BRIDGE AND SETON WATERSHEDS:
TAILED FROG ENVIRONMENTAL DNA ASSESSMENT

Fish and Wildlife Compensation Program: COA-F17-W-1286

Prepared for:
Fish and Wildlife Compensation Program, Coastal
11th Floor, 6911 Southpoint Drive
Burnaby, BC V3N 4XB

Prepared by:
Jared Hobbs
Hemmera Envirochem Inc.
18th Floor, 4730 Kingsway
Burnaby, BC V5H 0C6

Prepared with:
Financial support of the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and public stakeholders.

File: 1629-004.01
03-28-2017

FWCP Fish & Wildlife COMPENSATION PROGRAM

HEMMERA

ACKNOWLEDGEMENTS

This project was managed and delivered with financial support from the Fish and Wildlife Compensation Program (www.frcp.ca) and the British Columbia Ministry of Environment (BC MOE). BC MOE funding was provided from the Land-based Investment Strategy (LBIS) to support laboratory analysis by ecosystem biologist Kathy Paige. FWCP program partners include BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and the public; working together to conserve and enhance fish and wildlife impacted by the construction of BC Hydro dams.

In-kind support was also provided by several staff-members within BC MOE including Kathy Paige, John Surgenor, Bevan Ernst, and Purnima Govindarajulu. The Lillooet Naturalist Society (LNS) also provided in-kind support with event hosting, logistical support during field implementation, and work-planning. Split Rock Environmental also provided generous support by providing a field vehicle in addition to assistance with sampling by an enthusiastic and capable biologist, Iraleigh Anderson, as a volunteer for a full day of collection and filtration.

Project biologists, Jared Hobbs (Hemmera) and Jocelyn Garner (Spea Environmental), donated considerable in-kind (unbilled) time during project implementation and public outreach by waiving fees charged for travel and overtime associated with field sample collection.

We thank the local community members, landowners, St'at'imc Government Services, and the Lillooet Tribal Council for allowing access to their lands, welcoming us into their communities, and providing letters of support for the project. In particular, we acknowledge Matt Manuel and Darwin John of the Lillooet Tribal Council for their support during project implementation as they accompanied us for a day in the field. The opportunity to exchange information and foster improved knowledge and awareness of eDNA methods and tailed frog ecology and management is recognized as an important component of this project.

EXECUTIVE SUMMARY

Coastal tailed frog (*Ascaphus truei*) is provincially blue-listed by the Conservation Data Centre (CDC) and is listed on the Category of Species at Risk under the *Forests and Range Practices Act*. Federally, it is designated as Special Concern by the Committee on the Status of Endangered Wildlife in Canada and listed as Special Concern under Schedule 1 of the *Species at Risk Act*. In Canada, coastal tailed frog (herein referred to as "tailed frog") occurs only in British Columbia (BC). Within BC, the species is known to be widely occurring west of the Coast Mountain Ranges; with their range extending north almost as far as the Alaskan panhandle. It's occurrence on the east side of the Coast Mountain Ranges is less frequently documented with only a few known extant occurrences near Lytton and in the Shulaps (one suspected occurrence).

In 2016 suitable habitats within the Cayoosh, Bridge (Shulaps), Seton, Anderson, Carpenter, and Downton Lake drainages were strategically sampled using environmental DNA (eDNA) methods. Results from four earlier studies were used to guide study design for this project; these include:

1. MFLNRO (2013: 89 sites surveyed),
2. E. Wind and P. Friele (2009; 125 sites surveyed),
3. Rodway and Regehr (2006; 34 sites surveyed); and,
4. E. Leupin (2000; 43 sites surveyed).

Four non-consecutive years of previous inventory effort, using conventional time-constrained search (TCS) methods, allowed survey of 292 sites within the FWCP study area. Tailed frog was detected at three discrete sites in 2013 (MFLNRO), 11 detections at seven sites in 2009 (Wind and Friele), four detections at one site in 2006 (Rodway and Regehr), and four discrete sites in 2000 (Leupin). In total, tailed frog was detected 22 times, representing 15 spatially discrete stream reaches) in the four previous inventories. Average detection rate of coastal tailed frog across all TCS surveys was 5.1%.

Between August 13 and August 17, 2016, 72 locations were sampled within potential tailed frog habitats. These locations were selected to ensure sampling was conducted at many of the same stream reaches sampled in the four previous studies conducted within the FWCP study area. In total, tailed frog eDNA was detected in 34 discrete stream reaches at 28 new sites. Average detection rate, for the 2016 eDNA survey, was 47.2%.

By using more sensitive and efficient eDNA methods we were able to identify 28 new occurrence records for tailed frog within the FWCP study area (see **Results**). This result more than doubles the number of known sites within the Bridge-Coastal study area. We also confirmed a more widespread extant distribution at several previously unconfirmed areas within an apparently isolated metapopulation of tailed frog in the Shulaps drainage and in tributaries along Anderson Lake. This initiative partially addresses species based

actions identified for coastal tailed frog in the Bridge/Seton River Watershed Species of Interest Action Plan: to conduct "*inventory on secondary and tertiary streams, and baseline studies on existing streams to determine the extent of species habitat and distribution*" (FWCP 2011).

This project provides an improved understanding of coastal tailed frog distribution and habitat use within the Bridge-Seton Watersheds. More accurate information, with expanded geographic scope, is required (in future years) to capitalize on the efficiency of eDNA methods based on proven comparative efficiency of this method in this study (and others). Expansion of this work will be proposed again in future years to assist FWCP in meeting the program's stated objectives within the FWCP species action planning process. An accurate understanding of species' distribution and abundance is required to more efficiently ensure adequate management and effective conservation of coastal tailed frog within the Bridge-Seton FWCP area.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Appendices

1.0 INTRODUCTION

There are two species of tailed frog in the amphibian family Ascaphidae; both occur in British Columbia (BC). Rocky Mountain tailed frog (*Ascaphus montanus*) and coastal tailed frog (*A. truei*). Unless otherwise noted any reference made to tailed frog in this report is in reference to coastal tailed frog.

Tailed frogs represent a distinct and ancient lineage. Tailed frogs are unique among anurans as they are associated with mid- to high-elevation low-order mountain streams (COSEWIC 2011). Adult, sub-adult, and juvenile (terrestrial) tailed frogs are small, 2.2 – 5.1 cm snout to vent length (SVL), and inconspicuous, appearing on the surface during wet or cool conditions. Tailed frogs have been characterized to have a primitive hopping ability compared to other anurans (Essner et al. 2010). Tailed frog movements are thought to be highly localized and restricted to short-distance dispersal movements; typically remaining within 100 m of their natal streams. Adults have been found between 250 m to 500 m from perennial streams in old forests (Dupuis and Friele 2003). Movements are also likely seasonally restricted as adult tailed frogs are considered to be more susceptible to desiccation than other anurans (Claussen 1973, Brown 1975); however, adult females have been reported to migrate between upland habitat and lotic aquatic habitats during the breeding period (Dupuis 2004). Adults are predominantly nocturnal; foraging within riparian habitats along stream edges on a wide array of prey items (insects, spiders, arthropods, snails).

Breeding occurs in early fall via internal delayed fertilization. The following summer the female lays up to 85 colorless eggs, which hatch six weeks later. Embryos feed on yolk sacs (visible in **Figure 1**) through their first winter. By the following spring, the eggs transform into tadpoles with a white spot on their tail (ocellus) that may help distract predators by drawing attention to the waving tail. Tadpoles are approximately 11 mm Total Length (TL) upon hatching and can grow up to 6.5 cm TL before metamorphosis into a terrestrial form (Dupuis 2004). Tadpoles are also morphologically unique as they possess a large adhesive disk, or sucker, on their anterior ventral surface (**Figure 2**). This unique adaptation aids with foraging in fast flowing mountain streams where tadpoles feed on diatoms that they graze from rocks in both riffle and pool habitats.

Figure 1 Eggs are adhered to the underside of large rocks in stream pools

Figure 2 Tailed frog tadpoles have an adhesive oral-disc, or mouth, to attach to rocks in stream habitats

Metamorphosis into an adult form generally occurs within four years (minimum one year) of hatching (Dupuis 2004). Since maturation to adulthood requires several years, tailed frog populations can only persist in perennial lotic systems (Dupuis and Friele 2004). Sub-adults generally reach sexual maturity (as adults) at eight or nine years of age. Adult tailed frogs have large heads, vertical pupils, no tympana, and broad outer hind toes (**Figure 3**). Males have a short 'tail' to enable insemination (**Figure 4**). This is a necessary adaptation as the more typical anuran method of external fertilization would not be effective in fast-flowing water. These adaptations allow tailed frogs to flourish in cool fast-flowing mountain streams often in isolation from sympatric anurans. Tailed frogs are long-lived; adults may exceed 20 years of age in the wild (Daugherty and Sheldon 1982).

Figure 3 **Note the vertical pupils, lack of an external 'ear' membrane, and long outer hind toes**

Figure 4 **Male (right) and female (left) adult tailed frogs are sexually dimorphic – the 'tail' is visible on the adult male**

Suitable aquatic habitat occurs within lotic systems that feature a boulder or cobble substrate with abundant interstitial spaces, which provide security habitat for tadpoles and adults (i.e., refugia from predators and dynamic system events) (Frid et al. 2003, Dupuis and Friele 2004). Occupied streams generally occur in drainages with catchment basins ranging from 0.3-50 km^2 and stream reaches used for breeding are generally <10 km^2. Ideal lotic habitats feature step-pool or cascade-pool morphology. Terrestrial forms require mature forests that provide retreat sites (i.e., course woody debris) within a stable and moist microclimate (COSEWIC 2011) as tailed frogs have a narrow temperature tolerance (from 6°C to 18°C) (Dupuis and Friele 2004). Intact riparian vegetation likely helps maintain cool, clear, and silt-free water, and provide cooler microclimates for foraging adults (Dupuis and Friele 2004, Frid et al. 2003).

Globally, tailed frog occurs along both the west and east side of the Coast and Cascade mountain ranges in North America; from California extending northwards, almost reaching the Alaska panhandle in the coastal region of northern BC (COSEWIC 2011, Ritland et al. 2000). In BC, tailed frog occurrences are documented with a continuous distribution from the international BC: Washington (US) border extending north along the Cascades as far as Lytton (Merritt-Cascades Forest District) and along the coast mountain

range to at least Kitimat (**Figure 5**). Within the Cascade Mountain range in BC, occurrences in leeward drainages are thought to be uncommon (BC CDC 2014); however, increased inventory using novel and more effective eDNA methods (as applied in this project) will likely result in documentation of increased occurrence records for these areas.

Tailed frog is currently recognized as a species of conservation concern due to specialized habitat requirements and sensitivity to ongoing degradation and loss of habitat (e.g., stream sedimentation due to roads, logging, and fire) (COSEWIC 2011). In BC, tailed frog is provincially blue-listed (S3S4 – Vulnerable/ Apparently Secure) by the BC Conservation Data Centre (CDC). Globally the species is ranked as G4 (Apparently Secure). Tailed frog is designated as special concern (SC) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (BC CDC 2016) and federally listed as SC under Schedule 1 of the *Species at Risk Act* (*SARA*).

Threats to tailed frog include habitat loss and fragmentation (from activities such as road-building, power projects and forestry) and habitat degradation exacerbated by increased levels of stream siltation post-disturbance (COSEWIC 2011). Within BC, tailed frog has been designated as Identified Wildlife under the *Forest and Range Practices Act* (*FRPA*) to address concerns regarding the effects of forestry practices on the habitat and populations of this species. Under *FRPA*, Wildlife Habitat Areas (WHA) may be established along streams where tailed frogs have been confirmed. Within designated WHAs, General Wildlife Measures (GWMs) are mandated to ensure conservation of biodiversity values. For tailed frogs, these typically provide no-timber-harvesting zones of 30-m on both sides of the streams, and an additional 20-m zone of managed forest adjacent to the 30-m reserve zone. Tailed frogs are negatively affected by high road densities near occupied streams (BC Ministry of Environment 2014, Dupuis 2004). Natural processes in interior stream systems (e.g., debris torrents and sediment floods) may also contribute to local extinctions (Lamberti et al. 1991).

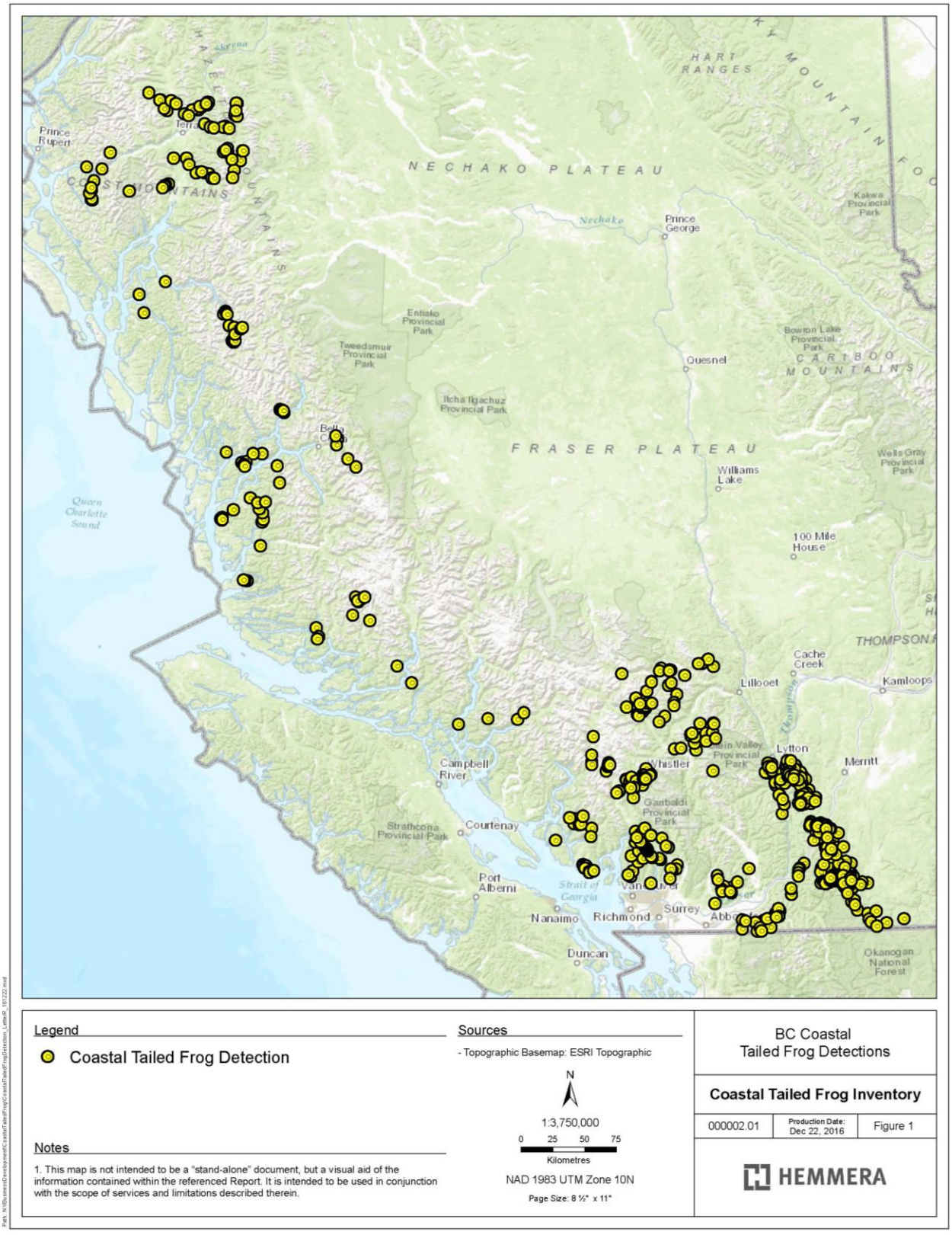

Figure 5 Coastal Tailed Frog Occurrence Records in BC (n=895)

1.1 GOALS AND OBJECTIVES

Hydroelectric development within the FWCP study area may have had undocumented negative effects on tailed frog population persistence in the Bridge, Seton, Anderson, Carpenter, and Downton watersheds. Hydroelectric development has influenced riparian and aquatic conditions within these watersheds; processes such as draw-down and flooding have resulted in alterations to riparian and aquatic (lotic) habitats. These alterations have likely negatively affected tailed frog populations at both the local and landscape level; with local effects on species mortality rates and landscape effects to species dispersal. Quantification of these effects is impossible as no baseline inventory data exists for these areas; however, previous hydroelectric development likely continues to influence species' distribution, population connectivity, productivity, survival, and dispersal within affected drainages. To inform management and recovery actions for tailed frog within the FWCP-Coastal Program area, a scientific baseline understanding of the extant distribution of this species is needed. Our main study objectives included:

1. To examine/assess tailed frog presence within the FWCP study area, including the Cayoosh, Bridge (Shulaps), Seton, Anderson, Carpenter, and Downton Lake drainages.
2. To re-visit and survey tributaries in the Shuswap Range (i.e., tributaries of the Yalakom River) where tailed frog presence was equivocal based on earlier survey work (P. Friele pers. comm), and assess tailed frog presence in the headwaters of Shulaps Creek (a tributary to the Yalakom River).
3. To provide the BC Ministry of Forests, Lands, and Natural Resources (MFLNRO) with the requisite information to enable species-specific conservation and management of all extant tailed frog occurrence records. This may be enabled via designation of new WHAs (for stream reaches with newly confirmed tailed frog presence) or via Forest Stewardship Planning under *FRPA*.

1.2 SUMMARY OF PREVIOUS INVENTORY INFORMATION

Prior to beginning this survey, we solicited and compiled all available tailed frog records from 13 previous surveys and/or existing databases (e.g., the Wildlife Species Inventory database (WSI)) within BC to document the current understanding of the species' distribution and abundance in the province. Within the FWCP study area, detailed survey results, including areas searched with no positive detection recorded, were analysed to inform study design (**Table 1**). This compilation of tailed frog occurrence records, and inventory effort, represents the most exhaustive up-to-date synthesis of information for tailed frog currently available. Data from this project and a concurrent volunteer project, led by Pierre Friele, have also been added to this database and are reported here (refer to **Figure 5**).

Table 1 Summary of Data Sources Compiled in Preparation for the 2016 FWCP funded eDNA Survey. Inventory Projects Completed within the 2016 FWCP Study Area are noted in bold

Data Source	Principal Investigator	Year(s)	# of Sites with Detections
Species Inventory Database	Various	1995-2002	496*
Southern Cascades TCS Surveys	L. Gyug	1996-2002	154
Cascades TCS Survey	**E. Leupin**	**1999-2000**	**11****
Goldbridge-Bralorne TCS Survey	**Rodway/Regehr**	**2006**	**4**
Boston Bar LBIS TCS Survey	J. Hobbs/F. Iredale	2009	31
Hurley River TCS Survey	**P. Friele/E. Wind**	**2009**	**11**
Sunshine Coast TCS Survey	E. Wind	2006-2013	33
Whistler Area TCS Survey	E. Wind	2010	35
Kwoiek Creek/Lytton TCS Survey	Confidential	2010	1
Mid-Cascades TCS Surveys (Merritt)	J. Hobbs/L. Gyug	2012	37
Hurley-Cascades Sp.@Risk TCS Survey	**J. Surgenor**	**2013**	**3**
Indian River TCS Survey	Confidential	2015	22
Cayoosh-Carpenter TCS Inventory (iButton)	P. Friele	2016	18
Cayoosh-Carpenter FWCP eDNA Inventory	J.Hobbs	2016	33
			889

*Records from the Wildlife Species Inventory Database (WSI) were not used in calculation of occupancy rate as null data was not consistently reported within the study area.
**only 4 (of 11) sites reported by E. Leupin were located within the FWCP study area. As such, occupancy rate was calculated for TCS using only those (four) sites.

2.0 STUDY AREA

The Bridge River and Seton, Anderson, Carpenter, and Downton Lake drainages (approximately 3700 km^2) are located in the xeric rain-shadow of the southern coastal mountains (FWCP 2011). The study area is located in the Interior Transition Ranges ecoregion within the Southern Interior Ecoprovince

Lower elevations within the study area occur within the Interior Douglas-Fir (IDF) bio-geoclimatic (BEC) zone and are characterized by warm dry summers, a fairly long growing season, and cool winters (Hope et al. 1991). The Coastal Western Hemlock (CWH) BEC zone occurs at low to mid-elevations (up to 990 m above sea level (ASL)) in the study area. The CWH has the highest level of annual rainfall of all the BEC zones in the study area with a cool meso-thermal climate (cool summers and mild winters) (Meidinger and Pojar 1991).

Upper elevation habitats occur within the Montane Spruce (MS) and Engelmann Spruce-Sub-alpine fir (ESSF) BEC zones before transitioning into the Interior Mountain-heather Alpine (IMA) BEC zone (Meidinger and Pojar 1991). The ESSF BEC zone occurs at elevations between 900-2100 m ASL (lower elevation limits vary by aspect), and is characterized to have a severe climate with long cold winters and short cool summers. Engelmann spruce (*Picea engelmannii*) and sub-alpine fir occur in wetter areas; whitebark pine (*Pinus albicaulis*) occurs on drier sites. Lodgepole pine (*Pinus contorta*) is an increaser species after disturbance (Meidinger and Pojar 1991). The MS BEC zone occurs at mid-elevations in the central interior, on the leeward side of the Coast Mountains. Cold winters and short warm summers are characteristic in these areas; forested areas are dominated by sub-alpine fir (*Abies lasiocarpa*). Tailed frogs are known to commonly occur in the MS, CWH, and ESSF BEC zones, and occur with lower (reported) frequency in the IDF BEC zone.

The Bridge and Seton watersheds are located within the territory of several First Nation groups that are St’át’imc member bands of the Lillooet Tribal Council. Other tenured stakeholders include MFLNRO and its clients (independent licensees and range tenure holders), the BC Ministry of Energy and Mines, and private landowners.

3.0 METHODS

3.1 ENVIRONMENTAL DNA

Environmental DNA is any trace fragment of exogenous DNA that is released by an organism into the environment (Herder et al. 2014). The reliable detection of aquatic vertebrate species (Ficetola et al. 2008, Jerde et al. 2011, Thomsen et al. 2012, Herder 2014, Fukumoto et al. 2015), including *Ascaphus* spp. (Goldberg et al. 2011, Steventon and Todd 2012, Pilliod et al. 2013a), using eDNA from a variety of freshwater systems has been confirmed. This method has, to date, been applied successfully by Hemmera Envirochem Inc. (led by Hemmera senior biologist: Jared Hobbs) on 23 projects since 2014; focused on 18 different target taxa in BC and the Yukon Territory. In addition, provincial standards have been authored to guide practitioners in the collection of surface water eDNA sampling methods in BC; these standards have been accepted as a new Resource Inventory Standards Committee (RISC) standard by BC MOE (Hobbs et al. 2016).

Ex-situ testing for the presence of a species' DNA using quantitative polymerase chain reaction (qPCR) methods requires development of a set of species-specific primers that target a small section of the mitochondrial DNA cytochrome *b* gene (Goldberg et al. 2011). Development of this species-specific qPCR assay for coastal tailed frog had already been completed, prior to this project and was made available by Dr. Caren Goldberg at Washington State University (WSU). The primer provided by WSU was adapted, in 2015, by Dr. Caren Helbing and Nik Veldhoen, at the University of Victoria genetic lab. Validation was required to ensure efficacy when testing site water in BC to exclude potential for false negatives due to geographic phylogenetic variation. Validation of the primer was completed prior to its use in testing the 2016 samples collected in this project (**Section 3.5** and **Appendix 3**). Primer validation was completed following standards outlined in Veldhoen et al. (2016).

3.2 SAMPLE SITE SELECTION

Sites were selected based on the consideration of several criteria including:

(i) Previous Time Constrained Search (TCS) methods applied. Sites with a documented positive detection of tailed frog were included during sample design and collection to provide a measure of comparison of methodological efficacy at known extant coastal tailed frog sites.

(ii) Communication and collaboration with Pierre Friele was integrated into sample design to ensure overlap of eDNA with sites selected by Pierre for stream temperature monitoring studies (as initiated by P. Friele. 2016).

(iii) Consideration of surficial geology, catchment size, and origin (lake fed vs. glacier fed) to ensure representation of habitat heterogeneity in sample site placement.

(iv) Expert-based on-site habitat suitability assessments were conducted at each site during sample collection. The assessments were based on previous field experience with both species of tailed frog in BC and extensive familiarity and local knowledge of the FWCP study area.

In all cases, in-field site selection targeted microsites within stream reaches immediately (~50 m) upstream of a confluence in a particular stream reach to ensure samples were not contaminated by water from effluent immediately downstream.

3.3 FIELD SAMPLE COLLECTION

Biologists collected two (i.e., duplicate) one litre (L) water samples at each sample location or 'site'. Sites near a stream confluence were sampled upstream of the confluence to eliminate ambiguity regarding eDNA source. Bottles were labelled with the site name, Universal Transverse Mercator (UTM) coordinate, collection time (24:00 hrs), date and name of collector. Biologists wore clean nitrile gloves to triple rinse the sample bottles with stream water, and each bottle was filled as close to the thalweg of the stream as possible. Thalwegs 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. 2013a).

The crew recorded a UTM coordinate using a Garmin Map60csx GPS unit set to collect in NAD 83 datum. The crew collected pertinent habitat data with an iPad Air 128gb V4 iPad. Habitat data parameters are described in **Appendix 1**. Once stream water is collected exonucleases may accelerate degradation of DNA in sample water if temperatures and/or exposure to ultraviolet rays (e.g., sunlight) are elevated relative to site conditions (Pilliod et al. 2014). To ameliorate degradation of DNA, prior to off-site filtration and preservation, collected samples were placed in an insulated cooler, in contact with ice, during field collection.

3.4 SAMPLE FILTRATION

Samples were processed following a standard eDNA protocol (Hobbs et al. 2015). Samples were stored in a refrigerator set to 4^{o}C during holding for filtering. Samples were processed within 24 hrs of collection; processing is recommended within 24 hrs of collection to ameliorate degradation of DNA (Pilliod et al. 2014) in the same chronological order as collected. Samples were poured into a 250 mL sterile polypropylene filter funnel with a 0.45 µm pore diameter cellulose nitrate membrane. The sample was filtered through the membrane using a 115 volt GAST alternating pressure to create a vacuum. On completion of filtration, the filter (cellulose membrane) was removed using sterile gloves and tweezers sterilized in a 50% bleach solution. The biologist placed the membrane into a 2 mL sterile polypropylene cryogenic vial and filled the vial with 95% molecular-grade ethanol. Vials were labelled and placed inside labelled whirl-pak storage bags for shipping.

One 1 L control sample (i.e., distilled water) was processed for each day of sample collection using the same filtration protocol as the site water to serve as a contamination test of both the filtration and laboratory analysis processes. Distilled water control samples were not identified to the lab prior to analysis. Preserved filter membranes were shipped to Dr. Caren Helbing for subsequent extraction and analysis.

3.5 LABORATORY ANALYSIS

Ex-situ testing of water samples collected during the 2016 field component of the project was completed by Jessica Round and Dr. Nik Veldhoen, University of Victoria.

In 2016, eDNA was isolated using an established protocol (Veldhoen et al. 2016). Each isolated DNA sample was assessed in eight replicate qPCR runs with each independent assay run to include both one positive and two negative PCR reaction controls. In addition, an internal control was used to confirm sample quality required for qPCR (i.e., tested to ensure sufficient DNA was recovered, after extraction, for assessment and/or to ensure that PCR inhibition was not occurring for each sample) (see **Section 3.6**). This internal control was added to eliminate potential for mistaken assignation of a negative result.

3.6 ANALYSIS OF LAB RESULTS

As a first step, before samples were tested for eDNA from the target taxa (i.e. tailed frog in this study), each sample was first tested for inhibition and to ensure field filtration methods effectively isolated eDNA from the sample. In this initial screening the lab runs an ePLANT (algae) probe to test PCR amplification. If eDNA is confirmed on the sample, we proceeded on the knowledge that inhibition would not affect results for that sample and with confirmation that eDNA has been captured during filtration. If the ePLANT probe tests were negative we disregarded the sample (i.e. the sample was not useable and was not used in site interpretation[1]).

Next, if the sample was confirmed to contain eDNA using the ePLANT probe, we conducted eight repeated qPCR analyses (hereafter referred to as runs) on each sample using the probe for the target taxa (i.e., tailed frog in this study). Eight runs have been determined to provide appropriate effort, based on power analysis, to provide adequate confidence to reduce the potential for error in a binomial analysis to below 11% (Helbing and Veldhoen unpubl. data). This approach provides a statistically robust assessment to determine presence of eDNA in a sample from the target species (Helbing and Veldhoen unpubl. data). qPCR results were analyzed and interpreted for each **sample** as follows:

- A positive result on a sample was assigned if ≥3 (of 8) runs return a positive result for the sample. This is accepted as positive evidence of the presence of DNA from the target taxa.
- A sample with ≤2 (of 8) positive results was accepted as negative for evidence of the presence of DNA from the target taxa.

[1] This did not occur to any samples collected in this study and is a very infrequent event.

Assignation of positive or negative values at the **site** level was based on consideration of both samples (in this study we collected two filtered 1 L water samples at each site) using the following decision criteria:

- If either of the two replicate samples yielded a positive qPCR result for three or more of eight runs (≥3/8) the site was categorized as **positive**, regardless of the other sample's score.
- If at least one of the samples yielded a positive qPCR result for exactly 2 of eight runs (=2/8) and the second replicate received a score of 1/8 (i.e., not 0) then the site was categorized as suspected. In this case, both replicates were considered and the site assignation was considered in the context of the species ecology with consideration of both habitat connectivity and quality and adjacency of extant sites.
- If both samples yielded a positive qPCR results for ≤1/8 runs, the site was categorized as **negative**.

4.0 RESULTS

Between August 13 and 18, 2016, we collected 144 water samples from 72 sites within the FWCP study area. Sites selected for survey using eDNA methods overlapped, geographically, with four independent previously completed formal studies. These (previous) studies used traditional TCS search methods and were conducted by E. Leupin (2000), Rodway and Regehr (2006), E. Winde and P. Friele (2009) and MFLNRO (2013). In total, these studies sampled 292 stream reaches within the 2016 FWCP study area (**Figure 6** and **Table 2**). Results from each of these previous studies were assessed during study design for this project and many of the streams that had been previously sampled using TCS methods were resampled in 2016 using eDNA methods to facilitate comparison of efficacy between both methods.

Table 2 Summary of previous study results the 2016 FWCP funded eDNA Survey.

Data Source (TCS)	Principal Investigator	Year	# TCS sites	# of Occurrences	Detection Frequency (%)
Cascades TCS Survey	E. Leupin	2000	44	4*	9 %
Goldbridge-Bralorne TCS Survey	Rodway/Regehr	2006	34	1	3 %
Hurley River TCS Survey	P. Friele/E. Wind	2009	125	7	5.6 %
Hurley-Cascades Sp.@Risk	J. Surgenor	2013	89	3	3.4 %
Summary	**All**	**All**	**292**	**15**	**5.1 %**

*only 4 (of 11) sites reported by E. Leupin were located within the FWCP study area. As such, occupancy rate was calculated for TCS using only those (four) sites.

Table 3 Summary of eDNA study results for comparative purpose.

Data Source (eDNA)	Principal Investigator	Year	# sample sites	# of Occurrences	Detection Frequency (%)
FWCP 2016 eDNA Survey	J. Hobbs	2016	72	34	47.2%

Previous formal/reported TCS study results within the Bridge-Coastal study area confirmed a total of 22 coastal tailed frog detections at 15 discrete stream reaches (of 291 reaches surveyed) within the 2016 FWCP project area. These earlier studies were used to guide study design; however, there was some overlap/repetition with previous studies; the 22 reported tailed frog detections represent only 15 independent stream reaches with confirmed extant occurrence of coastal tailed frog. These 15 stream reaches include all (reported) coastal tailed frog occurrence records in the study area prior the 2016 eDNA study. Overall, TCS surveys applied to the 2016 FWCP study area resulted in a 5.1% detection rate using traditional methods (**Table 2**).

In 2016, 72 independent stream reaches were sampled using eDNA methods. Three samples were collected at known extant sites from previous TCS surveys; these sites were included to provide a positive control to ensure eDNA methods were able to detect tailed frog at known sites. All three previously known

sites tested using eDNA had a positive result for the detection of tailed frog eDNA in the sample water collected at these sites. Additional TCS surveys were conducted at 25 sites by P. Friele in 2016 concurrent with this study. This work resulted in confirmation of an additional seven extant tailed frog sites; three of these newly confirmed extant sites were concomitantly tested with eDNA methods and two tested positive with eDNA[2]. By contrast five sites searched by P. Friele in 2016 tested positive using eDNA methods yet no tailed frogs were detected at these sites during TCS searches conducted concomitantly. In total, thirty-four of the 72 sites sampled using eDNA methods tested positive resulting in a 47.2% detection rate (**Table 3**).

Based on the results from this study tailed frog DNA was confirmed within virtually all higher-order watersheds in the FWCP study area (**Figure 7**). eDNA from coastal tailed frog was detected in 28 new locations including three creeks in the Shulaps, two creeks in the Upper Bridge River, two tributaries that flow into Carpenter Lake, one creek in the Cadwallader drainage, 12 creeks in the Hurley drainage, one creek in the Lillooet River Drainage (Railroad Creek), two creeks in the Anderson drainage, two creeks that flow into Haylemore Creek, and eight tributaries along Cayoosh Creek (**Figure 7**).

A brief analysis of associated coarse-scale landscape habitat parameters, considering both representative effort (number of sites) and detection rates (number and/or percentage of sites with positive detections), is synthesized as follows:

1. Bio-geoclimatic zone: We sampled four BEC zones including: CWH (5 sites), ESSF (17 sites), IDF (23 sites) and MS (27 sites). Tailed frog eDNA was detected at four sites in CWH (80% of sites sampled), 7 sites in ESSF (41% of sites sampled), 9 sites in IDF (39% of sites sampled) and 13 sites in MS (48% of sites sampled).
2. Elevation: We sampled sites between 255-1758 m ASL. Tailed frog eDNA was detected in sites from 470-1513 m ASL.
3. Distribution by watershed: We collected samples from 28 watersheds; tailed frog eDNA was detected in samples collected from 16 watersheds in the FWCP study area.

More precise site-level considerations are not synthesized in this report as 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 et al. 2014). Notwithstanding, detailed site data, including location, habitat quality parameters, sample dates, and conditions, as well as field expert-based estimates of habitat suitability are included in **Appendix 1**.

[2] Tailed frog was confirmed, using TCS methods, at Blowdown Creek (by P. Friele) on August 5, 2016, 11 days prior to eDNA sample collection.

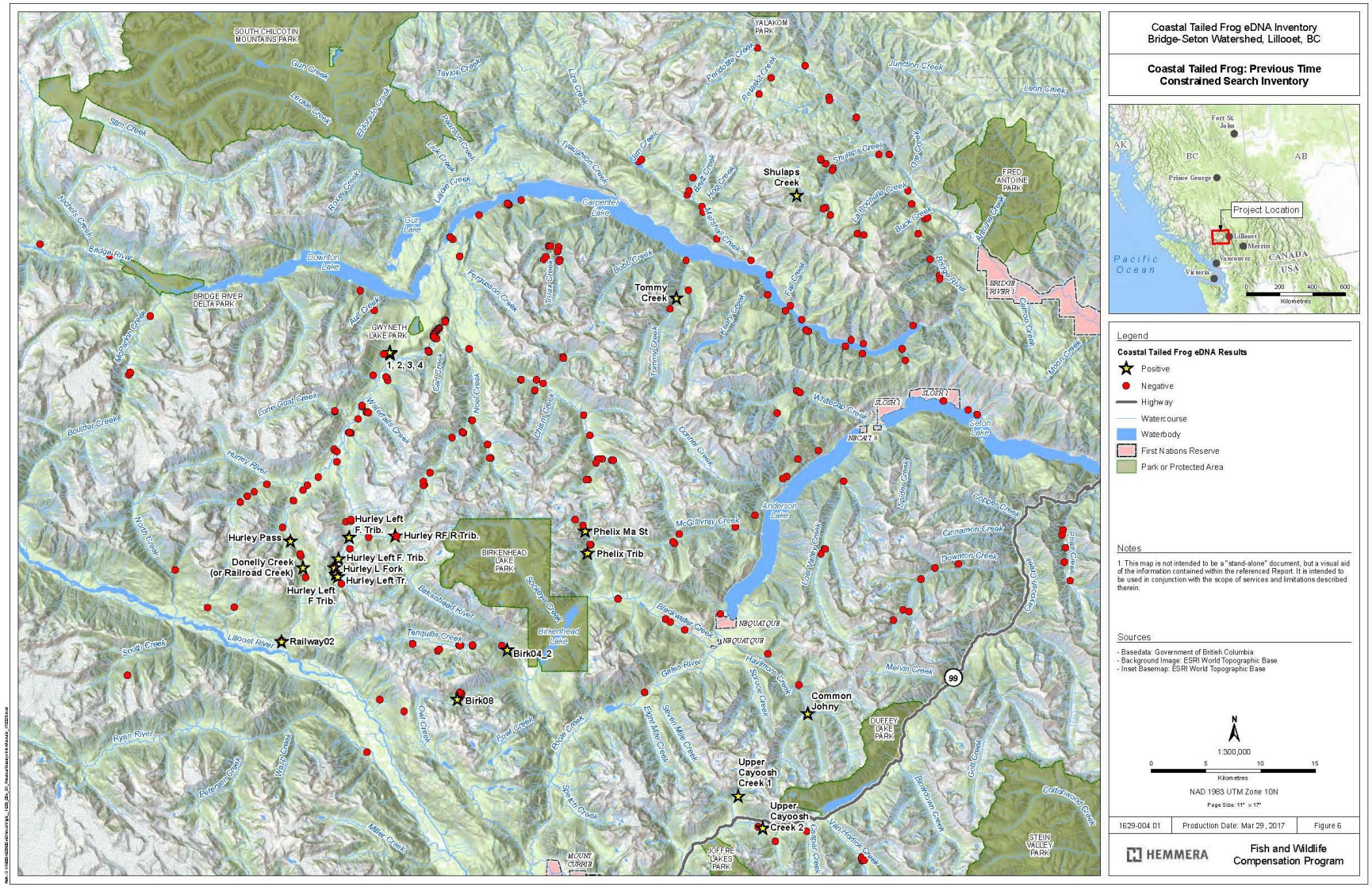

Figure 6 Previous Time Constrained Survey Stations and Results within the FWCP Study Area

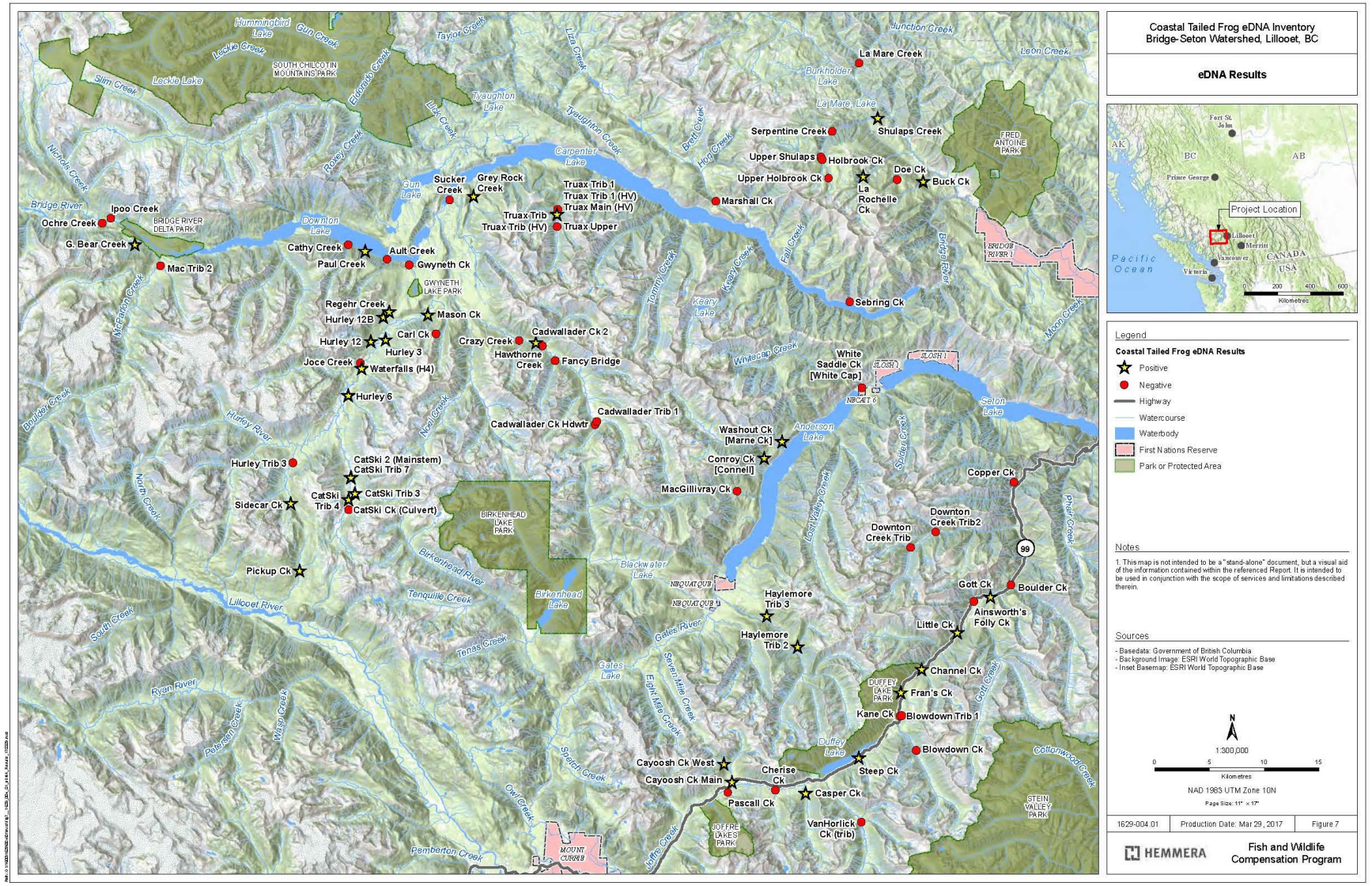

Figure 7 eDNA Sampling Stations and Results within the FWCP Study Area

5.0 DISCUSSION

The sensitivity of eDNA methods when applied towards the detection of inconspicuous species that feature discontinuous distributions, persist at low population densities (i.e., rare species), and/or live in habitats that are often challenging to survey, has gained increasing acceptance in BC. To date there have been over two-dozen eDNA studies completed in BC and Yukon and over a hundred published studies completed in the US and in Europe (Herder et al. 2014, Hobbs and Goldberg 2016, Goldberg 2015, Bohmann et al. 2014). Within BC, eDNA has been applied since 2014 to expand current understanding of the distribution of several amphibians including Oregon spotted frog (*Rana pretiosa*), Cascades frog (*Rana cascadia*), rocky mountain tailed frog, coastal tailed frog, red-legged frog (*Rana Aurora*), American bullfrog (*Lithobates catesbiena*), western toad (*Anaxyrus boreas*), coastal giant salamander (*Dicamptodon tenebrosus*) and Columbia spotted frog (*Rana luteiventris*) (Hobbs and Adams 2014, Hobbs and Adams 2016, Ovaska et al 2017). These gains are being achieved at a fraction of the cost relative to the use of conventional methods for surveying aquatic amphibian taxa. Adoption and application of eDNA methods is increasing with development of provincial standards for field collection (Hobbs and Goldberg 2016) and ongoing training provided to regulators within both the BC and Yukon provincial government, to staff within funding agencies, and to other qualified environmental professionals. Advances have also been achieved to improve rigour and ensure consistency in laboratory methods and interpretation (Hobbs and Goldberg 2016, Veldhoen et al. 2016). The inertia of applying eDNA methods in the survey of amphibians and fish in BC is growing rapidly. This project serves as yet another application of eDNA methods in environmental practice that effectively demonstrates proof-of-concept and confirms the ability of eDNA methods, when applied with rigour, to provide cost-effective survey of aquatic taxa in both lotic and lentic systems.

Prior to this FWCP-funded application of eDNA in the Bridge River system, there had been extensive previous studies using conventional methods to document presence and distribution of coastal tailed frog within the study area. Previous studies include: MFLNRO (2013; 89 sites), E. Wind and P. Friele (2009; 125 sites), Rodway and Regehr (2006; 34 sites) and E. Leupin (2000; 44 sites (within the FWCP study area)). In total, 292 sites had received previous inventory effort within the FWCP study area. Coastal tailed frog was detected at 3 discrete stream reaches 2013 (MFLNRO), 7 discrete stream reaches sites in 2009 (Wind and Friele), one discrete stream reach in 2006 (Rodway and Regehr), and four discrete stream reaches in 2000 (Leupin). In total, coastal tailed frog had been formally reported at only 15 discrete stream reaches in seven watersheds as a cumulative effort from all four previous TCS inventories. In 2016, we collected stream water samples from the same 28 previously sampled watersheds. Tailed frog eDNA was detected at 34 sites from nine additional new watersheds increasing the number of confirmed extant occurrences of coastal tailed frog to 16 watersheds within the FWCP study area at a fraction of the cost relative to the four previously funded formal TCS inventories (**Figure 6** and **Figure 7**).

Results from our 2016 eDNA survey provide evidence of tailed frog occurrence in the headwaters of the Bridge, Shulaps, Cayoosh, Hurley, Lillooet River, Downton and Anderson Lake watersheds. Conclusive evidence of an extant population of tailed frog in the Shulaps range, along the west-side of the Yalakom River drainage, is particularly significant as this population appears, based on current data, to be disjunct and geographically isolated from the larger relatively contiguous population elsewhere in the Bridge-Seton watershed. In addition to documenting tailed frog in nine new watersheds, additional occupied tributaries in each of the drainages where tailed frog was known to occur based on previous inventory were also documented during the 2016 eDNA study further augmenting our understanding of the distribution of coastal tailed frog within the FWCP study area.

6.0 MANAGEMENT RECOMMENDATIONS

The results of this study, when contrasted with results from previous studies using conventional methods, provide compelling support for the use of eDNA as an alternative method for detecting the presence of aquatic taxa, including coastal tailed frog. The rapid field collection associated with eDNA studies (relative to conventional TCS methods), the relatively low cost of filter materials, the elimination of observer bias, and relatively high efficacy (i.e., relatively greater detection probabilities) suggest that eDNA methods are more efficient and more effective for tailed frog inventory than the current RISC standard TCS methods.

Additional future eDNA studies are recommended range-wide to better document the current extent, abundance, and distribution of tailed frog in BC. Within the FWCP study area additional sites were mapped and proposed for inventory in 2017 using eDNA methods however this funding application was not successful. Resubmission in 2018 is anticipated.

All streams occupied by tailed frog are eligible for species specific management under the Government Actions Regulation of *FRPA*. We recommend establishment of new WHAs to afford species-specific focused management for tailed frog within the area. Foremost among these recommendations is protection for the newly confirmed population in the Shulaps as this population appears to be completely isolated from all other tailed frog occurrence locations within the FWCP study area.

7.0 COMMUNITY OUTREACH

The Bridge-Seton coastal tailed frog eDNA Project involved substantial community engagement during implementation. Outreach efforts were completed April 16 2017; this informative presentation ensured that the important knowledge gained during this study was shared with the local community, naturalists groups, biologists, and the public.

8.0 CLOSURE

We sincerely appreciate the opportunity to have assisted you with the anticipated information requirements for this project. If there are any questions, please do not hesitate to contact the undersigned by phone or email.

Report prepared by:
Hemmera Envirochem Inc.

Jared Hobbs, M.Sc., R.P.Bio.
Senior Biologist
250.889.2071
jhobbs@hemmera.com

Report reviewed by:
Hemmera Envirochem Inc.

Sarah Bowie, M.Sc. R.P.Bio.
Project Manager, Biologist
604.669.0424 (441)
sbowie@hemmera.com

9.0 REFERENCES

BC Conservation Data Centre. 2015. BC Species and Ecosystems Explorer. BC Ministry of Environment Victoria, BC. Available: http://a100.gov.bc.ca/pub/eswp/ (accessed Jan 7, 2015).

Bohmann, K., A. Evans, M.T.P. Gilbert, G.R. Carvalho, S. Creer, M. Knapp, D.W. Yu and M. de Bruyn. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution, 29:358-367.

COSEWIC. 2011. COSEWIC assessment and status report on the Coastal tailed frog *Ascaphus truei* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa, ON.

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

Dupuis, L. 2004. Coastal tailed frog. Accounts and measures for managing identified wildlife. British Columbia Ministry of Water, Land and Air Protection, Victoria, British Columbia. 12pp.

Dupuis, L. and P. Friele. 1996. Riparian management and the tailed frog. Report for B.C. Ministry of Forests, Smithers, BC. 18pp.

Essner Jr, R. L., D.J. Suffian, P.J. Bishop, and S.M. Reilly. 2010. Landing in basal frogs: evidence of saltational patterns in the evolution of anuran locomotion. Naturwissenschaften 97:935-939.

Frid, L., Friele, P. and L. Dupuis. 2003. Defining effective Wildlife Habitat Areas for tailed frog (Ascaphus truei) populations in coastal British Columbia. Essa Technologies Ltd. report to Ministry of Water, Land and Air Protection. Nanaimo, B.C. Ficetola, G.F., C. Miaud, F. Pompanon, and Taberlet. 2008. Species detection using environmental DNA from water samples. Biology Letters 4:423–425.

Fish and Wildlife Compensation Plan (FWCP). 2011. Bridge/Seton River Watershed Species of Interest Action Plan – Final Draft. 30pp.

Fukumoto, S., A. Ushimaru, and T. Minamoto. 2015. A basin-scale application of environmental DNA assessment for rare endemic species and closely related exotic species in rivers: a case study of giant salamanders in Japan. Journal of Applied Ecology 52: 358–365.

Goldberg, C.S., Pilliod, D.S., Arkle, R.S., and 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. and Strickler 2013. eDNA protocol; sample collection with cellulose nitrate filters. University of Idaho. 9pp.

Goldberg, C. S., Strickler and D. Pilliod 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biological Conservaiton. 183:1-3.

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

Hobbs, J. and C. Goldberg. 2016. Standard Operating Procedure. Environmental DNA Protocol for Freshwater Aquatic Ecosystems. Prepared for B.C. Ministry of Environment. 1-25.

Hobbs. J, E. Vincer and I. Adams 2015. Rocky Mountain Tailed Frog (Ascaphus montanus) Environmental DNA (eDNA) Inventory in the Flathead and Wigwam River Watersheds. Prepared for B.C. MLFNRO.

Hobbs. J and I. Adams 2016. Rocky Mountain Tailed Frog Environmental DNA Inventory ~ Flathead and Wigwam River Watersheds. Year Two (2015). Prepared for B.C. MLFNRO.

Hobbs. J and I.Adams 2017 (*in prep*). Rocky Mountain Tailed Frog Environmental DNA Inventory ~ Yahk and Moyie River Watersheds. Year Two (2015). Prepared for B.C. MLFNRO.

Ovaska et al 2017. eDNA Inventory of Endangered Frog Species in the Lower Mainland. Year one (of three). Prepared for HCTF.

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

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

Meidinger, D., and J. Pojar, editors. 1991. Ecosystems of British Columbia. British Columbia Ministry of Forests Special Report Series No. 6, Victoria, British Columbia, Canada. 330 pp.

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

Pilliod, D.S., Goldberg, C.S., Arkle, R.S. and L.P. Waits. 2013a. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Canadian Journal of Fisheries and Aquatic Science 70:1123-1130.

Pilliod, D.S., C.S. Goldberg, M.B. Laramie, and L.P. Waits. 2013b. Application of environmental DNA for inventory and monitoring of aquatic species. US Geological Survey Fact Sheet 2012-3416.

Ritland, K., L.A. Dupuis, F.L. Bunnell, W.L.Y. Hung, and J.E. Carlson. 2000. Phylogeography of the tailed frog (*Ascaphus truei*) in British Columbia. Can. J. Zool. 78:1749-1758.

Steventon, D. and M. Todd. 2012. Environmental DNA (eDNA) for Inventory and Monitoring of the Tailed-Frog (*Ascaphus truei*). FLNRO Research Project Workplan.

Thomsen, P.F., J. Kielgast, L.L. Iversen, C. Wiuf, M. Rasmussen, M.T.P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology 21:2565–2573.

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

APPENDIX 1

Sample Site Characteristics

Appendix 1: 2016 eDNA Sample Sites

Site Name	Sample ID	Zone	Easting	Northing	Elev	eDNA Result	Known Site	Aspect	BEC	Watershed	Watershed Area	Accuracy	Date	Time	Observers	Precip	Cloud Cover	Air Temp	Water Temp	Photo ID	Stream Order	Reach Desc.	Dominant Substrate	Subdominant Substrate	Embeddedness	Gradient	Channel Bed Stability	Logging Activity	Riparian Cover (within	Riparian Width (m)	ASTR Suit. Rating	Coastal tailed frog Adults	Coastal tailed frog Tadpoles	Fish Observed	WHA suitability	Comments
Sebring Ck	SEB	10	548481	5625237	661	Negative	No	10	IDF xc	Bridge River	7701	< 5 m	08/08/2016	14:35	JH/JG	LR	100	15	-	See iPad	S5	Cascade	Fines	Gravel	High	15	Low	No	60	Infinite	Low	No	No	No		
Pickup Ck	Pick	10	498236	5600828	993	Positive	Yes	262	ESSFmw	Railroad Creek	2665	< 5 m	13/08/2016	14:20	JH/JG	None	0	30	9	See iPad	S6	Run	Gravel	Cobble	Low	5	High	In area, not	60	RL Infinite; I	High	No	No	No		
Sidecar Ck	SCR	10	497434	5606939	1215	Positive	Yes	218	ESSFmw 2	Donelly Creek	3762	< 5 m	13/08/2016	15:00	JH/JG	None	0	28	10	See iPad	S6	Riffle	Cobble	Gravel	Medium	5	High	In area	30	RL Infinite; I	High	No	No	No		
Hurley Trib 3	HT3	10	497642	5610606	1202	Negative	No	288	MS mw 2	Donelly Creek	3762	< 5 m	13/08/2016	15:12	JH/JG	None	0	28	8	See iPad	S6	Riffle	Cobble	Gravel	High	6	High	In area	60	Infinite	Medium	No	No	No		
CatSki Ck (Culvert)	CCR	10	502739	5606331	1365	Negative	No	45	ESSFmw 2	Catski	5329	< 5 m	13/08/2016	16:05	JH/JG	None	0	26	12	See iPad	S6	Riffle	Fines	Gravel	High	5	High	Yes, RR log	<10	0	Low	No	No	No		Collected directly from culvert; possibly drop site, low suitability
CatSki Trib 4	CT4	10	502711	5607302	1387	Positive	Yes	87	ESSFmw 2	Catski	5329	< 5 m	13/08/2016	16:25	JH/JG	None	0	26	8	See iPad	S3	Riffle	Fines	Boulder	High	7	Low	In area	25	RL <10 m; R	Low	No	No	No		Point not on trim line; is ~ 500 m south of trim line
CatSki Trib 3	CT3	10	503308	5607852	1354	Positive	Yes	331	ESSFmw 2	Catski	2067	< 5 m	13/08/2016	16:37	JH/JG	None	0	25	10	See iPad	S3	Step/Riffle	Boulder	Fines	Medium	7	High	In area; bloc	25	RL <10 m; R	Low	No	No	No		Moss on boulders and large cobbles
CatSki 2 (Mainstem)	CC2	10	502942	5609315	1305	Positive	Yes	24	MS mw 2	Catski	5329	< 5 m	13/08/2016	16:50	JH/JG	None	0	23	-	See iPad	S2	Riffle	Fines	Cobble	High	3	Medium	No	10	Infinite	Low	No	No	No		High suspended solids
CatSki Trib 7	CT7	10	502917	5609316	1305	Positive	No	60	MS mw 2	Catski	5329	< 5 m	13/08/2016	17:00	JH/JG	None	0	20	6	See iPad	S6	Riffle	Gravel	Fines	High	5	High	In area, not	80	Infinite	Low	No	No	No		Wasps
Hurley 6	H6	10	502705	5616781	1073	Positive	Yes	315	MS dc 1	Hurley River	3297	< 5 m	13/08/2016	17:45	JH/JG	None	0	27	10	See iPad	S3	Riffle	Cobble	Gravel	High	8	High	Yes, runs th	30	0	High	No	No	No		
Sucker Creek	Skr	10	511957	5634532	-	Negative	No	63	IDF dc	Fergusson Creek	2882	< 5 m	14/08/2016	7:40	JH/JG	None	0	14	9	None taken	S3	Riffle	Cobble	Gravel	Low	8	High	No	60	Infinite	Medium	No	No	No		
Ipoo Creek	Ipoo	10	481016	5632853	866	Negative	No	174	MS mw 2	Bridge River	7792	< 5 m	14/08/2016	12:32	JH/JG	None	0	30	9	See iPad	S6	Cascade	Boulder	Fines	Medium	15	High	No	75	Infinite	Low	No	No	No		
Ochre Creek	OCH	10	480227	5632435	866	Negative	No	338	MS mw 2	Bridge River	7792	< 5 m	14/08/2016	12:41	JH/JG	None	0	30	9	See iPad	S6	Cascade	Boulder	Gravel	Low	12	High	No	85	Infinite	Low	No	No	No		Collected at lower crossing with Bridge Rd
G. Bear Creek	GBR	10	483205	5630527	996	Positive	No	172	MS mw 2	Bridge River	7792	< 5 m	14/08/2016	13:00	JH/JG	None	0	30	9	See iPad	S3	Riffle	Boulder	Gravel	Low	5	Medium	Yes; abover	30	5m above R	Medium	No	No	No		
Mac Trib 2	MAC2	10	485540	5628535	900	Negative	No	198	MS mw 2	McParlon Creek	6273	< 5 m	14/08/2016	13:30	JH/JG	None	0	30	8	See iPad	S2	Cascade	Boulder	Gravel	High	8	High	No	60	Infinite	Low	No	No	No		
Cathy Creek	Cathy	10	502682	5630448	799	Negative	No	310	IDF dc	Bridge River	8915	< 5 m	14/08/2016	14:05	JH/JG	None	0	30	-	See iPad	S3	Cascade	Boulder	Gravel	Medium	10	High	No	60	Infinite	Low	No	No	No		Transmission line crosses creek ~ 20m u/s of sample site
Paul Creek	Paul	10	504232	5629919	792	Positive	No	52	IDF dc	Bridge River	8915	< 5 m	14/08/2016	14:15	JH/JG	None	0	30	-	See iPad	S3	Cascade	Cobble	Gravel	Medium	12	High	No	60	Infinite	Medium	No	No	No		
Ault Creek	Ault	10	506237	5629120	675	Negative	No	8	IDF dc	Bridge River	8915	< 5 m	14/08/2016	14:30	JH/JG	None	0	30	-	See iPad	S3	Falls	Fractured be	Cobble	Medium	70	High	No	70	Infinite	Low	No	No	No		Low suitability at sample site; hoping better habitat upstream.
Gwyneth Ck	Gwyn	10	508268	5628612	888	Negative	No	349	IDF dc	Bridge River	8915	< 5 m	14/08/2016	14:45	JH/JG	None	0	30	-	See iPad	S6	Run	Boulder	Cobble	Medium	15	High	No	75	Infinite	Low	No	No	No		Low flow at sample site
Cadwallader Ck Hdwtr	Cad HD	10	525243	5614052	1420	Negative	No	11	ESSFmw 2	Cadwallader Creek	3202	< 5 m	14/08/2016	16:35	JH/JG	None	0	25	-	See iPad	S3	Riffle	Cobble	Fines	High	3	Medium	Yes, cutbloc	60	d/s of bridge	Low	No	No	No		
Cadwallader Trib 1	CT1	10	525408	5614376	1435	Negative	No	219	ESSFmw 2	Cadwallader Creek	3202	< 5 m	14/08/2016	16:45	JH/JG	None	0	25	-	See iPad	S3	Run	Boulder	Gravel	High	10	Medium	Yes, in area	60	20	Low	No	No	No		
Fancy Bridge	FABR	10	521597	5619908	1314	Negative	No	354	MS dc 1	Cadwallader Creek	6968	< 5 m	14/08/2016	17:15	JH/JG	None	0	23	-	See iPad	S5	Run	Cobble	Gravel	High	8	High	Yes, in area	60	Infinite	Low	No	No	No		
Hawthorne Creek	HAW	10	520410	5621247	1279	Negative	No	153	MS dc 1	Hawthorn Creek	3136	< 5 m	14/08/2016	17:26	JH/JG	None	0	23	-	See iPad	S2	Run	Boulder	Gravel	High	8	High	Yes, in area	70	Infinite	Low	No	No	No		
Cadwallader Ck 2	Cad 2	10	519817	5621578	1286	Positive	No	285	MS dc 1	Cadwallader Creek	5265	< 5 m	14/08/2016	17:31	JH/JG	None	0	23	-	See iPad	S6	Run	Cobble	Gravel	Medium	8	High	Yes, in area	80	Infinite	Medium	No	No	No		Some moss on rocks
Crazy Creek	CZY	10	518305	5621718	1271	Negative	No	10	MS dc 1	Cadwallader Creek	5265	< 5 m	14/08/2016	17:45	JH/JG	None	0	23	-	See iPad	S3	Run	Cobble	Gravel	Low	15	High	Yes, in area	70	10 m	Medium	No	No	No		
Grey Rock Creek	GRY	10	514123	5634913	887	Positive	No	294	IDF dc	Bridge River	4844	< 5 m	15/08/2016	7:50	JH	None	0	12	9	See iPad	S2	Riffle	Gravel	Fines	High	3	High	Yes, on wes	60	RL Infinite; I	Low	No	No	No		
Truax Upper	TRU	10	521767	5632099	1423	Negative	No	54	ESSFdv 1	Truax Creek	5289	< 5 m	15/08/2016	8:40	JH	None	0	9	6	See iPad	S3	Cascade	Cobble	Gravel	Medium	10	High	In area, not	60	Infinite	Medium	No	No	No		
Truax Trib	TT	10	521735	5633252	1513	Positive	No	77	ESSFdv 1	Truax Creek	5289	< 5 m	08/08/2016	8:45	JH	None	0	9	6	See iPad	S3	Cascade	Boulder	Cobble	Low	20	High	Yes, 20 yr c	30	RL Infinite; I	Medium	No	No	No		Flooded; very dilute; heavey rain event recently
Truax Trib 1	TT1	10	521810	5633670	1471	Negative	No	94	ESSFdv 1	Truax Creek	5289	< 5 m	08/08/2016	8:50	JH	None	0	9	9	See iPad	S5	Cascade	Cobble	Gravel	Low	15	High	In area, not	30	Infinite	High	No	No	No		Stream in flood
Carl Ck	Carl	10	510729	5622334	995	Negative	No	134	ESSFdvw	Hurley River	7625	< 5 m	15/08/2016	12:00	JH/JG	None	0	20	-	See iPad	S3	Run	Cobble	Gravel	Low	8	High	Yes; stream	30	0	Medium	No	No	No		
Mason Ck	Mas	10	509954	5624130	978	Positive	No	324	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	12:15	JH/JG	None	0	22	-	See iPad	S3	Run	Cobble	Gravel	Low	10	High	Yes; RR cut	85	RR 0; RL <1	High	No	No	No		
Hurley 3	HU3	10	506092	5621859	1034	Positive	No	3	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	12:30	JH/JG	None	0	25	-	See iPad	S3	Riffle	Cobble	Boulder	Low	15	Low	Yes; runs th	60	<10m abou	High	No	No	No		
Regehr Creek	Reg	10	506427	5624391	1179	Positive	Yes	208	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	12:50	JH/JG	None	0	25	-	See iPad	S6	Step-pool	Boulder	Gravel	Low	20	High	In area	70	Infinite	High	No	Yes	No		Tadpole 5m u/s from sample collection site
Hurley 12B	HU12B	10	505892	5623907	1152	Positive	No	121	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	13:00	JH/JG	None	0	25	-	See iPad	S6	Riffle	Cobble	Gravel	High	15	High	Yes; creek	30	0	Low	No	No	No		
Hurley 12	HU12	10	504738	5621701	1072	Positive	No	163	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	13:15	JH/JG	None	0	25	-	See iPad	S3	Falls/Casca	Gravel	Boulder	Medium	45	High	Yes, in area	70	Infinite	Low	No	No	No		High flow; turbid
Joce Creek	Joce	10	503823	5619707	1035	Negative	No	143	MS dc 1	Hurley River	7625	< 5 m	15/08/2016	13:40	JH/JG	None	0	30	-	See iPad	S6	Run	Cobble	Gravel	Medium	20	High	No; avalanc	70	Infinite	Low	No	No	No		
Waterfalls (H4)	WTF	10	503914	5619249	1029	Positive	No	270	MS dc 1	Hurley River	3297	< 5 m	15/08/2016	14:00	JH/JG	None	0	30		See iPad	S3	Run	Boulder	Gravel	High	5	High	No	60	Infinite	Medium	No	No	No		
Pascall Ck	Pas	10	537358	5580586	1237	Negative	No	354	ESSFmw 2	Cayoosh Creek	7294	< 5 m	16/08/2016	9:10	JH/JG	None	0	16	8	See iPad	S3	Cascade	Cobble	Boulder	Low	15	High	Yes, in area	70	RR Infinite;	High	No	No	No		Embeddedness is high at Rd crossing
Cayoosh Ck West	CayW	10	536987	5583234	1359	Positive	No	149	ESSFmw 2	Cayoosh Creek	7294	< 5 m	16/08/2016	10:20	JH/JG	None	0	20	10	See iPad	S3	Step-pool	Gravel	Cobble	Medium	10	High	Yes, in area	65	Infinite	Medium	No	No	No		
Cayoosh Ck Main	CayM	10	537721	5581574	1269	Positive	Yes	221	ESSFmw 2	Cayoosh Creek	7294	< 5 m	16/08/2016	11:00	JH/JG	None	0	25	11	See iPad	S2	Run	Cobble	Gravel	Medium	5	High	No	25	Infinite	Low	No	No	No		Collected under bridge at Hwy 99; dilution test b/c ASTR in Cay East (PF)
Cherise Ck	Cher	10	541719	5580823	1243	Negative	No	107	CWH ms 1	Haylmore Creek	8246	< 5 m	16/08/2016	11:20	JH/JG	None	0	22	9	See iPad	S2	Riffle	Cobble	Gravel	High	5	High	No	40	Infinite	Low	No	No	No		Hiked down Cherise Ck Summer trail; turbid
Casper Ck	Casp	10	544453	5580549	1255	Positive	No	309	CWH ms 1	Caspar Creek	2253	< 5 m	16/08/2016	11:55	JH/JG	None	0	25	-	See iPad	S3	Riffle	Cobble	Gravel	Low	5	High	In area	60	Infinite	High	No	No	No		
VanHorlick Ck (trib)	VHT1	10	549561	5577902	1314	Negative	No	288	ESSFmw 2	Van Horlick Creek	9550	< 5 m	16/08/2016	12:20	JH/JG	None	0	25	14	See iPad	S3	Step-pool	Cobble	Gravel	Medium	8	High	In area; cutb	60	Infinite	Low	No	No	No		Picked up trib; algae on rocks
Steep Ck	STP	10	549292	5583770	1173	Positive	Yes	323	CWH ms 1	Cayoosh Creek	4651	< 5 m	16/08/2016	13:00	JH/JG	None	0	25	13	See iPad	S3	Cascade	Cobble	Gravel	Medium	10	High	No	60	Infinite	High	No	No	No		
Kane Ck	Kane	10	553085	5587549	1117	Negative	No	272	MS mw 2	Cayoosh Creek	5177	< 5 m	16/08/2016	13:20	JH/JG	None	0	25	14	See iPad	S2	Falls	Cobble	Gravel	Medium	40	Medium	In area	65	Infinite	Medium	No	No	No		Collected at bottom of alls at hwy 99 crossing
Blowdown Ck	BlowM	10	554572	5584413	1367	Negative	Yes	255	ESSFdv 1	Blowdown Creek	5563	< 5 m	16/08/2016	14:00	JH/JG	None	0	28	14	See iPad	S2	Riffle	Boulder	Gravel	Medium	5	High	In area	40	Infinite	High	No	No	No		Known site, collected at PF's iButton site
Blowdown Trib 1	BT1	10	553243	5587584	1176	Negative	No	291	MS mw 2	Cayoosh Creek	5177	< 5 m	16/08/2016	15:00	JH/JG	None	0	25	14	See iPad	S6	Run	Cobble	Boulder	Low	25	High	In area	75	Infinite	High	No	No	No		Pierre has looked here
Fran's Ck	Fran	10	553144	5589725	1062	Positive	No	30	MS mw 2	Cayoosh Creek	5177	< 5 m	16/08/2016	15:15	JH/JG	None	0	25	12	See iPad	S3	Run	Cobble	Gravel	Low	10	High	No	70	Infinite	High	No	No	No		Native bridge
Channel Ck	Chan	10	555031	5591837	1033	Positive	No	325	IDF dc	Cayoosh Creek	5177	< 5 m	16/08/2016	15:30	JH/JG	None	0	25	16	See iPad	S3	Run	Boulder	Gravel	Low	15	High	No	70	Infinite	Medium	No	No	Yes		PF iButton site; good interstitial but lots of algae; fish swimming upstream under bridge
Little Ck	LC	10	558306	5595181	979	Positive	No	158	IDF dc	Cayoosh Creek	4214	< 5 m	16/08/2016	15:45	JH/JG	None	0	25	12	See iPad	S3	Step-pool	Cobble	Gravel	Low	10	High	No	75	Infinite	High	No	No	No		
Ainsworth's Folly Ck	AF	10	559809	5597971	891	Negative	No	31	IDF dc	Cayoosh Creek	4214	< 5 m	16/08/2016	16:00	JH/JG	None	0	25	13	See iPad	S3	Step-pool	Cobble	Gravel	Low	10	High	No	80	Infinite	High	No	No	No		PF iButton
Gott Ck	Gott	10	561321	5598445	833	Positive	No	340	IDF dc	Gott Creek	6958	< 5 m	16/08/2016	16:30	JH/JG	None	0	30	16	See iPad	S2	Riffle	Cobble	Gravel	High	4	High	No	40	Infinite	Low	No	No	No		Low gradient, large creek
Boulder Ck	BLDR	10	563219	5599470	825	Negative	No	260	IDF dc	Boulder	5485	< 5 m	16/08/2016	16:40	JH/JG	None	0	30	14	See iPad	S3	Step-pool	Boulder	Gravel	Low	15	High	No	70	Infinite	Low	No	No	No		Not suitable habitat at sample site; DNA
Copper Ck	CPR	10	563488	5608826	659	Negative	No	166	IDF xc	Copper Creek	2447	< 5 m	16/08/2016	16:45	JH/JG	None	0	30	10	See iPad	S3	Step-pool	Cobble	Gravel	Low	10	High	No	60	Infinite	High	No	No	No		
La Mare Creek	LAM	10	549345	5646965	869	Negative	No	85	IDF dk 1	Junction Creek	6221	< 5 m	16/08/2016	12:05	JH	None	0	23	8	See iPad	S3	Run	Gravel	Fines	Low	4	High	No	60	Infinite	Low	No	No	No		Light turbidity
Shulaps Creek	SHUL	10	551022	5642014	806	Positive	Yes	32	IDF xc	Shulaps Creek	6896	< 5 m	16/08/2016	12:45	JH	None	0	26	11	See iPad	S3	Run	Cobble	Boulder	Medium	4	High	No	60	Infinite	High	No	No	No		

Appendix 1: 2016 eDNA Sample Sites

Site Name	Sample ID	Zone	Easting	Northing	Elev	eDNA Result	Known Site	Aspect	BEC	Watershed	Watershed Area	Accuracy	Date	Time	Observers	Precip	Cloud Cover	Air Temp	Water Temp	Photo ID	Stream Order	Reach Desc.	Dominant Substrate	Subdominant Substrate	Embeddedness	Gradient	Channel Bed Stability	Logging Activity	Riparian Cover (within	Riparian Width (m)	ASTR Suit. Rating	Coastal tailed frog Adults	Coastal tailed frog Tadpoles	Fish Observed	WHA suitability	Comments
Serpentine Creek	SERP	10	546897	5640789	1224	Negative	No	185	IDF dc	Shulaps Creek	6896	< 5 m	16/08/2016	14:40	JH	None	0	30	12	See iPad	S3	Run	Boulder	Gravel	Medium	8	High	No	50	Infinite	High	No	No	No		local microhabitat temp ~10degrees; site located in an old burn
Marshall Ck	Mar	10	536264	5634411	824	Negative	No	171	IDF xc	Marshall Creek	6925	< 5 m	16/08/2016	11:16	JG	None	0	25	9	See iPad	S3	Step-pool	Cobble	Gravel	Low	5	High	No	70	RR Infinite;	High	No	No	No		ASTR reported in Marshall previously to Darwyn
White Saddle Ck [White (	WHT	10	549602	5617437	255	Negative	No	15	IDF xc	Whitecap Creek	7463	< 5 m	16/08/2016	14:00	JG	None	0	28	13	See iPad	S2	Step-run	Gravel	Fines	High	3	Low	No	20	Infinite	Low	No	No	No		Creek flooded 2 yrs ago; sample site blown out; turbed water; not suitable here but potentially way up higher elevation
Washout Ck [Marne Ck]	Wash	10	542303	5612586	492	Positive	No	149	IDF ww 1	Seton River	3683	< 5 m	16/08/2016	14:50	JG	None	0	30	14	See iPad	S3	Step-pool	Boulder	Fines	Low	15	Low	No	15	Infinite	Low	No	No	No		Frequent flooding/washouts at road; water is cool/clear and substrate is good; possible tehre is suitable habitat upstream
Conroy Ck [Connell]	Con	10	540654	5611093	470	Positive	No	85	IDF ww 1	Connel Creek	5785	< 5 m	16/08/2016	15:30	JG	None	0	28	14	See iPad	S2	Step-pool	Cobble	Boulder	Low	15	High	No	40	Infinite	Medium	No	No	No		Ravine at sample site
MacGillivray Ck	MacG	10	538211	5608006	664	Negative	No	20	IDF ww 1	McGillivray Creek	5231	< 5 m	16/08/2016	16:06	JG	None	0	28	12	See iPad	S2	Step-pool	Boulder	Fines	Medium	10	High	No	70	Infinite	Medium	No	No	No		Looks decent for ASTR, best so far on N side of Anderson Lake
Haylemore Trib 2	HayleT2	10	543713	5593884	1103	Positive	No	141	CWH ms 1	Haylmore Creek	8246	< 5 m	16/08/2016	17:44	JG	None	0	30	11	See iPad	S5	Step-pool	Boulder	Cobble	Low	20	High	No	70	Infinite	High	No	No	No		
Haylemore Trib 3	HayleT3	10	540901	5596703	837	Positive	No	182	CWH ds 1	Haylmore Creek	8246	< 5 m	16/08/2016	18:10	JG	None	0	30	15	See iPad	S5	Step-pool	Cobble	Gravel	Medium	20	High	Yes, in area	60	Infinite	Medium	No	No	No		
Upper Shulaps	USH	10	545847	5638438	1432	Negative	No	141	MS dc 3	Shulaps Creek	6896	< 5 m	16/08/2016	15:20	JH	None	0	30	12	See iPad	S3	Run	Boulder	Cobble	Low	6	High	Yes, cutblo	60	Infinite	High	No	No	No		Old fire nearby; rocj is fractured granite
Holbrook Ck	HOL	10	545974	5638199	1453	Negative	No	32	MS dc 3	Shulaps Creek	6896	< 5 m	16/08/2016	15:30	JH	None	0	30	11	See iPad	S3	Run	Cobble	Gravel	High	3	Low	No	60	Infinite	Medium	No	No	No		
Upper Holbrook Ck	HOLU	10	546566	5636501	1758	Negative	No	351	ESSFdv 2	Shulaps Creek	6896	< 5 m	16/08/2016	15:45	JH	None	0	30	12	See iPad	S3	Run	Boulder	Cobble	Medium	3	High	Logging dow	10	Patchy	High	No	No	No		Lake fed, riparien has patchy Piceeng & Abielas
La Rochelle Ck	Lar	10	549711	5636705	1513	Positive	No	135	MS dc 3	La Rochelle Creek	3104	< 5 m	16/08/2016	16:45	JH	None	0	30	11	See iPad	S3	Riffle	Cobble	Gravel	Medium	3	High	Yes, old cut	60	Infinite	Medium	No	No	No		
Doe Ck	Doe	10	552806	5636374	1412	Negative	No	345	IDF dc	Yalakom River	4242	< 5 m	16/08/2016	16:30	JH	None	0	30	8	See iPad	S2	Run	Cobble	Gravel	High	3	High	In area	-	-	Low	No	No	No		
Buck Ck	Buck	10	555163	5636258		Positive	No	76	IDF dc	Yalakom River	4242	< 5 m	16/08/2016	16:40	JH	None	0	30	11	See iPad	S2	Run	Boulder	Cobble	Low	7	High	In area	60	-	Medium	No	No	No		
Downton Creek Trib	DownT	10	554056	5602877	1475	Negative	No	101	MS dc 1	Downton Creek	9635	< 5 m	16/08/2016	20:50	JH	None	0	30	10	See iPad	S3	Cascade	Boulder	Cobble	Low	6	Medium	Yes	20	0	Medium	No	No	No		
Downton Creek Trib2	DownT2	10	556348	5604314		Negative	No	130	MS dc 1	Downton Creek	9635	< 5 m	16/08/2016	20:30	JH	None	0	30	10	See iPad	S3	Step-pool	Boulder	Cobble	Low	4	High	Yes	-	-	High	No	No	No		
Not tested																																				
Truax Main (HV)	TRM	10	521820	5633658	1423	NA	No					< 5 m	08/08/2016	17:35	JH/JG	None	100	12	8	See iPad	S3	Cascade	Cobble	Gravel	Medium	10	High	In area, not	60	Infinite	Medium	No	No	No		Stream in flood
Truax Trib 1 (HV)	TT1	10	521810	5633670	1471	NA	No					< 5 m	08/08/2016	17:45	JH/JG	None	100	12	9	See iPad	S5	Cascade	Cobble	Gravel	Low	15	High	In area, not	30	Infinite	High	No	No	No		Stream in flood
Truax Trib (HV)	TT	10	521735	5633252	1513	NA	No					< 5 m	08/08/2016	18:04	JH/JG	None	100	12	6	See iPad	S3	Cascade	Boulder	Cobble	Low	20	High	Yes, 20 yr c	30	RL Infinite;	Medium	No	No	No		Flooded; very dilute; heavey rain event recently

APPENDIX 2
eDNA qPCR Data

MasterSort	Location name	Site ID	Sample replicate	Lab Code	Collection date	Collected by	Collection Time	Zone	Easting	Northing	Filter Date	DPN	eDNA Recovered (ng/uL)	qPCR Viability Frequency	qPCR Viability Call	eFrog Frequency	eFrog Call	ASTR Frequency	Lab Call	Biol Call	Test for ASTR	Known ASTR	Filter start time	Filter end time	Filtering time (calc)	Sample Volume (ml)	Sample Contents	Filtered by	Test Priority	Comments	Project Number
1	Truax Main (HV)	TRM	A		08/08/2016	JH	17:35	10	521820	5633658	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	20:29	21:02	0:33	900	Site water	JH	DO NOT TEST	Flooded- superceeded by Truax Upper?	1629-004.01
2	Truax Main (HV)	TRM	B		08/08/2016	JH	17:35	10	521820	5633658	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	20:29	21:07	0:38	850	Site water	JH	DO NOT TEST	Flooded- superceeded by Truax Upper?	1629-004.01
3	Truax Trib (HV)	TT	A		08/08/2016	JH	18:04	10	521735	5633252	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	21:21	21:58	0:37	600	Site water	JH	DO NOT TEST	Flooded- superceeded	1629-004.01
4	Truax Trib (HV)	TT	B		08/08/2016	JH	18:04	10	521735	5633252	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	21:11	21:54	0:43	750	Site water	JH	DO NOT TEST	Flooded- superceeded	1629-004.01
5	Sidecar Ck	SCR	A	SCR-A	13/08/2016	JH	15:00	10	497434	5606939	August 13, 2016	12	1.4	4/4	Y	ND	ND	8/8	Y	Y	Yes	Yes	19:36	19:41	0:05	1100	Site water	JH	High		1629-004.01
6	Sidecar Ck	SCR	B		13/08/2016	JH	15:00	10	497434	5606939	August 13, 2016									Y	No	Yes	19:36	19:42	0:06	1050	Site water	JH	High		1629-004.01
7	CatSki Ck (Culvert)	CCR	A	CCR-A	13/08/2016	JH	16:05	10	502739	5606331	August 13, 2016	57	1.3	4/4	Y	0/8	N	1/8	N	N	Yes	Yes	19:51	19:55	0:04	1000	Site water	JH	Low	Collected from Culvert, headwaters, probably above occurrence	1629-004.01
8	CatSki Ck (Culvert)	CCR	B	CCR-B	13/08/2016	JH	16:05	10	502739	5606331	August 13, 2016	106	1.9	4/4	Y	1/8	N	0/8	N	N	Yes	Yes	19:50	19:55	0:05	1000	Site water	JH	Low	Collected from Culvert, headwaters, probably above occurrence	1629-004.01
9	CatSki Trib 3	CT3	A	CT3-A	13/08/2016	JH	16:37	10	503308	5607852	August 13, 2016	59	1.9	4/4	Y	3/8	Y	6/8	Y	Y	Yes	Yes	20:06	20:10	0:04	1000	Site water	JH	High	Testing Transport, PF detection 4km upstream	1629-004.01
10	CatSki Trib 3	CT3	B		13/08/2016	JH	16:37	10	503308	5607852	August 13, 2016									Y	No	Yes	20:06	20:10	0:04	1000	Site water	JH	High	Testing Transport, PF detection 4km upstream	1629-004.01
11	CatSki Trib 7	CT7	A	CT7-A	13/08/2016	JH	17:00	10	502917	5609316	August 13, 2016	55	1.5	4/4	Y	1/8	N	4/8	Y	Y	Yes	No	20:50	20:54	0:04	1100	Site water	JH	High		1629-004.01
12	CatSki Trib 7	CT7	B		13/08/2016	JH	17:00	10	502917	5609316	August 13, 2016									Y	No	No	20:50	20:54	0:04	1100	Site water	JH	High		1629-004.01
13	ARC	ARC	A	ARC-A	13/08/2016	JH	15:10	10	502000	5616000	August 13, 2016	18	0.9	4/4	Y	ND	ND	0/8	N	N	Yes	No	21:09	21:10	0:01	1000	DI	JH	High		1629-004.01
14	ARC	ARC	B		14/08/2016	JH	17:00	10	502000	5616000	August 14, 2016									N	No	No	22:26	22:27	0:01	750	DI	JH	High		1629-004.01
15	Ipoo Creek	Ipoo	A	Ipoo-A	14/08/2016	JH	12:32	10	481016	5632853	August 14, 2016	17	3.5	4/4	Y	ND	ND	1/8	N	N	Yes	No	19:31	19:35	0:04	1000	Site water	JH	High		1629-004.01
16	Ipoo Creek	Ipoo	B	Ipoo-B	14/08/2016	JH	12:32	10	481016	5632853	August 14, 2016	100	6.8	4/4	Y	1/8	N	0/8	N	N	Yes	No	19:31	19:35	0:04	1000	Site water	JH	High		1629-004.01
17	G. Bear Creek	GBR	A	GBR-A	14/08/2016	JH	13:00	10	483205	5630527	August 14, 2016	6	4.2	4/4	Y	1/8	N	5/8	Y	Y	Yes	No	19:44	19:48	0:04	1000	Site water	JH	High		1629-004.01
18	G. Bear Creek	GBR	B		14/08/2016	JH	13:00	10	483205	5630527	August 14, 2016									Y	No	No	19:44	19:48	0:04	1000	Site water	JH	High		1629-004.01
19	Cathy Creek	Cathy	A	Cathy-A	14/08/2016	JH	14:05	10	502682	5630448	August 14, 2016	63	1.1	4/4	Y	3/8	Y	1/8	N	N	Yes	No	20:01	20:06	0:05	1000	Site water	JH	High		1629-004.01
20	Cathy Creek	Cathy	B	Cathy-B	15/08/2016	JH	14:05	10	502682	5630448	August 14, 2016	108	2.7	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:01	20:06	0:05	1000	Site water	JH	High		1629-004.01
21	Ault Creek	Ault	A	Ault-A	14/08/2016	JH	14:30	10	506237	5629120	August 14, 2016	3	3.4	4/4	Y	1/8	N	1/8	N	N	Yes	No	20:18	20:22	0:04	1000	Site water	JH	High		1629-004.01
22	Ault Creek	Ault	B	Ault-B	14/08/2016	JH	14:30	10	506237	5629120	August 14, 2016	99	1.7	4/4	Y	1/8	N	0/8	N	N	Yes	No	20:18	20:22	0:04	1000	Site water	JH	High		1629-004.01
23	Cadwallader Ck Hdwtr	Cad HD	A	CadHD-A	14/08/2016	JH	16:35	10	525243	5614052	August 14, 2016	69	1.8	4/4	Y	3/8	Y	0/8	N	N	Yes	No	20:38	21:10	0:32	850	Site water	JH	High		1629-004.01
24	Cadwallader Ck Hdwtr	Cad HD	B	CadHD-B	14/08/2016	JH	16:35	10	525243	5614052	August 14, 2016	113	4.2	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:38	21:10	0:32	900	Site water	JH	High		1629-004.01
25	Fancy Bridge	FABR	A	FABR-A	14/08/2016	JH	17:15	10	521597	5619908	August 14, 2016	16	3.0	4/4	Y	ND	ND	0/8	N	N	Yes	No	21:41	21:45	0:04	1100	Site water	JH	High		1629-004.01
26	Fancy Bridge	FABR	B	FABR-B	14/08/2016	JH	17:15	10	521597	5619908	August 14, 2016	83	1.3	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:41	21:46	0:05	1000	Site water	JH	High		1629-004.01
27	Cadwallader Ck 2	Cad 2	A	Cad2-A	14/08/2016	JH	17:31	10	519817	5621578	August 14, 2016	41	1.5	4/4	Y	7/8	Y	5/8	Y	Y	Yes	No	22:11	22:16	0:05	1000	Site water	JH	High		1629-004.01
28	Cadwallader Ck 2	Cad 2	B		14/08/2016	JH	17:31	10	519817	5621578	August 14, 2016									Y	No	No	22:11	22:16	0:05	1000	Site water	JH	High		1629-004.01
29	Grey Rock Creek	GRY	A	GRY-A	15/08/2016	JH	7:50	10	514123	5634913	August 15, 2016	13	5.5	4/4	Y	ND	ND	4/8	Y	Y	Yes	No	9:31	9:41	0:10	950	Site water	JH	High		1629-004.01
30	Grey Rock Creek	GRY	B		15/08/2016	JH	7:50	10	514123	5634913	August 15, 2016									Y	No	No	9:31	9:42	0:11	950	Site water	JH	High		1629-004.01
31	Truax Trib	TT	A	TT-A	15/08/2016	JH	8:45	10	521735	5633252	August 15, 2016	33	0.5	4/4	Y	1/8	N	3/8	Y	Y	Yes	No	10:05	10:46	0:41	750	Site water	JH	High		1629-004.01
32	Truax Trib	TT	B		15/08/2016	JH	8:45	10	521735	5633252	August 15, 2016									Y	No	No	10:05	10:46	0:41	750	Site water	JH	High		1629-004.01
33	Carl Ck	Carl	A	Carl-A	15/08/2016	JH	12:00	10	510729	5622334	August 15, 2016	66	1.1	4/4	Y	0/8	N	0/8	N	N	Yes	No	16:48	16:52	0:04	1000	Site water	JH	High		1629-004.01
34	Carl Ck	Carl	B	Carl-B	15/08/2016	JH	12:00	10	510729	5622334	August 15, 2016	92	2.2	4/4	Y	0/8	N	0/8	N	N	Yes	No	16:48	16:52	0:04	1100	Site water	JH	High		1629-004.01
35	Hurley 3	HU3	A	HU3-A	15/08/2016	JH	12:30	10	506092	5621859	August 15, 2016	4	3.7	4/4	Y	0/8	N	2/8	N	N	Yes	No	17:09	17:45	0:36	950	Site water	JH	High		1629-004.01
36	Hurley 3	HU3	B	HU3-B	15/08/2016	JH	12:30	10	506092	5621859	August 15, 2016	96	3.5	4/4	Y	0/8	N	0/8	N	N	Yes	No	17:09	17:51	0:42	925	Site water	JH	High		1629-004.01
37	Hurley 12B	HU12B	A	HU12B-A	15/08/2016	JH	13:00	10	505892	5623907	August 15, 2016	5	7.7	4/4	Y	0/8	N	8/8	Y	Y	Yes	No	18:00	18:04	0:04	1000	Site water	JH	High		1629-004.01
38	Hurley 12B	HU12B	B		15/08/2016	JH	13:00	10	505892	5623907	August 15, 2016									Y	No	No	18:00	18:04	0:04	1000	Site water	JH	High		1629-004.01
39	Joce Creek	Joce	A	Joce-A	15/08/2016	JH	13:40	10	503823	5619707	August 15, 2016	10	1.0	4/4	Y	6/8	Y	0/8	N	N	Yes	No	18:41	18:45	0:04	1000	Site water	JH	High		1629-004.01
40	Joce Creek	Joce	B	Joce-B	15/08/2016	JH	13:40	10	503823	5619707	August 15, 2016	94	2.1	4/4	Y	5/8	Y	0/8	N	N	Yes	No	18:41	18:45	0:04	1000	Site water	JH	High		1629-004.01
41	Pascall Ck	Pas	A	Pas-A	16/08/2016	JH	9:10	10	537358	5580586	August 16, 2016	24	1.7	4/4	Y	1/8	N	1/8	N	N	Yes	No	18:39	18:41	0:02	1000	Site water	JH	High		1629-004.01
42	Pascall Ck	Pas	B	Pas-B	16/08/2016	JH	9:10	10	537358	5580586	August 16, 2016	78	1.2	4/4	Y	0/8	N	0/8	N	N	Yes	No	18:39	18:41	0:02	900	Site water	JH	High		1629-004.01
43	Cayoosh Ck Main	CayM	A	CayM-A	16/08/2016	JH	11:00	10	537721	5581574	August 16, 2016	43	2.1	4/4	Y	3/8	Y	5/8	Y	Y	Yes	Yes	19:25	19:29	0:04	1000	Site water	JH	High	Known ASTR 3km upstream in Cayoosh East	1629-004.01
44	Cayoosh Ck Main	CayM	B		16/08/2016	JH	11:00	10	537721	5581574	August 16, 2016									Y	No	Yes	19:25	19:29	0:04	1000	Site water	JH	High		1629-004.01
45	Casper Ck	Casp	A	Casp-A	16/08/2016	JH	11:55	10	544453	5580549	August 16, 2016	36	3.0	4/4	Y	3/8	Y	3/8	Y	Y	Yes	No	19:39	19:45	0:06	1000	Site water	JH			1629-004.01
46	Casper Ck	Casp	B		16/08/2016	JH	11:55	10	544453	5580549	August 16, 2016									Y	No	No	19:39	19:45	0:06	1000	Site water	JH			1629-004.01
47	Steep Ck	STP	A	STP-A	16/08/2016	JH	13:00	10	549292	5583770	August 16, 2016	42	2.9	4/4	Y	0/8	N	5/8	Y	Y	Yes	Yes	19:55	19:59	0:04	1000	Site water	JH	High		1629-004.01
48	Steep Ck	STP	B		16/08/2016	JH	13:00	10	549292	5583770	August 16, 2016									Y	No	Yes	19:55	20:00	0:05	1000	Site water	JH	High		1629-004.01
49	Blowdown Ck	BlowM	A	BlowM-A	16/08/2016	JH	14:00	10	554572	5584413	August 16, 2016	25	7.1	4/4	Y	2/8	N	1/8	N	N	Yes	Yes	20:09	20:15	0:06	1000	Site water	JH	High		1629-004.01
50	Blowdown Ck	BlowM	B	BlowM-B	16/08/2016	JH	14:00	10	554572	5584413	August 16, 2016	110	2.5	4/4	Y	0/8	N	0/8	N	N	Yes	Yes	20:09	20:15	0:06	1000	Site water	JH	High		1629-004.01
51	Fran's Ck	Fran	A	Fran-A	16/08/2016	JH	15:15	10	553144	5589725	August 16, 2016	23	1.8	4/4	Y	1/8	N	3/8	Y	Y	Yes	No	20:16	20:21	0:05	1000	Site water	JH	High		1629-004.01
52	Fran's Ck	Fran	B		16/08/2016	JH	15:15	10	553144	5589725	August 16, 2016									Y	No	No	20:16	20:21	0:05	1000	Site water	JH	High		1629-004.01
53	Little Ck	LC	A	LC-A	16/08/2016	JH	15:45	10	558306	5595181	August 16, 2016	19	1.6	4/4	Y	ND	ND	8/8	Y	Y	Yes	No	20:37	20:43	0:06	1000	Site water	JH	High		1629-004.01
54	Little Ck	LC	B		16/08/2016	JH	15:45	10	558306	5595181	August 16, 2016									Y	No	No	20:37	20:43	0:06	1100	Site water	JH	High		1629-004.01
55	Gott Ck	Gott	A	Gott-A	16/08/2016	JH	16:30	10	561321	5598445	August 16, 2016	34	4.8	4/4	Y	1/8	N	4/8	Y	Y	Yes	No	20:53	20:56	0:03	900	Site water	JH	High		1629-004.01
56	Gott Ck	Gott	B		16/08/2016	JH	16:30	10	561321	5598445	August 16, 2016									Y	No	No	20:53	20:57	0:04	1000	Site water	JH	High		1629-004.01
57	Copper Ck	CPR	A	CPR-A	16/08/2016	JH	16:45	10	563488	5608826	August 16, 2016	61	0.6	0/4	N	0/8	N	0/8	N	N	Yes	No	21:05	21:11	0:06	1050	Site water	JH	High		1629-004.01
58	Copper Ck	CPR	B	CPR-B	16/08/2016	JH	16:45	10	563488	5608826	August 16, 2016	88	0.8	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:05	21:11	0:06	1050	Site water	JH	High		1629-004.01
59	La Mare Creek	LAM	A	LAM-A	17/08/2016	JH	12:05	10	549345	5646965	August 17, 2016	54	7.9	4/4	Y	1/8	N	1/8	N	N	Yes	No	19:33	19:37	0:04	1000	Site water	JH	High		1629-004.01
60	La Mare Creek	LAM	B	LAM-B	17/08/2016	JH	12:05	10	549345	5646965	August 17, 2016	114	11.5	4/4	Y	0/8	N	0/8	N	N	Yes	No	19:33	19:37	0:04	1000	Site water	JH	High		1629-004.01
61	Serpentine Creek	SERP	A	SERP-A	17/08/2016	JH	14:40	10	546897	5640789	August 17, 2016	53	1.8	4/4	Y	3/8	Y	2/8	N	N	Yes	No	19:50	19:54	0:04	1000	Site water	JH	High		1629-004.01
62	Serpentine Creek	SERP	B	SERP-B	17/08/2016	JH	14:40	10	546897	5640789	August 17, 2016	97	3.0	4/4	Y	0/8	N	0/8	N	N	Yes	No	19:50	19:54	0:04	1000	Site water	JH	High		1629-004.01
63	White Saddle Ck [White Cap]	WHT	A	WHT-A	17/08/2016	JG	14:00	10	549602	5617437	August 17, 2016	1	9.3	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:40	22:17	0:37	1000	Site water	JG	High		1629-004.01
64	White Saddle Ck [White Cap]	WHT	B	WHT-B	17/08/2016	JG	14:00	10	549602	5617437	August 17, 2016	109	12.8	4/4	Y	0/8	N	1/8	N	N	Yes	No	21:40	22:17	0:37	1000	Site water	JG	High		1629-004.01
65	Conroy Ck	Con	A	Con-A	17/08/2016	JG	15:30	10	540654	5611093	August 17, 2016	67	1.2	4/4	Y	2/8	N	0/8	N	Y	Yes	No	22:29	22:33	0:04	1000	Site water	JG	High		1629-004.01
66	Conroy Ck	Con	B	Con-B	17/08/2016	JG	15:30	10	540654	5611093	August 17, 2016	116	2.8	4/4	Y	1/8	N	5/8	Y	Y	Yes	No	22:29	22:33	0:04	1000	Site water	JG	High		1629-004.01
67	Haylemore Trib 2	HayleT2	A	HayleT2-A	17/08/2016	JG	17:44	10	543713	5593884	August 17, 2016	39	2.7	4/4	Y	2/8	N	7/8	Y	Y	Yes	No	22:46	22:50	0:04	1000	Site water	JH	High		1629-004.01
68	Haylemore Trib 2	HayleT2	B		17/08/2016	JG	17:44	10	543713	5593884	August 17, 2016									Y	No	No	22:46	22:50	0:04	1000	Site water	JH	High		1629-004.01
69	Upper Shulaps	USH	A	USH-A	17/08/2016	JH	15:20	10	545847	5638438	August 17, 2016	58	2.0	4/4	Y	1/8	N	0/8	N	N	Yes	Yes	23:02	23:07	0:05	1000	Site water	JH	High		1629-004.01
70	Upper Shulaps	USH	B	USH-B	17/08/2016	JH	15:20	10	545847	5638438	August 17, 2016	87	2.4	4/4	Y	0/8	N	0/8	N	N	Yes	Yes	23:02	23:07	0:05	1000	Site water	JH	High		1629-004.01
71	Upper Holbrook Ck	HOLU	A	HOLU-A	17/08/2016	JH	15:35	10	546566	5636501	August 17, 2016	9	5.0	1/4	N	0/8	N	0/8	N	N	Yes	No	23:18	23:24	0:06	1000	Site water	JH	High		1629-004.01
72	Upper Holbrook Ck	HOLU	B	HOLU-B	17/08/2016	JH	15:35	10	546566	5636501	August 17, 2016	90	3.4	4/4	Y	0/8	N	0/8	N	N	Yes	No	23:18	23:24	0:06	1000	Site water	JH	High		1629-004.01
73	Doe Ck	Doe	A	Doe-A	17/08/2016	JH	16:30	10	552806	5636374	August 17, 2016	73	0.9	4/4	Y	1/8	N	0/8	N	N	Yes	No	23:33	23:38	0:05	1000	Site water	JH	High		1629-004.01
74	Doe Ck	Doe	B	Doe-B	17/08/2016	JH	16:30	10	552806	5636374	August 17, 2016	101	2.6	4/4	Y	0/8	N	0/8	N	N	Yes	No	23:33	23:38	0:05	1000	Site water	JH	High		1629-004.01
75	Downton Creek Trib	DownT	A	DownT-A	17/08/2016	JH	20:50	10	554056	5602877	August 17, 2016	40	3.4	4/4	Y	1/8	N	0/8	N	N	Yes	No	23:48	23:54	0:06	1000	Site water	JH	High		1629-004.01
76	Downton Creek Trib	DownT	B	DownT-B	17/08/2016	JH	20:50	10	554056	5602877	August 17, 2016	98	3.1	4/4	Y	0/8	N	0/8	N	N	Yes	No	23:48	23:54	0:06	1000	Site water	JH	High		1629-004.01
77	Sebring Ck	SEB	A	SEB-A	08/08/2016	JH	14:35	10	548481	5625237	August 8, 2016	28	1.1	4/4	Y	1/8	N	0/8	N	N	Yes	No	19:50	19:55	0:05	1050	Site water	JH	Mod	Flooded	1629-004.01
78	Sebring Ck	SEB	B	SEB-B	08/08/2016	JH	14:35	10	548481	5625237	August 8, 2016	95	4.5	4/4	Y	0/8	N	0/8	N	N	Yes	No	19:50	19:54	0:04	1050	Site water	JH	Mod	Flooded	1629-004.01
79	Truax Trib 1 (HV)	TT1	A		08/08/2016	JH	17:45	10	521810	5633670	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	21:08	21:19	0:11	950	Site water	JH	DO NOT TEST	Flooded- superceeded	1629-004.01
80	Truax Trib 1 (HV)	TT1	B		08/08/2016	JH	17:45	10	521810	5633670	August 8, 2016	~	~	~	~	~	~	~	~	~	No	No	21:58	22:12	0:14	950	Site water	JH	DO NOT TEST	Flooded- superceeded	1629-004.01
81	Pickup Ck	Pick	A	Pick-A	13/08/2016	JH	14:20	10	498236	5600828	August 13, 2016	44	1.3	4/4	Y	1/8	N	3/8	Y	Y	Yes	Yes	19:22	19:26	0:04	950	Site water	JH	High		1629-004.01
82	Pickup Ck	Pick	B		13/08/2016	JH	14:20	10	498236	5600828	August 13, 2016									Y	No	Yes	19:22	19:27	0:05	1000	Site water	JH	High		1629-004.01
83	Hurley Trib 3	HT3	A	HT3-A	13/08/2016	JH	15:12	10	497642	5610606	August 13, 2016	30	0.8	4/4	Y	0/8	N	3/8	Y	Y	Yes	Yes	19:43	19:47	0:04	1100	Site water	JH	High		1629-004.01
84	Hurley Trib 3	HT3	B		13/08/2016	JH	15:12	10	497642	5610606	August 13, 2016									Y	No	Yes	19:43	19:47	0:04	1100	Site water	JH	High		1629-004.01
85	CatSki Trib 4	CT4	A	CT4-A	13/08/2016	JH	16:25	10	502711	5607302	August 13, 2016	27	1.5	4/4	Y	4/8	Y	5/8	Y	Y	Yes	Yes	20:00	20:04	0:04	1000	Site water	JH	High		1629-004.01
86	CatSki Trib 4	CT4	B		13/08/2016	JH	16:25	10	502711	5607302	August 13, 2016									Y	No	Yes	20:00	20:04	0:04	1000	Site water	JH	High		1629-004.01
87	CatSki 2 (Mainstem)	CC2	A	CC2-A	13/08/2016	JH	16:50	10	502942	5609315	August 13, 2016	47	2.1	4/4	Y	3/8	Y	6/8	Y	Y	Yes	In Tribs	20:12	20:46	0:34	950	Site water	JH	High	Testing transport and dilution from tribs	1629-004.01
88	CatSki 2 (Mainstem)	CC2	B		13/08/2016	JH	16:50	10	502942	5609315	August 13, 2016									Y	No	In Tribs	20:12	20:46	0:34	950	Site water	JH	High	Testing transport and dilution from tribs	1629-004.01
89	Hurley 6	H6	A	H6-A	13/08/2016	JH	17:45	10	502705	5616781	August 13, 2016	38	3.1	4/4	Y	2/8	N	7/8	Y	Y	Yes	Yes	20:58	21:01	0:03	900	Site water	JH	High		1629-004.01
90	Hurley 6	H6	B		13/08/2016	JH	17:45	10	502705	5616781	August 13, 2016									Y	No	Yes	20:58	21:01	0:03	1000	Site water	JH	High		1629-004.01
91	Sucker Creek	Skr	A	Skr-A	14/08/2016	JH	7:40	10	511957	5634532	August 14, 2016	15	8.8	4/4	Y	ND	ND	0/8	N	N	Yes	No	7:48	7:53	0:05	950	Site water	JH	Low		1629-004.01
92	Sucker Creek	Skr	B	Skr-B	14-Aug-16	JH	7:40	10	511957	5634532	August 14, 2016	82	4.8	4/4	Y	0/8	N	0/8	N	N	Yes	No	7:48	7:53	0:05	950	Site water	JH	Low		1629-004.01
93	Ochre Creek	OCH	A	OCH-A	14/08/2016	JH	12:41	10	480227	5632435	August 14, 2016	52	1.4	4/4	Y	1/8	N	1/8	N	N	Yes	No	19:38	19:42	0:04	1100	Site water	JH	High		1629-004.01
94	Ochre Creek	OCH	B	OCH-B	14/08/2016	JH	12:41	10	480227	5632435	August 14, 2016	117	2.4	4/4	Y	0/8	N	0/8	N	N	Yes	No	19:38	19:42	0:04	1000	Site water	JH	High		1629-004.01
95	Mac Trib 2	MAC2	A	MAC2-A	14/08/2016	JH	13:30	10	485540	5628535	August 14, 2016	48	3.1	4/4	Y	8/8	Y	1/8	N	N	Yes	No	19:51	19:59	0:08	1000	Site water	JH	High		1629-004.01
96	Mac Trib 2	MAC2	B	MAC2-B	14/08/2016	JH	13:30	10	485540	5628535	August 14, 2016	76	1.9	4/4	Y	0/8	N	0/8	N	N	Yes	No	19:51	19:59	0:08	1000	Site water	JH	High		1629-004.01
97	Paul Creek	Paul	A	Paul-A	14/08/2016	JH	14:15	10	504232	5629919	August 14, 2016	35	2.9	4/4	Y	1/8	N	3/8	Y	Y	Yes	No	20:12	20:15	0:03	1000	Site water	JH	High		1629-004.01
98	Paul Creek	Paul	B		14/08/2016	JH	14:15	10	504232	5629919	August 14, 2016									Y	No	No	20:12	20:15	0:03	1000	Site water	JH	High		1629-004.01
99	Gwyneth Ck	Gwyn	A	Gwyn-A	14/08/2016	JH	14:45	10	508268	5628612	August 14, 2016	50	4.9	4/4	Y	4/8	Y	2/8	N	N	Yes	No	20:32	20:36	0:04	1000	Site water	JH	High		1629-004.01
100	Gwyneth Ck	Gwyn	B	Gwyn-B	14/08/2016	JH	14:45	10	508268	5628612	August 14, 2016	105	4.4	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:32	20:36	0:04	1000	Site water	JH	High		1629-004.01
101	Cadwallader Trib 1	CT1	A	CT1-A	14/08/2016	JH	16:45	10	525408	5614376	August 14, 2016	70	1.8	4/4	Y	3/8	Y	2/8	N	N	Yes	No	21:12	21:38	0:26	850	Site water	JH	High		1629-004.01
102	Cadwallader Trib 1	CT1	B	CT1-B	14/08/2016	JH	16:45	10	525408	5614376	August 14, 2016	102	2.8	4/4	Y	1/8	N	0/8	N	N	Yes	No	21:12	21:38	0:26	975	Site water	JH	High		1629-004.01
103	Hawthorne Creek	HAW	A	HAW-A	14/08/2016	JH	17:26	10	520410	5621247	August 14, 2016	51	5.1	4/4	Y	2/8	N	1/8	N	N	Yes	No	21:48	22:08	0:20	925	Site water	JH	High		1629-004.01
104	Hawthorne Creek	HAW	B	HAW-B	14/08/2016	JH	17:26	10	520410	5621247	August 14, 2016	103	2.6	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:48	22:09	0:21	900	Site water	JH	High		1629-004.01

Appendix 2: PCR – Final Results

MasterSort	Location name	Site ID	Sample replicate	Lab Code	Collection date	Collected by	Collection Time	Zone	Easting	Northing	Filter Date	DPN	eDNA Recovered (ng/uL)	qPCR Viability Frequency	qPCR Viability Call	eFrog Frequency	eFrog Call	ASTR Frequency	Lab Call	Biol Call	Test for ASTR	Known ASTR	Filter start time	Filter end time	Filtering time (calc)	Sample Volume (ml)	Sample Contents	Filtered by	Test Priority	Comments	Project Number
105	Crazy Creek	CZY	A	CZY-A	14/08/2016	JH	17:45	10	518305	5621718	August 14, 2016	14	12.2	4/4	Y	ND	ND	0/8	N	N	Yes	No	22:18	22:22	0:04	1000	Site water	JH	High		1629-004.01
106	Crazy Creek	CZY	B	CZY-B	14/08/2016	JH	17:45	10	518305	5621718	August 14, 2016	111	3.6	4/4	Y	0/8	N	0/8	N	N	Yes	No	22:18	22:22	0:04	1000	Site water	JH	High		1629-004.01
107	Truax Upper	TRU	A	TRU-A	15/08/2016	JH	8:40	10	521767	5632099	August 15, 2016	71	3.2	4/4	Y	3/8	Y	1/8	N	N	Yes	No	9:45	10:04	0:19	1000	Site water	JH	High		1629-004.01
108	Truax Upper	TRU	B	TRU-B	15/08/2016	JH	8:40	10	521767	5632099	August 15, 2016	77	5.5	4/4	Y	2/8	N	0/8	N	N	Yes	No	9:45	10:04	0:19	1000	Site water	JH	High		1629-004.01
109	Truax Trib 1	TT1	A	TT1-A	15/08/2016	JH	8:50	10	521810	5633670	August 15, 2016	2	5.2	4/4	Y	0/8	N	1/8	N	N	Yes	No	10:48	10:53	0:05	1000	Site water	JH	High		1629-004.01
110	Truax Trib 1	TT1	B	TT1-B	15/08/2016	JH	8:50	10	521810	5633670	August 15, 2016	104	2.8	4/4	Y	1/8	N	1/8	N	N	Yes	No	10:48	10:55	0:07	1000	Site water	JH	High		1629-004.01
111	Mason Ck	Mas	A	Mas-A	15/08/2016	JH	12:10	10	509954	5624130	August 15, 2016	8	3.2	4/4	Y	0/8	N	1/8	N	Y	Yes	No	17:00	17:04	0:04	950	Site water	JH	High		1629-004.01
112	Mason Ck	Mas	B	Mas-B	15/08/2016	JH	12:10	10	509954	5624130	August 15, 2016	112	2.6	4/4	Y	1/8	N	3/8	Y	Y	Yes	No	17:00	17:04	0:04	950	Site water	JH	High		1629-004.01
113	Regehr Creek	Reg	A	Reg-A	15/08/2016	JH	12:50	10	506427	5624391	August 15, 2016	65	1.5	4/4	Y	1/8	N	2/8	N	Y	Yes	Yes	17:52	17:57	0:05	1000	Site water	JH	High		1629-004.01
114	Regehr Creek	Reg	B	Reg-B	15/08/2016	JH	12:50	10	506427	5624391	August 15, 2016	75	2.8	4/4	Y	2/8	N	4/8	Y	Y	Yes	Yes	17:52	17:57	0:05	1000	Site water	JH	High		1629-004.01
115	Hurley 12	HU12	A	HU12-A	15/08/2016	JH	13:15	10	504738	5621701	August 15, 2016	72	2.9	4/4	Y	4/8	Y	8/8	Y	Y	Yes	No	18:06	18:39	0:33	1000	Site water	JH	High		1629-004.01
116	Hurley 12	HU12	B		15/08/2016	JH	13:15	10	504738	5621701	August 15, 2016									Y	No	No	18:06	18:39	0:33	950	Site water	JH	High		1629-004.01
117	Waterfalls (H4)	WTF	A	WTF-A	15/08/2016	JH	14:00	10	503914	5619249	August 15, 2016	7	3.2	4/4	Y	8/8	Y	7/8	Y	Y	Yes	No	18:55	19:00	0:05	1000	Site water	JH	High		1629-004.01
118	Waterfalls (H4)	WTF	B		15/08/2016	JH	14:00	10	503914	5619249	August 15, 2016									Y	No	No	18:55	19:00	0:05	1000	Site water	JH	High		1629-004.01
119	Cayoosh Ck West	CayW	A	CayW-A	16/08/2016	JH	10:20	10	536987	5583234	August 16, 2016	26	1.2	4/4	Y	1/8	N	3/8	Y	Y	Yes	No	19:15	19:20	0:05	1100	Site water	JH	High		1629-004.01
120	Cayoosh Ck West	CayW	B		16/08/2016	JH	10:20	10	536987	5583234	August 16, 2016									Y	No	No	19:15	19:20	0:05	1100	Site water	JH	High		1629-004.01
121	Cherise Ck	Cher	A	Cher-A	16/08/2016	JH	11:20	10	541719	5580823	August 16, 2016	20	2.7	4/4	Y	ND	ND	2/8	N	N	Yes	No	19:32	19:38	0:06	1000	Site water	JH	High		1629-004.01
122	Cherise Ck	Cher	B	Cher-B	16/08/2016	JH	11:20	10	541719	5580823	August 16, 2016	81	1.0	4/4	Y	1/8	N	0/8	N	N	Yes	No	19:32	19:38	0:06	1000	Site water	JH	High		1629-004.01
123	VanHorlick Ck (trib)	VHT1	A	VHT1-A	16/08/2016	JH	12:20	10	549561	5577902	August 16, 2016	11	1.4	4/4	Y	ND	ND	1/8	N	N	Yes	Yes	19:48	19:53	0:05	1000	Site water	JH	High		1629-004.01
124	VanHorlick Ck (trib)	VHT1	B	VHT1-B	16/08/2016	JH	12:20	10	549561	5577902	August 16, 2016	91	2.1	4/4	Y	2/8	N	0/8	N	N	Yes	Yes	19:48	19:53	0:05	1000	Site water	JH	High		1629-004.01
125	Kane Ck	Kane	A	Kane-A	16/08/2016	JH	13:20	10	553085	5587549	August 16, 2016	22	3.4	4/4	Y	3/8	Y	0/8	N	N	Yes	No	20:02	20:06	0:04	900	Site water	JH	High		1629-004.01
126	Kane Ck	Kane	B	Kane-B	16/08/2016	JH	13:20	10	553085	5587549	August 16, 2016	80	1.7	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:02	20:07	0:05	1000	Site water	JH	High		1629-004.01
127	Blowdown Trib 1	BT1	A	BT1-A	16/08/2016	JH	15:00	10	553243	5587584	August 16, 2016	68	1.1	4/4	Y	1/8	N	0/8	N	N	Yes	No	20:23	20:28	0:05	1000	Site water	JH	High		1629-004.01
128	Blowdown Trib 1	BT1	B	BT1-B	16/08/2016	JH	15:00	10	553243	5587584	August 16, 2016	89	2.5	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:23	20:28	0:05	1000	Site water	JH	High		1629-004.01
129	Channel Ck	Chan	A	Chan-A	16/08/2016	JH	15:30	10	555031	5591837	August 16, 2016	56	2.8	4/4	Y	0/8	N	4/8	Y	Y	Yes	No	20:30	20:35	0:05	900	Site water	JH	High		1629-004.01
130	Channel Ck	Chan	B		16/08/2016	JH	15:30	10	555031	5591837	August 16, 2016									Y	No	No	20:30	20:35	0:05	1100	Site water	JH	High		1629-004.01
131	Ainsworth's Folly Ck	AF	A	AF-A	16/08/2016	JH	16:00	10	559809	5597971	August 16, 2016	62	0.4	4/4	Y	1/8	N	0/8	N	N	Yes	No	20:46	20:50	0:04	900	Site water	JH	High		1629-004.01
132	Ainsworth's Folly Ck	AF	B	AF-B	16/08/2016	JH	16:00	10	559809	5597971	August 16, 2016	84	2.5	4/4	Y	2/8	N	2/8	N	N	Yes	No	20:46	20:50	0:04	950	Site water	JH	High		1629-004.01
133	Boulder Ck	BLDR	A	BLDR-A	16/08/2016	JH	16:40	10	563219	5599470	August 16, 2016	49	2.3	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:59	21:04	0:05	1000	Site water	JH	High		1629-004.01
134	Boulder Ck	BLDR	B	BLDR-B	16/08/2016	JH	16:40	10	563219	5599470	August 16, 2016	79	1.2	4/4	Y	0/8	N	0/8	N	N	Yes	No	20:59	21:04	0:05	1000	Site water	JH	High		1629-004.01
135	DFR Ck	DFR	A	DFR-A	16/08/2016	JH	17:00	10	563000	5608000	August 17, 2016	46	0.8	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:13	21:16	0:02	1000	DI	JH	High		1629-004.01
136	DFR Ck	DFR	B		16/08/2016	JH	17:00	10	563000	5608000	August 17, 2016									N	No	No	0:05	0:07	0:02	900	DI	JH	High		1629-004.01
137	Shulaps Creek	SHUL	A	SHUL-A	17/08/2016	JH	12:45	10	551022	5642014	August 17, 2016	31	1.1	4/4	Y	0/8	N	7/8	Y	Y	Yes	Yes	19:43	19:48	0:05	1000	Site water	JH	High		1629-004.01
138	Shulaps Creek	SHUL	B		17/08/2016	JH	12:45	10	551022	5642014	August 17, 2016									Y	No	Yes	19:43	19:48	0:05	1000	Site water	JH	High		1629-004.01
139	Marshall Ck	Mar	A	Mar-A	17/08/2016	JG	11:15	10	536264	5634411	August 17, 2016	74	2.2	4/4	Y	1/8	N	0/8	N	N	Yes	No	21:29	21:34	0:05	1000	Site water	JG	High		1629-004.01
140	Marshall Ck	Mar	B	Mar-B	17/08/2016	JG	11:15	10	536264	5634411	August 17, 2016	86	2.6	4/4	Y	0/8	N	0/8	N	N	Yes	No	21:29	21:34	0:05	1000	Site water	JG	High		1629-004.01
141	Washout Ck [Marne Ck]	Wash	A	Wash-A	17/08/2016	JG	14:50	10	542303	5612586	August 17, 2016	32	2.3	4/4	Y	3/8	Y	0/8	N	Y	Yes	No	22:19	22:24	0:05	1000	Site water	JG	High		1629-004.01
142	Washout Ck [Marne Ck]	Wash	B	Wash-B	17/08/2016	JG	14:50	10	542303	5612586	August 17, 2016	107	2.0	4/4	Y	0/8	N	3/8	Y	Y	Yes	No	22:19	22:24	0:05	1000	Site water	JG	High		1629-004.01
143	MacGillivray Ck	MacG	A	MacG-A	17/08/2016	JG	16:06	10	538211	5608006	August 17, 2016	21	2.0	4/4	Y	1/8	N	1/8	N	N	Yes	No	22:38	22:42	0:04	1000	Site water	JH	High		1629-004.01
144	MacGillivray Ck	MacG	B	MacG-B	17/08/2016	JG	16:06	10	538211	5608006	August 17, 2016	93	2.6	4/4	Y	0/8	N	0/8	N	N	Yes	No	22:38	22:42	0:04	1000	Site water	JH	High		1629-004.01
145	Haylemore Trib 3	HayleT3	A	HayleT3-A	17/08/2016	JG	18:10	10	540901	5596703	August 17, 2016	64	2.3	4/4	Y	4/8	Y	5/8	Y	Y	Yes	No	22:53	22:58	0:05	1000	Site water	JH	High		1629-004.01
146	Haylemore Trib 3	HayleT3	B		17/08/2016	JG	18:10	10	540901	5596703	August 17, 2016									Y	No	No	22:53	22:58	0:05	1000	Site water	JH	High		1629-004.01
147	Holbrook Ck	HOL	A	HOL-A	17/08/2016	JH	15:30	10	545974	5638199	August 17, 2016	45	0.7	0/4	N	6/8	Y	0/8	N	N	Yes	No	23:10	23:16	0:06	1000	Site water	JH	High		1629-004.01
148	Holbrook Ck	HOL	B	HOL-B	17/08/2016	JH	15:30	10	545974	5638199	August 17, 2016	85	1.2	4/4	Y	0/8	N	0/8	N	N	Yes	No	23:10	23:16	0:06	1000	Site water	JH	High		1629-004.01
149	La Rochelle Ck	Lar	A	Lar-A	17/08/2016	JH	16:15	10	549711	5636705	August 17, 2016	29	2.8	4/4	Y	2/8	N	8/8	Y	Y	Yes	No	23:27	23:32	0:05	1000	Site water	JH	High		1629-004.01
150	La Rochelle Ck	Lar	B		17/08/2016	JH	16:15	10	549711	5636705	August 17, 2016									Y	No	No	23:27	23:32	0:05	1000	Site water	JH	High		1629-004.01
151	Buck Ck	Buck	A	Buck-A	17/08/2016	JH	16:40	10	555163	5636258	August 17, 2016	37	5.1	4/4	Y	4/8	Y	6/8	Y	Y	Yes	No	23:43	23:47	0:04	900	Site water	JH	High		1629-004.01
152	Buck Ck	Buck	B		17/08/2016	JH	16:40	10	555163	5636258	August 17, 2016									Y	No	No	23:43	23:47	0:04	1000	Site water	JH	High		1629-004.01
153	Downton Creek Trib2	DownT2	A	DownT2-A	17/08/2016	JH	20:30	10	556348	5604314	August 17, 2016	60	1.2	4/4	Y	0/8	N	1/8	N	N	Yes	No	23:55	23:59	0:04	1000	Site water	JH	High		1629-004.01
154	Downton Creek Trib2	DownT2	B	DownT2-B	17/08/2016	JH	20:30	10	556348	5604314	August 17, 2016	115	2.1	4/4	Y	0/8	N	1/8	N	N	Yes	No	23:55	23:59	0:04	1000	Site water	JH	High		1629-004.01

ND= Not Determined, multiple tests conducted which did not pass laboratory QC

Laboratory Report of qPCR run controls performed for the eDNA assay

- ☑ qPCR Positive Controls for correct assembly of assay reactions were successful for all technical plate runs
- ☑ qPCR Negative Controls for detection of assay contamination were successful for all technical plate runs

Biologist Status Assignation-RULES:

-Site = **Yes**, if lab call is Yes (3/8 or higher) for at least 1 replicate;
-Site = **Suspected** (S) if result is 2/8 for both replicates, also suspected if 2/8 for one rep and 1/8 for the other.
-Site = **No** if result is 0/8 or 1/8 for both replicates. If result is 2/8 for one AND 0/8 for the other

APPENDIX 3
Assay Information

Helbing Laboratory eDNA Technical Bulletin

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity.

Bulletin Version: 81716

General eDNA Assay Information

Target Species : Pacific (Coastal) Tailed Frog (*Ascaphus truei*)

Species Abbreviation : ASTR

eDNA qPCR Tool : eASTR4

eDNA qPCR Format : TaqMan

eDNA Assay Specificity Tests

A. qPCR Activity : Multi-species analysis of eDNA tool efficacy

Multiple qPCR reactions (n=25) performed per target DNA. Detection within the standardized eDNA qPCR assay = Yes

ASMO	ASTR	ANBO-VI	LICA	PSRE	RAAU	RALU	XELA	TAGR	HOSA	NTC
No	Yes	No	No	No	No	No	No	No	No	No

B. Confirmation of gene-specificity in eDNA assay : ☑

C. Field Validation Completed : ☑

eDNA Assay Sensitivity Test

DNA (ug/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	1	0.00
1	1	0.00
0.2	0.96	0.07
0.04	0.64	0.17
0.008	0.32	0.16
0	0	0.00

Appendix: Abbreviations

Rocky Mountain Tailed Frog (*Ascaphus montanus*)	ASMO	
Pacific (Coastal) Tailed Frog (*Ascaphus truei*)	ASTR	
Western Toad (*Anaxyrus (Bufo) boreas*)	ANBO-VI	Sourced from Vancouver Island (VI)
Bullfrog (*Lithobates (Rana) catesbeiana*)	LICA	
Pacific Chorus Frog (*Pseudacris (Hyla) regilla*)	PSRE	
Northern Red-legged Frog (*Rana aurora*)	RAAU	
Columbia Spotted Frog (*Rana luteiventris*)	RALU	
African Clawed Frog (*Xenopus laevis*)	XELA	
Rough-skinned Newt (*Taricha granulosa*)	TAGR	
Human (*Homo sapiens*)	HOSA	
qPCR no template control	NTC	
quantitative real-time polymerase chain reaction	qPCR	
environmental DNA	eDNA	