Thomas W. Franklin[1], **Taylor M. Wilcox**, **Kevin S. McKelvey**, **Samuel E. Greaves**, **Joseph C. Dysthe**, **Michael K. Young**, and **Michael K. Schwartz**, US Department of Agriculture, Forest Service, National Genomics Center for Wildlife and Fish Conservation, Rocky Mountain Research Station, 800 E. Beckwith Avenue, Missoula, Montana 59801

and

Jason Lindstrom, Montana Fish, Wildlife and Parks, PO Box 24, Anaconda, Montana 59711

Repurposing Environmental DNA Samples to Verify the Distribution of Rocky Mountain Tailed Frogs in the Warm Springs Creek Basin, Montana

Abstract

Rocky Mountain tailed frogs (*Ascaphus montanus*) were thought to exist exclusively in two tributaries of Warm Springs Creek watershed—Storm Lake Creek and Twin Lakes Creek, based on opportunistic observations of tailed frogs during fish sampling rather than formal basin-wide sampling for frogs. We used extant environmental DNA (eDNA) samples originally collected to delineate bull trout (*Savelinus confluentus*) occupancy to determine whether tailed frogs reside outside of their current known distribution in the Warm Springs Creek watershed. We were able to rapidly confirm tailed frog occupancy in these two tributaries of Warm Springs Creek watershed, and located tailed frogs throughout the mainstem of Warm Springs Creek where their presence was previously unknown. Repurposing eDNA samples provides a sensitive and extremely cost effective way to determine species distributions, because existing samples can continue to be retested for unrelated taxa without repeating field collections.

Keywords: amphibian, environmental DNA, species distribution, repurposing

Introduction

Rocky Mountain tailed frogs (*Ascaphus montanus*; hereafter, tailed frogs) are associated with cold montane streams of western Montana, southeastern Washington, northeastern Oregon, central and northern Idaho, and extreme southern British Columbia (Leonard et al. 1993, Nielson et al. 2001). Although common throughout much of their range, the fine-scale distribution of Rocky Mountain tailed frogs and the factors affecting their distribution are not always well understood (Hayes and Quinn 2015). Relatively few surveys have targeted tailed frogs, but their benthic tadpoles are common bycatch during stream electrofishing for fishes. Bycatch patterns suggest an unexplained pattern of habitat occupancy for tailed frogs in western Montana. Tailed frogs have only been documented in two adjacent tributaries of Warm Springs Creek, Storm Lake Creek and Twin Lakes Creek (Jason Lindstrom, Montana Fish, Wildlife and Parks, personal observation), despite extensive backpack electrofishing surveys in adjacent tributaries with similar habitat characteristics. The lack of sampling directed at tailed frogs may have limited their detection in other small, intermediate-gradient, forested streams nearby. Targeted efforts to detect tailed frogs are necessary to examine the potential range limitations of this species which may help elucidate the conservation status for Rocky Mountain tailed frogs and the factors influencing their distribution.

Here, we investigate the putative distribution of tailed frogs within the Warm Springs Creek watershed by analyzing archived samples collected via environmental DNA (eDNA) sampling. Environmental DNA sampling uses genetic material in the environment to infer species presence without physically observing the target organism (Jerde et al. 2011). Frequently, eDNA surveys are conducted to determine the presence of single

[1]Author to whom correspondence should be addressed. Email: thomas.franklin@usda.gov

species (e.g., Young et al. 2017). However, each eDNA sample theoretically contains DNA from all species present within the waterbody (Taberlet et al. 2012), offering the potential to detect other organisms of interest. One recent study demonstrated the utility of repurposing eDNA samples collected targeting a large, highly mobile fish species (bull trout, *Salvelinus confluentus*) to detect a small, mostly sedentary mollusk (western pearlshell, *Margaritifera falcata*; Dysthe et al. 2018). We apply a similar approach to detect tailed frogs within the Warm Springs Creek watershed by repurposing samples collected as part of a range-wide bull trout eDNA survey (Young et al. 2017). This survey included eDNA sampling of all cold-water streams in the Upper Clark Fork 8-digit hydrologic unit at 1-km intervals, including the entire Warm Springs Creek watershed. We selected samples collected in the Warm Springs Creek watershed and analyzed them with an eDNA assay that has previously been shown to have very high detection probabilities for tailed frogs (Pilliod et al. 2013). Without doing any additional field work, it was possible to provide insight on the distribution of a small, under-recorded amphibian by leveraging archived eDNA samples from a large-scale sampling effort for a charismatic, federally-listed salmonid.

Methods

In 2015 and 2016, eDNA samples were collected streamside by filtering 5-L of water through a 1.5 µM pore, 47 mm diameter glass microfiber filter using protocols detailed in Carim et al. (2016a). We extracted DNA from one half of each filter using the QIAGEN DNeasy Blood and Tissue kit with modifications as described in Carim et al. (2016b) in a dedicated laboratory space at the National Genomics Center for Wildlife and Fish Conservation (NGCWFC; US Forest Service, Missoula, Montana). The DNA and the remaining filter half were archived at –20 °C for future analysis. Range extension of tailed frogs would most likely occur via the known populations in the headwater tributaries of the Warm Springs Creek watershed because their dispersal is primarily confined to stream basins (Hayes and Quinn 2015, but see Spear and Storfer 2010). Therefore, we reanalyzed extant samples (n = 39) collected at approximately 2-km intervals across the entire Warm Springs Creek watershed (Table 1). Although samples for the range-wide bull trout eDNA survey were collected on 1-km interval, we reanalyzed extant samples collected at approximately 2-km intervals in order to rapidly and cost-effectively inventory the watershed for tailed frog DNA.

We used an *Ascaphus*-specific hydrolysis assay to test for the presence of *Ascaphus montanus* (Goldberg et al. 2011, Pilliod et al. 2013). In contrast to Goldberg et al. (2011) and Pilliod et al. (2013), we optimized the primer and probe concentrations and analyzed with TaqMan Environmental Mastermix 2.0 (Life Technologies) rather than QuantiTect Multiplex PCR Mix (Qiagen, Inc.). We tested the specificity by screening DNA extracted from 20 tissues from seven non-target amphibian species and one non-target reptile species (Table 2). All samples used in this study were from existing collections acquired under appropriate sampling permits. Tissues were extracted with the DNeasy Tissue and Blood Kit (Qiagen, Inc.) following the manufacturer's protocol. We screened the *Ascaphus* assay *in vitro* with tissue-derived DNA in a single qPCR reaction. Screening was performed on a QuantStudio 3 Real-time PCR System (Life Technologies) in 15 µl reactions containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies), 300 nM forward primer, 600 nM reverse primer, 250 nM probe, 4 µl DNA template (~0.4 ng), and 2.75 µl deionized water. Thermocycler conditions included 95 °C for 10 min followed by 45 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. To minimize the risk of sample cross contamination, all qPCR tests were set up inside a UV hood where consumables and pipettes were irradiated with UV light for 1 h prior to each test. Each test included a no-template control with distilled water used in place of DNA template.

We tested the sensitivity of the assay by performing standard curve experiments with a synthetic plasmid DNA fragment from Integrated DNA Technologies containing the 90-bp target amplicon sequence. The plasmid was linearized using PvuI (New England BioLabs), purified us-

TABLE 1. Locations and detection results for 39 repurposed environmental DNA samples used to analyze for Rocky Mountain tailed frogs in the Warm Springs Creek watershed, Montana. Samples are arranged with downstream locations first. Rocky Mountain tailed frogs detections are noted with a "+" in the result tab; no detections are noted with "-". The # of positive wells refers to the number of wells in which Rocky Mountain tail frog DNA amplified in each triplicate. Site ID links to data in Young et al. (2017). Datum used for UTM coordinates is NAD83.

Stream	Site ID	Date Collected	UTM Zone	UTM Easting	UTM Northing	Result	# Of Positive Wells
Warm Springs Cr	489-13	9/29/2015	12	353877	5111071	+	1
Warm Springs Cr	489-15	9/29/2015	12	352157	5110718	-	0
Warm Springs Cr	489-19	9/29/2015	12	348499	5110969	+	1
Warm Springs Cr	497-1	9/29/2015	12	346861	5111716	-	0
Warm Springs Cr	533-2	9/29/2015	12	341719	5113581	+	1
Warm Springs Cr	533-4	9/29/2015	12	340126	5114309	+	1
Warm Springs Cr	539-3	9/29/2015	12	337193	5114093	-	0
Warm Springs Cr	541-1	9/2/2015	12	336217	5114438	+	2
Warm Springs Cr	558-1	9/1/2015	12	333622	5115028	-	0
Warm Springs Cr	619-2	9/1/2015	12	333478	5116043	-	0
Warm Springs Cr	619-6	9/1/2015	12	332222	5119402	-	0
Warm Springs Cr	642-3	9/1/2015	12	333434	5123642	-	0
WF Warm Springs Cr	673-1	9/1/2015	12	333673	5124115	-	0
WF Warm Springs Cr	673-3	9/1/2015	12	333701	5125988	-	0
MF Warm Springs Cr	683-1	9/1/2015	12	335270	5125328	-	0
MF Warm Springs Cr	683-2	9/1/2015	12	335226	5126252	-	0
EF Warm Springs Cr	678-1	9/1/2015	12	335367	5125294	-	0
EF Warm Springs Cr	678-2	9/1/2015	12	335676	5126143	-	0
Barker Cr	469-1	9/5/2015	12	336051	5109780	-	0
Barker Cr	469-4	9/5/2015	12	334321	5107477	-	0
Barker Cr	494-1	9/5/2015	12	335818	5111886	-	0
Nelson Gulch	485-1	9/8/2015	12	335695	5111854	-	0
Foster Cr	496-1	9/2/2015	12	336264	5114577	-	0
Foster Cr	496-5	9/2/2015	12	334823	5117722	-	0
Foster Cr	623-2	9/2/2015	12	336605	5120939	-	0
Foster Cr	676-2	9/2/2015	12	337720	5124049	-	0
Twin Lakes Cr	442-1	9/10/2015	12	328197	5104073	-	0
Twin Lakes Cr	471-1	9/10/2015	12	329334	5108993	+	3
Twin Lakes Cr	495-3	9/8/2015	12	332247	5113740	+	3
Twin Lakes Cr	495-6	9/8/2015	12	330188	5111959	+	3
EF Twin Lakes Cr	436-1	9/10/2015	12	330417	5104534	-	0
EF Twin Lakes Cr	467-2	9/10/2015	12	329437	5108019	+	3
Aqueduct	454-1	9/2/2015	12	331528	5115489	-	0
Cable Cr	578-3	9/2/2015	12	331355	5116768	-	0
Storm Lake Cr	106-3	7/12/2016	12	328860	5112895	+	3
Storm Lake Cr	106-5	7/12/2016	12	327947	5111252	+	3
Storm Lake Cr	106-7	7/12/2016	12	326674	5109657	+	3
Storm Lake Cr	106-9	7/12/2016	12	325531	5108154	+	3
Storm Lake Cr	106-11	7/12/2016	12	324885	5106536	+	2

TABLE 2. List of species used for *in vitro* screening of the *Ascaphus* assay. Origin refers to the state or province the sample was collected in. The qPCR detection results are reported as Y = yes (detected), N = no (not detected).

Species name	Common name	Origin	Detected? (Y/N)
Ascaphus montanus	Rocky Mountain tailed frog	MT	Y
Anaxyrus boreas	western toad complex (western toad)	ID	N
Anaxyrus boreas boreas	western toad complex (boreal toad)	WY	N
Anaxyrus boreas boreas	western toad complex (boreal toad)	UT	N
Anaxyrus boreas boreas	western toad complex (boreal toad)	CO	N
Anaxyrus boreas boreas	western toad complex (boreal toad)	MT	N
Anaxyrus woodhousii	Woodhouse's toad	CO	N
Anaxyrus woodhousii	Woodhouse's toad	CO	N
Anaxyrus woodhousii	Woodhouse's toad	CO	N
Ascaphus truei	coastal tailed frog	OR	N
Chelydra serpentina	common snapping turtle	MT	N
Chelydra serpentina	common snapping turtle	MT	N
Dicamptodon aterrimus	Idaho giant salamander	ID	N
Lithobates [*Rana*] *sylvaticus*	wood frog	SK	N
Lithobates [*Rana*] *sylvaticus*	wood frog	SK	N
Lithobates [*Rana*] *sylvaticus*	wood frog	SK	N
Pseudacris maculata	boreal chorus frog	WY	N
Pseudacris maculata	boreal chorus frog	WY	N
Pseudacris maculata	boreal chorus frog	WY	N
Rana luteiventris	Columbia spotted frog	WY	N
Rana luteiventris	Columbia spotted frog	WY	N

ing PureLink™ PCR Micro Kit (Invitrogen) and quantified with a Qubit 2.0 Fluorometer (ThermoFisher Scientific). From this stock, we prepared a five-level standard curve dilution series (6250, 1250, 250, 50, and 10 copies per 4 µl) in sterile TE, and analyzed three replicates of each dilution.

The eDNA samples were analyzed on a StepOne Plus Real-time PCR Instrument (Life Technologies) in triplicate 15-µL reactions using the same PCR recipe and cycling conditions described above. These samples had previously been tested for the presence of PCR inhibitors when analyzed for bull trout DNA via an internal positive control (Wilcox et al. 2016). Each qPCR plate included a triplicate positive control sample (tissue-extracted genomic DNA) and a triplicate no-template control (distilled water). We considered any sample with detectable fluorescence in at least one PCR well to be positive for the presence of *Ascaphus* DNA. Positive eDNA detections were considered "expected" if historical electrofishing surveys had previously detected tailed frogs (J. Lindstrom, personal observation). "Unexpected" detections occurred where the historical electrofishing surveys did not previously detect tailed frogs, but eDNA analyses detected tailed frog DNA. Samples labeled as "no detection" occurred where both eDNA and previous electrofishing surveys did not detect tailed frogs.

Results

We did not detect *Ascaphus* DNA in any of the 20 non-target tissues we tested (Table 2). The standard curve analysis resulted in an amplification efficiency of 101.77% (standard curve y-intercept = 35.667, r^2 = 0.998). The limit of detection (lowest concentration with > 95% amplification success; Bustin et al. 2009) for each marker was 10 mitochondrial DNA copies/reaction (mtDNA copies/rxn) with successful detection of target DNA in all three replicates. We detected *Ascaphus* DNA in 14 out of 39 eDNA samples. As expected from previous tailed frog detections by Montana Fish, Wildlife and Parks (J. Lindstrom, personal observation), four sites in Twin Lakes Creek tested positive along with all five sites in Storm Lake

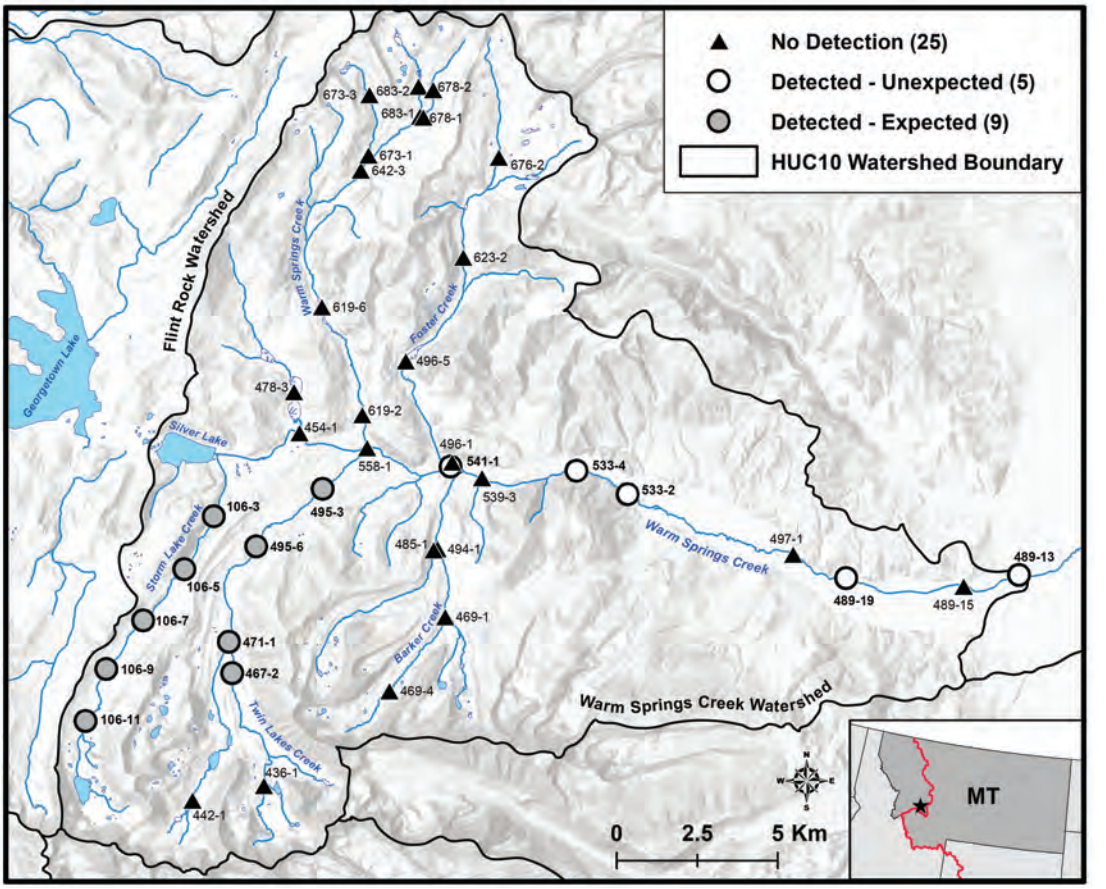

Figure 1. Locations of 39 repurposed environmental DNA samples analyzed for Rocky Mountain tailed frog DNA in the Warm Springs watershed, Montana. Site IDs correspond to data in Table 1 and Young et al. (2017). Positive eDNA detections are indicated with gray or white circles. Detections were considered "expected" if historical electrofishing surveys had previously detected tailed frogs (J. Lindstrom, personal observation). "Unexpected" detections occurred where the historical electrofishing surveys did not previously detect tailed frogs, but eDNA analyses detected tailed frog DNA. Samples labeled as "no detection" are indicated by black triangles and occurred where both eDNA and previous electrofishing surveys did not detect tailed frogs. The continental divide is displayed as a red line on the inset map.

Creek. However, five additional sites on Warm Springs Creek downstream from the confluence with Foster Creek also tested positive for *Ascaphus* DNA (Table 1, Figure 1). These were considered unexpected detections of *Ascaphus* DNA as there were no previous records of tailed frogs here. We did not detect tailed frogs in any other tributaries to Warm Springs Creek. All negative control wells tested negative for *Ascaphus* DNA.

Discussion

Collecting eDNA samples can be easy and quick, allowing large watersheds to be exhaustively sampled in short periods of time (e. g. McKelvey et al. 2016). This approach provides high probability of detection estimates if based on assays that are highly sensitive and taxon-specific, which is important when documenting rare and cryptic species (see McKelvey et al. 2008). Another advantage unique to eDNA sampling is the ability to repurpose samples. They potentially contain DNA from all organisms in the waterbody (Taberlet et al. 2012) and can therefore be queried for any taxa suspected to be present. Repurposing samples maintains the inherent strengths of eDNA sampling. Further, it allows for samples to be rapidly

analyzed for additional species at much reduced costs because the most expensive portions of an eDNA-based survey, sample collection and DNA extraction (Smart et al. 2016), have already been completed. In this case, repurposing eDNA samples to determine the extent of tailed frogs in the Warm Springs Creek watershed cost approximately $800, including labor. Metabarcoding of eDNA samples, the simultaneous analyses of the presence of many species, may remove the need to do sequential analyses for species presence, but does not yet afford the sensitivity or cost-effectiveness provided by single-species analyses (Simmons et al. 2015, Harper et al. 2018). Should those difficulties be overcome, the archived samples could instead by analyzed with that approach, again providing substantial cost savings.

Due to the high sensitivity, ease of collection, and ability to be repurposed, eDNA sampling excels at efficiently and affordably confirming the distributions of rare species or fine-tuning range boundaries at high spatial resolution (McKelvey et al. 2016). Further, eDNA sampling and analyses can also reveal additional, previously unknown populations (cf. McKelvey et al. 2016). In this study, we detected tailed frogs in samples collected throughout the Storm Lake Creek and Twin Lakes Creek watersheds where they were known to be present, and we detected tailed frogs at sampling sites in the mainstem Warm Springs Creek where their presence was previously unknown. In situations where there is an upstream source of target DNA, downstream occurrences may indicate DNA drift rather than the presence of local organisms. In some cases (e. g., Deiner and Altermatt 2014), downstream drift has produced positive detections up to 10 km below the putative source. Only extensive electrofishing of the mainstem of Warm Springs Creek could independently validate the presence of tailed frogs in the mainstem, but there are several reasons why we believe that downstream drift is an unlikely cause for the mainstem detections. First, while some downstream transport of DNA from tailed frogs in Twin Lakes Creek would be expected (Jane et al. 2015, Deiner et al. 2016), this eDNA would most likely be detected, if at all, in the mainstem sample closest to the confluence of the two streams. However, this sample (Warm Springs Cr, 539-3) tested negative. Further, downstream drift distances are limited in small streams (Pont et al. 2018). For example, Levi et al. (2018) found no indications of residual DNA from a lake containing thousands of migrating salmon when sampling eDNA at a weir approximately 1 km below the lake. Pont et al. (2018) modeled downstream DNA drift as a function of stream velocity and depth. Based on equations in Pont et al. (2018), and incorporating day-of-sampling USGS stream gauge data associated with Warm Springs Creek (USGS 12323770 located ~10 km downstream from our lowest site; https://waterdata.usgs.gov/mt/nwis/uv?site_no=12323770), downstream eDNA drift would not be expected to exceed ~10 km given an extremely strong eDNA source (2000 copies/L). Given that our furthest downstream detection of tailed frogs was > 22 km downstream from the mouth of Twin Lakes Creek (Figure 1), we feel that the likelihood that this occurrence was due to downstream DNA drift alone is extremely low.

The cause of the limited distribution of tailed frogs at the eastern edge of their historical range is unknown. Tailed frogs are a cold-water-limited species with optimal temperatures for embryo development being approximately 12 °C (Isaak et al. 2017, Wernz and Storm 1969). Yet this species is absent from most headwater streams east of the Continental Divide in Montana, despite being common in comparable habitats west of that divide (Werner et al. 2004). A similar pattern is evident in the Warm Springs Creek watershed even though it is west of the Continental Divide in Montana (Figure 1). Tailed frogs may be absent from the presumably suitable habitat in this watershed because overland dispersal may be difficult, especially over steep mountain ridges such as those in upper Warm Springs Creek (Figure 1; Hayes and Quinn 2015, but see Spear and Storfer 2010). The dispersal of this species from the adjacent Flint-Rock watershed into the Warm Springs Creek watershed may have been facilitated by the low topographic divide (< 10 m) and the abundant wetlands near the watershed boundary with Storm Lake Creek and Silver Lake (Figure 1). Silver Lake currently drains only into

the Warm Springs Creek watershed, but previously it had been rerouted into the Flint-Rock watershed via a pipeline to Georgetown Lake, which could have facilitated dispersal of tailed frogs into the Warm Springs Creek watershed. Regardless, the apparent failure of tailed frogs to expand beyond this area into nearby tributaries such as Barker Creek or Foster Creek is curious, particularly given the hydrologic connectivity of these tributaries to mainstem Warm Springs Creek where tailed frogs are present (Figure 1). Further work on the habitat associations of tailed frogs may help illuminate the answer and, with rapidly accumulating archives of eDNA samples across the northern Rocky Mountains, repurposed eDNA samples may be a cost-effective approach for doing so.

Literature Cited

Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, and G.L. Shipley. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55:611-622.

Carim, K. J., K. S. McKelvey, M. K. Young, T. M. Wilcox, and M. K. Schwartz. 2016a. A protocol for collecting environmental DNA samples from streams. USDA Forest Service General Technical Report RMRS-GTR-355. Rocky Mountain Research Station, Fort Collins, CO.

Carim, K. J., J. C. Dysthe, M. K. Young, K. S. McKelvey, and M. K. Schwartz. 2016b. An environmental DNA assay for detecting Arctic grayling in the upper Missouri River basin, North America. Conservation Genetics Resources 8:197–199.

Deiner, K., and F. Altermatt. 2014. Transport distance of invertebrate environmental DNA in a natural river. PLoS ONE 9:e88786.

Deiner, K., E. Fronhover, E. Machler, J. C. Walser, and F. Altermatt. 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications 12544.

Dysthe, J. C., T. Rodgers, T. W. Franklin, K. J. Carim, M. K. Young, K. S. McKelvey, K. E. Mock, and M. K. Schwartz. 2018. Repurposing environmental DNA samples: detecting the western pearlshell (*Margaritifera falcata*) as a proof of concept. Ecology and Evolution 8:2659-2670.

Goldberg, C. S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PloS ONE 6:p.e22746.

Hayes, M. P., and T. Quinn. 2015. Review and synthesis of literature on tailed frogs (genus *Ascaphus*) with special reference to managed landscapes. Cooperative Monitoring Evaluation and Research Report CMER 01-107. Washington State Forest Practices Adaptive Management Program. Washington Department of Natural Resources, Olympia.

Harper L. R., L. L. Handley, C. Hahn, N. Boonham, H. C. Rees, K. C. Gough, E. Lewis, I. P. Adams, P. Brotherton, S. Phillips, and B. Haenfling. 2018. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (*Triturus cristatus*). Ecology and Evolution 8:6330-6341.

Isaak, D. J., S. J. Wenger and M. K. Young. 2017. Big biology meets microclimatology: Defining thermal niches of ectotherms at landscape scales for conservation planning. Ecological Applications 27:977-990.

Jane, S. F., T. M. Wilcox, K. S. McKelvey, M. K. Young, M. K. Schwartz, W. H. Lowe, B. H. Letcher, and A. R. Whiteley. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Molecular Ecology Resources 15:216-227.

Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D. M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4:150-157.

Leonard, W. P., H. A. Brown, L. L. Jones, K. R. McAllister, and R. M. Storm. 1993. Amphibians of Washington and Oregon. Seattle Audubon Society, Seattle, WA.

Levi, T., J. M. Allen, D. Bell, J. Joyce, J. R. Russell, D. A. Tallmon, S. C. Vulstek, C. Yang, and D. W. Yu. 2018. Environmental DNA for the enumeration and management of Pacific salmon. Molecular Ecology Resources 19:597-608.

McKelvey, K. S., K. B. Aubry, and M. K. Schwartz. 2008. The use of anecdotal occurrence data for rare or elusive species: the illusion of reality and a call for evidentiary standards. Bioscience 58:549-555.

McKelvey, K. S., M. K. Young, E. L. Knotek, T. M. Wilcox, K. J. Carim, T. M. Padgett, and M. K. Schwartz. 2016. Sampling large geographic areas for rare species using eDNA: a preliminary study of bull trout *Savelinus confluentus* occupancy in western Montana. Journal of Fish Biology 88:1215-1222.

Nielson, M., K. Lohman, and J. Sullivan. 2001. Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. Evolution 55:147-160.

Pilliod, D. S., C. S. Goldberg, R. S. Arkle, and L. P. Waits. 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.

Pont, D., M. Rocle, A. Valentini, R. Civade, P. Jean, A. Maire, N. Roset, M. Schabuss, H. Zornig, and T. Dejean. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Scientific Reports 8:1-13.

Simmons, M., A. Tucker, W. L. Chadderton, C. L. Jerde, and A. R. Mahon. 2015. Active and passive environmental DNA surveillance of aquatic invasive species. Canadian Journal of Fisheries and Aquatic Sciences 73:76-83.

Smart, A. S., A. R. Weeks, A. R. van Rooyen, A. Moore, M. A. McCarthy, and R. Tingley. 2016. Assessing the cost-efficiency of environmental DNA sampling. Methods in Ecology and Evolution 7:1291-1298.

Spear, S. F., and A. Storfer. 2010. Anthropogenic and natural disturbance lead to differing patterns of gene flow in the Rocky Mountain tailed frog, *Ascaphus montanus*. Biological Conservation 143:778-786.

Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. Towards next generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21:2045-2050.

Werner J. K., B. A. Maxell, P. Hendricks, and D. L. Flath. 2004. Amphibians and reptiles of Montana. Mountain Press, Missoula, MT.

Wernz, J. G., and R. M. Storm. 1969. Pre-hatching stages of the tailed frog, *Ascaphus truei* Stejneger. Herpetologica 25:86-93.

Wilcox, T. M., K. S. McKelvey, M. K. Young, A. J. Sepulveda, B. B. Shepard, S. F. Jane, A. R. Whiteley, W. H. Lowe, and M. K. Schwartz. 2016. Understanding environmental DNA detection probabilities: A Case Study Using a Stream-Dwelling Char (*Salvelinus fontinalis*). Biological Conservation 194:209-16.

Young M. K., D. J. Isaak, K. S. McKelvey, M. K. Schwartz, K. J. Carim, W. Fredenberg, T. M. Wilcox, T. Franklin, G. L. Chandler, D. E. Nagel, S. L. Parkes-Payne, D. L. Horan, S. P. Wollrab. 2017. Species occurrence data from the Range-Wide Bull Trout eDNA Project. Forest Service Research Data Archive, Fort Collins, CO.

Received 01 June 2018
Accepted 22 January 2019