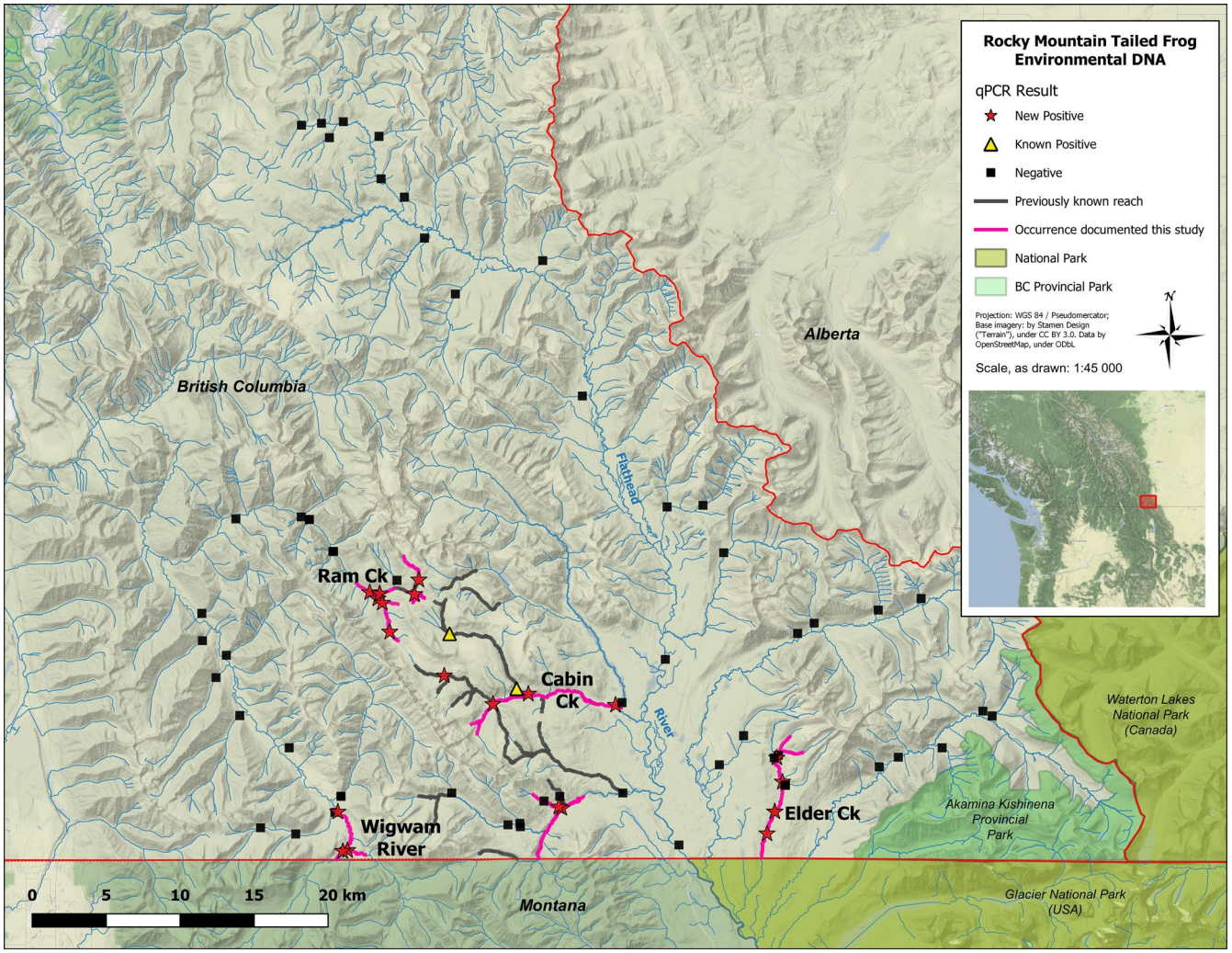

FIGURE 2 eDNA analyses expand the known range of Rocky Mountain tailed frog in BC to the east of the Koocanusa Reservoir including the Flathead (Elder Creek, Cabin Creek) and Wigwam (Ram Creek, Wigwam River) drainages. Areas with previously known occurrences are indicated by the dark gray lines. eDNA test site results are indicated by a red star (new positive), yellow triangle (known positive), or black square (negative). The new drainages documented with Rocky Mountain tailed frog occurrences are indicated by the fuschia lines. Map source: OGC web map service https://www.opengeospatial.org/standards/wms

remove any residual bleach. Each bottle was then filled with surface water as close to the thalweg of the stream as possible. Thalwegs may concentrate particulate matter, including DNA, into a narrow stream channel, thereby theoretically raising the probability of a positive test if the targeted species is present (Pilliod et al., 2013). A field negative control in which 1 L of distilled water was filtered as above was taken at the conclusion of each day of sampling. The field sampling crew recorded a UTM coordinate using an iPad and/or hand-held Garmin Map60CSX GPS unit set to collect in NAD 83 datum and collected pertinent habitat data with an iPad Air 128GB V4 iPad. Once stream water has been collected, nucleases may accelerate the degradation of DNA in the sample water if exposed to elevated temperatures and/or ultraviolet rays (e.g., sunlight) (Pilliod, Goldberg, Arkle, & Waits, 2014). To limit DNA degradation prior to off-site filtration and preservation, collected samples were placed in an insulated cooler in direct contact with crushed ice during field collection.

Samples were processed following an established eDNA protocol (Hobbs et al., 2015). Samples were stored at approximately 4°C during holding for filtering and processed within 24 hr in the same order as they were collected. This is also recommended to mitigate degradation of DNA (Pilliod et al., 2013). Samples were poured into a 250-ml single-use polypropylene filter funnel with a 0.45 µm pore size cellulose nitrate membrane. The sample was filtered through the membrane using a 115-volt alternating current Masterflex L/S Economy variable speed drive motor (Year 1) and a GAST Vacuum/pressure diaphragm pump (Year 2–5) to create a vacuum. When the entire 1L sample had passed through the filter, the filter membrane was removed using tweezers sterilized in a 50% bleach solution (immediately before use) and subsequently triple rinsed in distilled water. Filters were then placed in a 2 ml sterile polypropylene cryogenic vial filled with 95% molecular grade ethanol (Fisher Scientific; Years 1–3) or placed in a coin envelope in a sealing bag with blue self-indicating silica bead

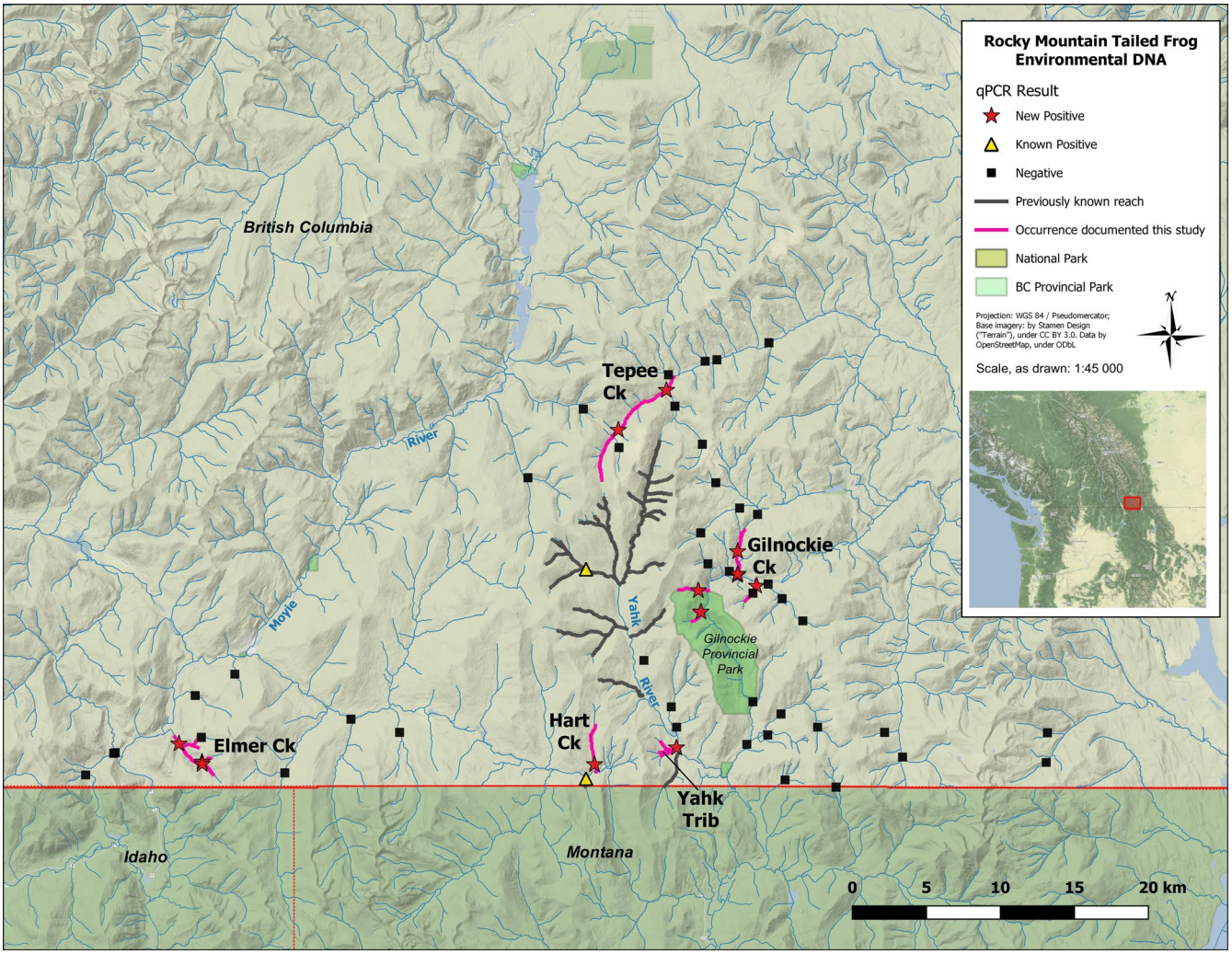

FIGURE 3 eDNA analyses expand the known range of Rocky Mountain tailed frog in BC to the west of the Koocanusa Reservoir including the Yahk (Gilnockie Creek, Hart Creek, and Yahk Trib), Tepee (Tepee Creek), and Moyie (Elmer Creek) watersheds. Areas with previously known occurrences are indicated by the dark gray lines. eDNA test site results are indicated by a red star (new positive), yellow triangle (known positive), or black square (negative). The new drainages documented with Rocky Mountain tailed frog occurrences are indicated by the fuschia lines. Map source: OGC web map service https://www.opengeospatial.org/standards/wms

desiccant (Dry & Dry (Silica gel Factory) via Amazon.ca; Years 4–5) (Hobbs et al., 2015, 2019).

Because stream levels were elevated due to rainfall immediately prior to collection of the water samples from the Gilnockie 7 site in 2018, three 1 L samples were filtered through one filter to concentrate any DNA material in an attempt to increase the likelihood of detection of tailed frog eDNA.

2.3 | Isolation of DNA from the filter membrane

DNA was isolated from the preserved filters using the Qiagen DNeasy Blood and Tissue DNA extraction kit in conjunction with the Qiagen Qiashredder as described previously (Goldberg, Pilliod, Arkle, & Waits, 2011). In Years 2–5, filter samples were randomized before processing and analysis to reduce technical bias as per recommendations outlined in Hobbs et al. (2019).

2.4 | eDNA assay setup and data analysis

2.4.1 | Year 1

Sample testing and data analysis followed the protocol outlined in Pilliod et al. (2013) using an eDNA test designed to be specific for tailed frogs. This test was originally designed to target both coastal (*Ascaphus truei*) and Rocky Mountain tailed frog species (*Ascaphus montanus*), but further characterization revealed that it does not amplify all haplotypes of coastal tailed frog. Each isolated DNA sample was assessed using three technical qPCR replicates with an exogenous internal positive control (TaqMan™ Exogenous Internal Positive Control, Catalogue #4308323, Thermo Fisher Scientific) to detect PCR inhibition. No samples were inhibited in this project year. When triplicate wells did not test consistently (i.e., one or two samples tested positive), the sample was rerun in triplicate to confirm the result. All three wells testing positive in the original run or at

least one in each independent run testing positive were considered as a positive result. A standard curve consisting of gDNA extracted from a tissue sample diluted 10^{-3} through 10^{-6} in duplicate and a negative no-template PCR control (NTC) were run on each plate.

2.4.2 | Years 2–5

In these years, an IntegritE-DNA™ test was applied to each sample preceding qPCR evaluation for eDNA from the focal taxa (i.e., tailed frog) (Hobbs et al., 2019; Veldhoen et al., 2016). The IntegritE-DNA™ assay simultaneously tests for sample inhibition and degradation and is an effective means for mitigating false-negative eDNA results (Hobbs et al., 2019). The IntegritE-DNA™ test is based upon the detection of endogenous plant/algae DNA in each sample and is used to assess sample quality (Hobbs et al., 2019; Veldhoen et al., 2016). In Year 2, each sample was tested with eight technical replicates using the ePlant5 primer/probe set. In subsequent study years, the number of technical replicates for the IntegritE-DNA™ test was reduced to four to increase cost savings and streamline data generation based on the consistency and strengths of positive signals above background. Typical site samples produced C_t values of 22.66 ± 0.05 (n = 964 technical replicates) over the course of the multi-year project while NTCs consistently had C_t values of 37.46 ± 0.40 (n = 82 technical replicates) that were clearly distinguishable from the site samples (Table S1). Forty distilled water field negative controls produced a C_t = 31.81 ± 0.26. We used a C_t cutoff of <30 to indicate a positive hit to trigger further processing using the Zymo OneStep™ PCR Inhibitor Removal Kit (Cedarlane) and retesting as described previously (Hobbs et al., 2019; Veldhoen et al., 2016). If the sample failed the IntegritE-DNA™ retest, then that sample failed quality control due to persistent inhibitor presence or degradation and was not deemed a reliable sample for tailed frog eDNA assessment (Hobbs et al., 2019; Veldhoen et al., 2016).

A new Rocky Mountain tailed frog primer/probe set (eASMO9) was designed and validated in Veldhoen et al. (2016). This eASMO9 eDNA test does not amplify coastal tailed frog DNA using reaction conditions outlined previously (Veldhoen et al., 2016). All primers and the probe containing a 5'FAM reporter dye and 3'ZEN/Iowa Black FQ quencher were ordered from Integrated DNA Technologies (IDT).

The eASMO9 primer/probe set sensitivity was further empirically established using a five-fold serial dilution of a 176 bp double-stranded synthetic DNA fragment corresponding to the target mitochondrial *cytochrome b* DNA sequence (Table S2) and measuring the C_t values obtained using the method outlined in Hobbs et al. (2019) (Figure S1A). The modeled and discrete limit of detection (LOD) as defined by Klymus et al. (2019) was 5.74 and 20 copies/reaction, respectively. The modeled and discrete limit of quantitation (LOQ) was 50 and 100 copies/reaction, respectively (Klymus et al., 2019). The highest binomial error was between 0.16 and 4 copies per reaction (Figure S1B). At n = 3 technical replicates, the highest percent binomial standard error was 28.8%. This was reduced to 17.7% at n = 8 and 10.2% at n = 24 (Figure S1B).

Samples were run in eight technical replicates as a reasonable compromise between detection sensitivity and cost (Hobbs et al., 2019; Veldhoen et al., 2016). Each plate also included two positive (synthetic DNA at 20 copies per reaction) and eight negative (NTC) PCR controls. Throughout the course of the project including validation and inter-laboratory comparisions (see below), a total of 233 NTC and 40 distilled water field negative control reactions were run with no amplification indicating that the background noise for the eASMO9 test was zero. This type of background was routinely possible through the implementation of careful field sample collection and handling techniques, filter processing in a laminar flow hood with a HEPA filter, bleaching of work surfaces and forceps, the use of dedicated electronic pipettors with filter tips and careful pipetting technique, and physical separation of amplified samples from qPCR setup areas. Further confidence in qPCR results was obtained through sample randomization (Hobbs et al., 2019). A sample was scored as positive if at least one replicate (1/8) produced a C_t value below 50 following the refinements presented in Hobbs et al. (2019).

An inter-laboratory comparison of results starting from portions of the same 2018 filters was performed between the University of Victoria (UVic) and Bureau Veritas Laboratories (BVL). Participating laboratories used the IntegritE-DNA™ and eASMO9 tests as per the described protocols above.

3 | RESULTS

3.1 | eDNA sample quality assessment

In the entire set of samples, only six site samples failed the initial IntegritE-DNA™ test. One sample from the Flathead watershed failed the subsequent IntegritE-DNA™ retest after inhibitor cleanup was performed (i.e., Couldrey Trib 5; Table S3) indicating that this sample was not reliable for subsequent tailed frog eDNA assessment. The remaining five samples, all from the Yahk watershed in the Gilnockie drainage, passed the IntegritE-DNA™ test after inhibitor cleanup suggesting the presence of inhibiting contaminants in the initial DNA preparations (Table S3).

3.2 | Project overview

Overall, 140 sites in the Flathead, Wigwam (Figure 2), Tepee, Moyie, Kootenay, and Yahk (Figure 3) watersheds were tested over 17 days of sampling between July 2014 and September 2018 (Table 1). Thirty-two

TABLE 1 Project overview

Year	Number of sampling days	Number of sites examined	Number of new occurrences
2014	4	49	9
2015	4	30	9
2016	3	30	4
2017	4	22	7
2018	2	9	3
Total	17	140	32

new occurrences of Rocky Mountain tailed frog were recorded (Table 1). A detailed classification of all eDNA results by watershed, sub-watershed, site, and collection date/time is provided in Table S3.

3.3 | Tailed frog occurrence in the Flathead and Wigwam watersheds

Forty-nine sites were examined in the Flathead watershed between 2014 and 2015 (Table 2). Two distinct drainages, Elder and Cabin Creeks, where tailed frog had not previously been reported returned positive results (Table 2; Figure 2). These findings were confirmed by eDNA samples from subsequent years and through visual confirmation in both drainages (Table 2). Both positive field control sites (i.e., water collected from known extant sites; Storm 1 & 2) returned positive results (Table 2).

Twenty-seven sites were examined in the Wigwam watershed between 2014 and 2016 between two distinct drainages: Ram Creek and Upper Wigwam (Table S3). Rocky Mountain tailed frog had not previously been reported from the Upper Wigwam, and tailed frog occurrence was greatly expanded in the Ram Creek drainage (Table 2; Figure 2). These findings were also confirmed using eDNA in subsequent years and through visual detection (Table 2). One negative site at the mouth of Desolation Creek was tested in two successive years with the same result confirmed (Desolation 1; Table S3).

3.4 | Tailed frog occurrence in the Yahk watershed and adjacent Kootenay, Moyie, and Tepee drainages

In the third to fifth years of the study, the focus of the surveys shifted toward inventory of streams adjacent to or connected to extant sites reported for the Yahk watershed and adjacent Kootenay, Moyie, and Tepee drainages. Four sites in the Kootenay watershed were all negative

TABLE 2 Mode of Rocky Mountain tailed frog detection and type of occurrence at the indicated sites in the Flathead and Wigwam watersheds. These sites along with additional sites where tailed frog was not detected are indicated in Figure 2

Watershed	Site Name	Date	Tailed frog detection[a]	Occurrence[b]
Flathead	Elder 1	08/25/2014	eDNA + Visual	New
	Elder 2	08/25/2014	eDNA	New
		07/21/2015	eDNA + Visual	Confirmed
	Elder 3	08/25/2014	eDNA	New
	Elder East Fork 2	07/20/2015	eDNA	New
	Cabin 1	08/24/2014	eDNA	Yes
		07/21/2015	eDNA	Confirmed
	Cabin Button	08/27/2014	eDNA	New
	Cabin Trib 1[c]	07/22/2015	eDNA + Visual	New
	Couldrey 2	07/26/2015	eDNA	New
	Storm 1	08/24/2014	eDNA	Known
	Storm 2[c]	08/24/2014	eDNA	Known
		07/21/2015	eDNA	Confirmed
Wigwam	Ram 1	08/28/2014	eDNA	New
	Ram North Fork 1	08/28/2014	eDNA	New
		07/21/2015	eDNA	Confirmed
	Ram North Fork 3	07/21/2015	eDNA	New
	Ram North Fork Trib 2	07/22/2015	eDNA + Visual	New
	Ram South Fork 1	07/22/2015	eDNA	New
	Ram South Fork 2	07/21/2015	eDNA	New
	Ram South Fork 3	08/28/2014	eDNA	New
	Weasel 1	07/22/2015	eDNA	New
	Wigwam 1	07/22/2015	eDNA	New
	Wigwam 2	08/28/2014	eDNA	New

[a]A positive detection is indicated as either eDNA and/or visually observed (visual) on the indicated date.

[b]Site occurrences are classified as "New" if this was the first record, "Known" if a site had previous records of Rocky Mountain tailed frog, and "Confirmed" where consistent results were obtained upon reevaluation of the same site in a subsequent year within the present study. Site location, sampling, and eDNA result details are presented in Table S3.

[c]Data from 2015 were reported in Veldhoen et al. (2016) but are part of the present full study.

(Table S3). Three of 15 sites tested in the Moyie watershed, all in Elmer Creek drainage, were positive (Table 3). Two additional sites, Elmer North Fork 1 and Elmer East Fork 1, were not sampled for eDNA because 81 tadpoles plus one adult and one tadpole were observed at each site, respectively, at the time of sampling (Table 3). Two of nine sites in the Tepee watershed were positive with visual confirmation of a tadpole at the Tepee 1 site in the year after an eDNA test was performed (Table 3).

Twenty-eight sites were examined in the Yahk watershed between 2015 and 2018 (Figure 3; Table S3). Two known extant tailed frog sites (Screw Creek and Malpass Creek) returned positive eDNA results (Table 3; Figure 3). Two new additional sites (Hart 1 and Yahk Trib 4) were positive (Table 3; Figure 3). The eDNA inventory was also expanded to potential suitable lotic habitats in the Gilnockie Creek drainage (Table 3). Tailed frog DNA was detected at five of 17 sites. Two sites (Gilnockie Trib 7 and Trib 8) are within the Gilnockie Provincial Park boundary, and the other three (Gilnockie 1, 4, and Trib 5.2) are outside of the park on Crown land (Figure 3; Table 3). The Gilnockie 1 site tested positive in two successive years (Table 3).

3.5 | Inter-laboratory comparison of eDNA results

The Gilnockie drainage proved to be particularly challenging due to the apparent presence of inhibiting substances in the water samples and high flow from recent rainfall. To gain confidence in the results, and to test the robustness of the eDNA tests, we performed an inter-laboratory comparison of the samples collected in 2018 (Table 4). The number of hits per total technical replicates was very consistent from sample to sample between the laboratories as were the C_t values from the detections obtained (Table 4). A marked improvement was realized in C_t values of most sample IntegritE-DNA™ test results from before inhibitor cleanup (>42) to after (~20) (bold in Table 4). The typical tailed frog detection C_t was consistently within the low to mid-40s (Table 4).

Samples from the Gilnockie Trib 7 and Trib 8 sites were particularly challenging. Seven of the eight technical replicates initially completely failed the IntegritE-DNA™ test (0/4; Table 4). After inhibitor cleanup and IntegritE-DNA™ retesting, the C_t values passed for all but one replicate (Gilnockie Trib 7A, UVic; Table 4), and these values tended to be higher than other samples (Table 4). Nevertheless, the inter-laboratory concordance for the IntegritE-DNA™ and the tailed frog tests were consistent for all but one sample. This sample, Gilnockie Trib 7A, had borderline performance on the IntegritE-DNA™ test (Average C_t for hits per sample replicate—34.61 ± 1.22 (UVic) and 28.05 ± 0.49 (BVL)) where subsequent tailed frog eDNA testing had no detection (UVic) or one detection (BVL) (Table 4).

TABLE 3 Mode of Rocky Mountain tailed frog detection and type of occurrence at the indicated sites in the Moyie, Tepee, and Yahk watersheds. These sites along with additional sites where tailed frog was not detected (including within the Kootenay watershed) are indicated in Figure 3

Watershed	Site name	Date	Tailed frog detection[a]	Occurrence[b]
Moyie	Elmer 1	07/15/2016	eDNA	New
	Elmer East Fork 1	08/09/2017	Visual	New
	Elmer East Fork 3	08/09/2017	eDNA	New
	Elmer East Fork 4	08/09/2017	eDNA	New
	Elmer North Fork 1	08/09/2017	Visual	New
Tepee	Tepee 1	07/14/2016	eDNA	New
		08/08/2017	Visual	Confirmed
	Tepee 2	08/08/2017	eDNA	New
Yahk	Gilnockie 1	08/10/2017	eDNA	New
		09/13/2018	eDNA	Confirmed
	Gilnockie 4	09/13/2018	eDNA	New
	Gilnockie Trib 5.2	08/10/2017	eDNA	New
	Gilnockie Trib 7	09/12/2018	eDNA	New
	Gilnockie Trib 8	09/12/2018	eDNA	New
	Hart 1	07/15/2016	eDNA	New
	Screw 1	07/15/2016	eDNA	Known
	Malpass 1	07/14/2016	eDNA	Known
	Yahk Trib 4	07/14/2016	eDNA	New

[a]A positive detection is indicated as either eDNA and/or visually observed (Visual) on the indicated date.

[b]Site occurrences are classified as "New" if this was the first record, "Known" if a site had previous records of Rocky Mountain tailed frog, and "Confirmed" where consistent results were obtained upon reevaluation of the same site in a subsequent year within the present study. Site location, sampling, and eDNA result details are presented in Table S3.

TABLE 4 Inter-laboratory comparison between the University of Victoria (UVic) and Bureau Veritas Laboratories (BVL) of C_t and binomial results including the impact of inhibitor cleanup on the 2018 samples. Bold text indicates that the sample was subjected to the inhibitor cleanup procedure because it failed the initial IntegritE-DNA™ test and the post-cleanup results are shown. Gilnockie Trib 7 replicate A failed post-cleanup in the UVic laboratory but passed post-cleanup when processed by BVL. "–", no signal

		Number of hits/total technical replicates				Average C_t for hits per sample replicate			
		IntegritE-DNA™		Tailed frog		IntegritE-DNA™		Tailed frog	
Site name	Replicate	UVic	BVL	UVic	BVL	UVic	BVL	UVic	BVL
Gilnockie 1	A	4/4	4/4	6/8	7/8	19.98 ± 0.03	20.54 ± 0.06	41.04 ± 0.74	45.09 ± 0.27
Gilnockie 1	B	4/4	4/4	5/8	3/8	20.73 ± 0.14	21.15 ± 0.03	44.81 ± 0.63	46.73 ± 1.16
Gilnockie 4	A	4/4	4/4	8/8	8/8	19.09 ± 0.07	19.91 ± 0.04	38.71 ± 0.64	42.21 ± 0.32
Gilnockie 4	B	4/4	4/4	5/8	5/8	20.56 ± 0.20	20.81 ± 0.05	40.24 ± 0.60	45.31 ± 0.35
Gilnockie 5	A	4/4	4/4	0/8	0/8	20.15 ± 0.07	20.72 ± 0.04	–	–
Gilnockie 5	B	4/4	4/4	0/8	0/8	21.10 ± 0.06	21.17 ± 0.07	–	–
Gilnockie 5.1	A	**4/4**	4/4	**0/8**	0/8	**19.48 ± 0.09**[a]	24.05 ± 0.78	**–**	–
Gilnockie 5.1	B	4/4	4/4	0/8	0/8	22.75 ± 0.13	21.78 ± 0.10	–	–
Gilnockie Trib 7	A	**0/4**	**4/4**	**0/8**	**1/8**	**34.61 ± 1.22**[b]	**28.05 ± 0.49**[b]	**–**	**44.52**[c]
Gilnockie Trib 7	B	**4/4**	**4/4**	**0/8**	**0/8**	**24.47 ± 0.28**[b]	**21.20 ± 0.29**[b]	**–**	**–**
Gilnockie Trib 8	A	**4/4**	4/4	**0/8**	0/8	**22.76 ± 0.27**[b]	24.65 ± 0.17	**–**	–
Gilnockie Trib 8	B	**4/4**	**3/4**	**1/8**	**1/8**	**24.84 ± 0.42**[b]	**21.43 ± 0.70**[b]	**43.97**[c]	**47.49**[c]
Gilnockie Trib 10	A	4/4	4/4	0/8	0/8	24.10 ± 0.24	23.33 ± 0.08	–	–
Gilnockie Trib 10	B	4/4	4/4	0/8	0/8	22.25 ± 0.14	22.32 ± 0.06	–	–
Gilnockie Trib 14	A	4/4	4/4	0/8	0/8	20.20 ± 0.10	20.41 ± 0.06	–	–
Gilnockie Trib 14	B	4/4	4/4	0/8	0/8	20.57 ± 0.09	21.33 ± 0.04	–	–
Gilnockie Trib 15	A	4/4	4/4	0/8	0/8	21.78 ± 0.09	21.56 ± 0.04	–	–
Gilnockie Trib 15	B	4/4	4/4	0/8	0/8	22.37 ± 0.15	22.23 ± 0.09	–	–
Distilled water	A	0/4	0/4	0/8	0/8	32.80 ± 0.13[d]	33.89 ± 0.15	–	–
Distilled water	B	0/4	0/4	0/8	0/8	33.35 ± 0.10[e]	33.74 ± 0.16	–	–

[a]Before cleanup C_t value 42.12 ± 1.44.

[b]Before cleanup C_t value N/A. The C_t from a second aliquot of the original eDNA isolate that was cleaned up was 23.32 ± 0.24.

[c]A single replicate was positive.

[d]After cleanup C_t value 33.19 ± 0.04.

[e]After cleanup C_t value 33.85 ± 0.16.

4 | DISCUSSION

4.1 | eDNA data quality considerations

The documented sensitivity of eDNA methods over traditional field methods has contributed to an increasing acceptance of this method to inform conservation decision-making processes, especially when applied toward the detection of inconspicuous species that feature discontinuous distributions, persist at low population densities (i.e., rare or invasive species), or live in habitats that are challenging to survey (Jerde, Mahon, Chadderton, & Lodge, 2011). The greater sensitivity of eDNA methods comes at a fraction of the cost relative to the use of conventional methods for surveying aquatic amphibian taxa, given the personnel costs associated with biophysical inventory for tailed frog (i.e., physical search surveys completed according to previously established government survey protocol) (Helbing & Hobbs, 2019).

Standard methods (e.g., physical searches and trapping) regularly used to detect amphibians can be prone to type 1 (false-positive) and type 2 (false-negative) errors due to misidentification or difficulty in observing the target taxa, respectively (Herder et al., 2014). Environmental DNA methods are similarly subject to these errors; however, careful study design and rigorous adherence to accepted eDNA standard operating procedures greatly reduce the probability of both error types. The positive detection of DNA from the target taxon using qPCR analysis techniques indicates that a specimen of the target species was very likely to have recently been present in the sampled medium (Goldberg et al., 2016). All known positive samples were detected by qPCR and all negative control samples tested negative for the target

species. Positive eDNA results in previously undocumented drainages were supported with subsequent or simultaneous visual observations in Elder, Tepee, and Elmer Creeks.

In the present study, we applied eDNA tests that had been validated against tissue samples from sympatric and parapatric species to mitigate the possibility of false-positive detection from closely related nontarget taxa (Pilliod et al., 2013; Veldhoen et al., 2016). As such, qPCR results allow confident inference of the extant occurrence of tailed frog when its DNA is detected in sample water. Tailed frog tadpoles or adults were observed during sample collection at many of the sites that tested positive for tailed frog DNA during laboratory testing. This provides further positive confirmation that eDNA methods can effectively detect tailed frog in this region.

In contrast, negative qPCR results indicate that the DNA of the target species was not detected in a sample. However, a negative result from eDNA methods (or conventional methods) should not be used to conclude species absence as negative results can arise for two reasons: The species was truly absent from the site during or immediately preceding the time of sample collection, or the species was present but sampling methods failed to detect the species' DNA. With eDNA methods, many factors could influence eDNA detection probabilities including filter type, volume of water filtered, extraction method, and assay quality (Goldberg et al., 2016; Helbing & Hobbs, 2019). Failed detection may also be attributed to degradation or dilution of eDNA in the system being sampled. The timeframe and concentration at which DNA persists in the environment depends on several factors, including the mechanism of DNA transport in the system (e.g., lotic or lentic) and system volume (high or low water levels) (Goldberg et al., 2016). All of the locations tested in the present study were lotic systems in which current velocity or system volume levels could affect eDNA detection (Fremier, Strickler, Parzych, Powers, & Goldberg, 2019; Wood, Erdman, York, Trial, & Kinnison, 2020). While we did not measure these parameters in the present study, our results obtained after a recent rainfall event in the Gilnockie drainage demonstrated that these factors could influence the ability to detect target taxon eDNA. Here, the IntegritE-DNA™ test was particularly useful in identifying sample integrity and assisting in appropriate sample interpretation. In this case, the IntegritE-DNA™ test was able to mitigate an incorrect attribution of a negative result. It was noted that this sample was, in fact, a composite of three 1 L water samples on the same filter. Given its poorer performance across two independent laboratories, the data suggest that a greater quantity of inhibitory contaminants may have been concentrated on the filter as a result of increased water volume passed through the filter.

The rate of eDNA degradation also strongly influences the amount of eDNA present in a sample and therefore its detectability (Barnes et al., 2014; Strickler, Fremier, & Goldberg, 2015). Ultraviolet rays, water temperature, pH, salinity, substrate type, and microbial community activity together affect degradation rates (Barnes et al., 2014; Strickler et al., 2015). Sample replication at the site (n = 2, for the present study) and technical levels (n = 3 in 2014; and n = 8 in 2015–18) increase the likelihood of target taxon eDNA detection at low concentrations. The use of controls such as IPCs and the IntegritE-DNA™ test reduces the potential for false negatives enabling detection of compromised samples.

This broader distribution documented for tailed frog in BC is substantive when compared to the previously known range. More precise site-level considerations are not generated because eDNA transport in lotic systems is inevitable. As such, analysis of sample site characteristics does not necessarily confer meaningful or representative information regarding tailed frog habitat characteristics (Deiner & Altermatt, 2014; Hobbs et al., 2019).

While estimating organismal abundance has been successful under certain circumstances (Doi et al., 2017; Maruyama, Sugatani, Watanabe, Yamanaka, & Imamura, 2018; Wilcox et al., 2016), there are challenges with using eDNA to estimate abundance particularly at low DNA concentrations (Goldberg et al., 2016; Klymus et al., 2019; Spear, Groves, Williams, & Waits, 2015). Thus, while the broader distribution and increased number of known occurrences of tailed frogs in BC are important to better enable species' conservation, the number of tailed frogs in each of these newly discovered occurrences currently remains unknown.

4.2 | Expanding the known range of Rocky Mountain tailed frog

The results of the present study represent a substantial advance in the understanding of current Rocky Mountain tailed frog distribution in BC (and Canada) by greatly expanding the previously accepted distribution of the species. The present study documents tailed frog occurrence in five previously unrecorded drainages in Canada: Elder Creek, Upper Wigwam River, Tepee Creek, Gilnockie Creek, and Elmer Creek.

Elder Creek represents the first (and to date only) record of tailed frog on the east side of the Flathead River watershed in Canada. It is isolated from all known tailed frog occurrences in Canada and the United States. The closest known occurrence is at Ford Creek in Montana, at least 15 km to the south (B. Hossack, Pers. Comm. to ITA, 2013). In Canada, Elder Creek is 16 km east of the closest occurrence (Burnham Creek, a tributary of Couldrey Creek) on the other side of the Flathead River mainstem.

Elmer Creek, a tributary of Moyie River, is now the furthest west known tailed frog occurrence in Canada. It is the only documented occurrence of the species in the Moyie River drainage in Canada and, like Elder Creek, is isolated from all other known tailed frog occurrences in Canada. The closest known occurrence to Elmer Creek is Copper Creek located 4 km to the south in Idaho, USA. The nearest Canadian location is Screw Creek situated 25 km to the east in the West Yahk River drainage.

Samples collected on Gilnockie Creek, a tributary of Yahk River, demonstrated a fragmented distribution of tailed frog detection. Tailed frogs were detected in some reaches while others that appeared suitable tested negative for the species' eDNA. This may be at least partly attributed to sub-optimal sampling conditions as it rained heavily prior to and during sample collection. The occurrence in Gilnockie

Provincial Park has significance for the species' conservation as they are the only records of tailed frog in a provincial park in Canada.

In addition to newly documented tailed frog occurrences in drainages where tailed frog had not been detected previously, the present study also demonstrates a greater distribution of the species within previously documented Flathead and Wigwam drainages including Cabin Creek and Ram Creek. The confirmation of tailed frog DNA using eDNA methods at many sites where time-constrained survey methods had been previously applied with a negative result suggests that physical search methods are not as effective as eDNA methods for survey of tailed frog occurrence. Indeed, 23% (32/138) sites tested positive for tailed frog eDNA compared to only 5% (7/140) where tailed frogs were detected through visual surveys during the study period. While our tailed frog eDNA tests proved effective in detecting this species in the regions surveyed, the discovery of tailed frog in several new geographically isolated drainages prompt further questions regarding the genetic relatedness and degree of genetic isolation of these populations.

5 | CONCLUSION

In summary, five brief tailed frog eDNA field surveys (17 days of total field work) were completed between 2014 and 2018. The focus of each year of this study was to gain an improved and more accurate understanding of tailed frog distribution; a species considered well-studied with extensive prior visual physical search-based occurrence studies conducted between 1996 and 2004. In a much shorter time span, with far less funding, the present study more efficiently documented tailed frog in the Flathead, Wigwam, Moyie, Tepee, and Yahk watersheds adding five new drainages with no previously documented occurrences in Canada. These newly identified inhabited stream reaches have been accepted by the Province of British Columbia for additional designation as WHAs, and for future mapping of Critical Habitat to further conserve Rocky Mountain tailed frog habitat.

The results of the present study build on previous work and provide further support for the use of eDNA as an effective and efficient method for detecting tailed frog presence in lotic systems. The rapid field collection associated with eDNA studies (relative to conventional surveying methods), the relatively low cost of filter materials, the reduced time required for field sampling (relative to physical search methods), and greater detection probabilities suggest that this technique is more efficient and more effective for tailed frog inventory than the current surveying techniques.

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AUTHOR CONTRIBUTIONS

The authors contributed to the study as follows: (i) the conception or design of the study: JH, ITA, CSG, CCH, (ii) the acquisition, analysis, or interpretation of the data: all authors; and (iii) writing and editing of the manuscript: all authors.

DATA AVAILABILITY STATEMENT

The data supporting the present study are presented as Supporting Information in Appendix S1.

ORCID

Caren C. Helbing https://orcid.org/0000-0002-8861-1070

REFERENCES

Barnes, M. A., Turner, C. R., Jerde, C., Renshaw, M. A., Chadderton, W. L., & Lodge, D. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*, 1819–1827.

BC Conservation Data Centre (2018). *BC species and ecosystems explorer*. BC Ministry of Environment Victoria, BC. Retrieved from http://a100.gov.bc.ca/pub/eswp/

BC Ministry of Environment (2014). *Recovery plan for the Rocky Mountain tailed frog* (Ascaphus montanus*) in British Columbia* (p. 37). Victoria, BC: Prepared for the BC Ministry of Environment.

COSEWIC (2013). *COSEWIC assessment and status report on the Rocky Mountain tailed frog* Ascaphus montanus *in Canada*. Ottawa, ON: Committee on the Status of Endangered Wildlife in Canada.

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE*, *9*, e88786.

Doi, H., Inui, R., Akamatsu, Y., Kanno, K., Yamanaka, H., Takahara, T., & Minamoto, T. (2017). Environmental DNA analysis for estimating the abundance and biomass of stream fish. *Freshwater Biology*, *62*, 30–39.

Dupuis, L., & Bunnell, F. (1997). *Status and distribution of the tailed frog in British Columbia*. Centre for Applied Conservation Biology. Final report for Forest Renewal British Columbia, 50 pp.

Dupuis, L., & Friele, P. (2004a). *Protection and management measures for the maintenance of* Ascaphus montanus *populations in the Border Ranges, based on habitat and landscape associations*. Report for BC Ministry of Water, Land & Air Protection. Nelson, BC.

Dupuis, L., & Friele, P. (2004b). *Survey for the Rocky Mountain tailed frog (*Ascaphus montanus*) in Dominion coal blocks, Contract 23145-05-0050090*. Final Report for Arthur Robinson, Natural Resources Canada, 506 West Burnside Road, Victoria, BC.

Dupuis, L., & Friele, P. (2005). *Rocky Mountain tailed frog conservation analysis*. Final report to BC Ministry of Water, Land and Air Protection, and BC Ministry of Forests. Victoria, BC.

Dupuis, L., & Friele, P. (2006a). The distribution of the Rocky Mountain tailed frog (*Ascaphus montanus*) in relation to the fluvial system: Implications for management and conservation. *Ecological Research*, *21*, 489–502.

Dupuis, L., & Friele, P. (2006b). *Post-fire assessment of tailed frog abundance in Storm and Leslie Creeks, following the 2003 Ram-Cabin Fire*. Report to Biodiversity Branch, BC Ministry of Water, Land and Air Protection, Victoria, BC. 12 pp.

Dupuis, L., & Wilson, K. (1999). *Status, distribution, and management needs of the tailed frog in the east Kootenays*. Report to BC Ministry of Environment, Lands and Parks. Nelson, BC. 19 pp.

Environment Canada (2015). *Recovery strategy for the Rocky Mountain tailed frog (*Ascaphus montanus*) in Canada*. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa. 20 pp + Annex.

Fremier, A. K., Strickler, K. M., Parzych, J., Powers, S., & Goldberg, C. S. (2019). Stream transport and retention of environmental DNA pulse releases in relation to hydrogeomorphic scaling factors. *Environmental Science & Technology*, *53*, 6640–6649.

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS ONE*, *6*(7), e22746.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*, 1299–1307.

Green, D. M., Weir, L. A., Casper, G. S., & Lannoo, M. J. (2013). *North American Amphibians: Distribution and diversity*. Berkeley, CA: University of California Press.

Helbing, C. C., & Hobbs, J. (2019). *Environmental DNA standardization needs for fish and wildlife population assessments and monitoring* (p. 41). Toronto, ON: Canadian Standards Association.

Herder, J. E., Valentini, A., Bellemain, E., Dejean, T., van Delft, J. J. C. W., Thomsen, P. F., & Taberlet, P. (2014). *Environmental DNA – A review of the possible applications for the detection of (invasive) species*. Stichting RAVON, Nijmegen. Report 2013–104.

Hobbs, J., Round, J. M., Allison, M. J., & Helbing, C. C. (2019). Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada using robust eDNA detection methods. *PLoS ONE*, *14*, e0213849.

Hobbs, J., Vincer, E., Adams, I., & Goldberg, C. S. (2015). *Rocky Mountain tailed frog (*Ascaphus montanus*) environmental DNA (eDNA) inventory in the Flathead and Wigwam River watersheds*. Prepared for: Ministry of Forests, Lands and Natural Resource Operations. 24 pp + appendices.

Jerde, C. L., Mahon, A. R., Chadderton, L. W., & Lodge, D. M. (2011). 'Sight-unseen' detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*, 150–157.

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., … Richter, C. (2019). Reporting the limits of detection (LOD) and quantification (LOQ) for environmental DNA assays. *Environmental DNA*, 1-12. in press.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., Wildman, R. C., & Moore, K. M. S. (1991). Stream ecosystem recovery following a catastrophic debris flow. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*, 196–208.

Maruyama, A., Sugatani, K., Watanabe, K., Yamanaka, H., & Imamura, A. (2018). Environmental DNA analysis as a non-invasive quantitative tool for reproductive migration of a threatened endemic fish in rivers. *Ecology and Evolution*, *8*, 11964–11974.

Matsuda, B., Green, D. M., & Gregory, P. T. (2006). *The amphibians and reptiles of British Columbia* (vi + 266 pp). Victoria, BC: Royal British Columbia Museum.

Meidinger, D., & Pojar, J. (Eds.) (1991). *Ecosystems of British Columbia*. British Columbia Ministry of Forests Special Report Series No. 6, Victoria, British Columbia, Canada. 330 pp.

Murray, B., & Flores, A. (2013). *Environmental DNA analysis for tailed frog distribution in northwestern BC – Protocol development and initial results*. Internal report prepared for University of Northern British Columbia. 17 pp.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*, 1123–1130.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2014). Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources*, *14*, 109–116.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., Gough, K. C., & Crispo, E. (2014). The detection of aquatic animal species using environmental DAN – A review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*, 1450–1459.

Spear, S. F., Groves, J. D., Williams, L. A., & Waits, L. P. (2015). Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biological Conservation*, *183*, 38–45.

Strickler, K., Fremier, A. K., & Goldberg, C. (2015). Quantifying the effects of UV, temperature, and pH on degradation rates of eDNA in aquatic microcosms. *Biological Conservation*, *183*, 85–92.

Veldhoen, N., Hobbs, J., Ikonomou, G., Hii, M., Lesperance, M., & Helbing, C. C. (2016). Implementation of novel design features for qPCR-based eDNA assessment. *PLoS ONE*, *11*, e0164907.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. F., Shepard, B. B., Jane, S. F., … Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216.

Wood, Z. T., Erdman, B. F., York, G., Trial, J. G., & Kinnison, M. T. (2020). Experimental assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. *Environmental DNA*, 1-11.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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