Assessing eDNA as a tool to evaluate Chinook Salmon distribution in Yukon Territory

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Technology Innovation
Yukon Research Centre, Yukon College
500 College Drive
PO Box 2799
Whitehorse, Yukon Y1A 5K4
867.668.8895 or 1.800.661.0504

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Authors

Hobbs, Jared M.Sc., RPBio	Hemmera, Victoria, Canada
Kanary, Lisa PhD, Applied Mathematics	Yukon Research Centre, Yukon, Canada

Contributors

Goldberg, Dr. Caren	Washington State University, Washington, USA
Muller, Michael	Hemmera, Yukon, Canada
Beckman, Kristina	Hemmera, Yukon, Canada
Gordon, Denise	Yukon College, Yukon, Canada
Ness, Isobel	Yukon Research Centre, Yukon, Canada

Technical Editing and Production

Lalena, Guin	Lalena Designs, Yukon, Canada
Damude, Kirstin	Yukon Research Centre, Yukon, Canada

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EXECUTIVE SUMMARY

As aquatic or semi-aquatic species move through the water DNA is shed exogenously and suspended in the aquatic habitat. The suspended DNA can be collected and detected using environmental DNA (eDNA) methods. eDNA methods use quantitative Polymerase Chain Reaction (qPCR) genetic analysis techniques to extract and detect DNA of selected target taxa from environmental samples. In this study, surface water was sampled to collect and detect Chinook salmon (*Oncorhynchus tshawytscha*) DNA deposited in the water column.

This method relies on aquatic organisms shedding exogenous genetic material (i.e., DNA) into their environment through feces, exfoliation, mucus and urine. A positive result from qPCR analysis suggests use, by the target taxa, of aquatic features at the sample site at, or shortly preceding, the time of sample collection. The positive detection of target taxa DNA in the aquatic features used as habitat by the species can be used to establish species presence.

In Yukon, Chinook have great subsistence, recreation, and economic value. The use of eDNA detection methods provides a non-invasive, efficient and accurate method to examine the presence of Chinook salmon DNA in aquatic environments. These methods were applied in southern Yukon in August, 2015. In total, 30 sites were sampled within four river drainages (Nisutlin, Yukon, Teslin and Kusawa) to assess use by, and evaluate distribution of, Chinook salmon as indicated by the presence of Chinook DNA in the lotic system.

Chinook salmon DNA was confirmed at 12 of 13 known extant (currently occupied) Chinook sample locations (sites) within southern Yukon aquatic environments using qPCR lab methods[1]. Chinook salmon DNA was not detected, using qPCR lab methods, at any sites where salmon are excluded and cannot occur (n=3) due to existing natural barriers (e.g., waterfalls) or artificial barriers (e.g. hanging culverts). Fourteen sites with no previous data on salmon use (classified as 'unknown' in this report) were also tested using eDNA methods. Within these 14 sites with unknown occupancy status, one site (Upper Sidney Creek) tested positive for Chinook salmon eDNA. This site was beyond the previously understood distributional limit of Chinook in the Sidney Creek drainage and provides a further example of the utility of eDNA methods for Chinook in Yukon rivers.

Study results provide insight regarding effects of dilution in high volume lotic systems. A single known extant high-volume (lake) site (Kusawa outflow) tested negative, however, five sites, collected in high-volume lotic systems, tested positive in addition to positive results from an additional eight sites collected in low volume systems.

Results from the qPCR analysis, when tested at known extant sites, demonstrate that eDNA is a highly effective way to test for the presence of Chinook salmon as results confirmed a 94.6% detection rate when testing for the presence of Chinook eDNA within known occupied streams. This research provides further demonstration for the applicability of eDNA methods as applied to Chinook salmon in Yukon.

[1] It should be noted that at the single site (Kusawa Outflow) that tested negative (for Chinook) eDNA sample water was collected well *above* (i.e., upstream of) the spawning area. This site was classified as a known positive site as salmon are known to enter Kusawa Lake; however, DNA would have been extremely dilute at the sample location. River hazards (submerged rocks) prevented collection, by boat, at a more favorable location downstream of the known spawning location at the "Kusawa Outflow" sample site.

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1. INTRODUCTION

Salmon are a commercially and culturally significant aquatic species in Yukon. Regional salmon population declines have increased conservation attention and further motivated the need for active management. Salmon population decline has been associated with disease prevalence (Zuray et al., 2012), decline in natural levels of species production (Murphy et al., 2013), bycatch (Stram & Ianelli, 2009), and decreased marine survival rates (Kilduff et al., 2014). Accurate data regarding distribution and abundance of salmon in Yukon is required to ensure appropriate conservation and management for this species.Chinook salmon (*Oncorhynchus tshawytscha)* (also referred to as King Salmon, and in this report referred to as 'Chinook') are a culturally and commercially significant species in Yukon. Life expectancy is three to seven years, with Chinook migrating through the Yukon River system from the Pacific Ocean to Southern Yukon (Department of Fisheries and Oceans, 2014) experiencing some of the longest recorded freshwater salmon migrations (Eiler et al., 2013). Immature Chinook feed on terrestrial and aquatic insects, amphipods and other crustaceans; adults are piscivorous (Department of Fisheries and Oceans, 2014; National Oceanic and Atmospheric Administration, 2015).

Declines in Yukon salmonid populations motivated the development of the 2001 Yukon River Salmon Agreement (YRSA) to address all fish related conservation and management issues. The YRSA prompted the creation of the Fish Habitat Management System (FHMS) which classifies fish habitat by quality, population sensitivity, productive capacity and suitability. The FHMS supports a goal of no net loss of fish habitat (Energy Mines and Resources, 2011).The Yukon Placer Stream Classification (YPSC) was developed as part of the FHMS at the Yukon Placer Secretariat, Yukon Government. The YPSC was intended to provide information to facilitate a sustainable placer mining industry in Yukon, as well as to conserve and protect fish and supporting fisheries (Energy Mines and Resources, 2011). This YPSC was incorporated in the Yukon Placer Watershed Atlas (YPWA) to provide detailed information about habitat suitability classification to placer mining operations (Energy Mines and Resources, 2012). The YPWA is a web based resource (http:/geolocator.yesab.ca) used to characterize species' distributions for certain aquatic species and provides access to data within Placer Watershed Boundaries in Yukon.

This report describes results from the application of a relatively new method increasingly being applied to study the distribution and occurrence of aquatic species in both lotic and lentic systems globally. This new method relies on the detection of environmental DNA, or eDNA, released by aquatic or semi-aquatic species as they complete their life history processes (within aquatic systems). When species are present in an aquatic system exogenous DNA is released into the surrounding water from feces, urine, mucus, and skin cells and suspended temporarily in the water column. This suspended DNA can be filtered from water samples collected in natural aquatic environments and detected in a lab using quantitative polymerase chain reaction (qPCR) methods; this method is termed environmental DNA (eDNA). eDNA detection methods can be used to reliably detect presence of DNA from target taxa, including Chinook, in aquatic environments (Herder et al., 2014; Laramie et al., 2015). eDNA has been tested and validated as a reliable and cost effective method to collect information regarding the distribution, or presence, of many aquatic species. It has been used to identify species at risk (Hobbs et al., 2015), invasive species (Herder et al., 2014), and evaluate habitat quality for Asian carp (Darling et al., 2011) and Slackwater darter (Janosik & Johnston, 2015).

In 2001, the Alaska Department of Fish and Game and the Department of Fisheries and Oceans Canada recommended that Yukon First Nations develop community harvest plans to address declining salmon numbers and promote conservation of Chinook. The high cost associated with conventional methods used for inventory of aquatic species, particularly in remote northern environments, can be prohibitive for fisheries managers (Goldberg et al., 2015). This study investigated the application of eDNA methods as an alternative or complementary method to assess Chinook distribution in several selected drainages in Yukon.

eDNA methods provide an alternative, efficient, non-invasive and highly effective method, relative to methods used in previous studies (e.g. electro-shocking, aerial survey, etc.) to assess Chinook occurrence and distribution in Yukon. These more traditional accepted methods are referred to as conventional methods in this report. Noted advantages of eDNA methods include:

- extended periods for survey timing,
- ability to allow post-hoc examination of samples, for multiple species, months or even years after collection,
- reduced disturbance (i.e. stress) to target taxa,
- reduced cost associated with inventory programs (costs associated with conventional inventory methods typically increase as species detectability diminishes); and,
- reduced probability for transfer of pathogens between isolated aquatic systems.

In August 2015, surface water samples were collected from known occupied (extant) Chinook occurrence sites (n=13), from areas known to be isolated from Chinook (n=3) (i.e., above topographic or man-made barriers) and from sites within potentially suitable habitats but with no previous understanding or knowledge of Chinook occurrence (n=14). Specific study objectives included:

1. To test the accuracy and efficacy of eDNA detection methods for determining presence of Chinook within a previously untested salmonid population,
2. To compare the current, known distribution of the species in selected drainages, and associated tributaries, with results from eDNA sampling,
3. To gain a more accurate and current understanding of the distribution of Chinook salmon in selected drainages in southern Yukon; and,
4. To investigate system dependent comparative relationships between DNA concentrations and selected environmental factors.

2. STUDY AREA

Yukon is part of the Canadian cordilleran region characterized by mountainous terrain, glaciers and icefields (Energy, Mines and Resources, 2015). It shares its borders with British Columbia (south), Alaska (west), and Northwest Territories (east). Yukon falls within the Western Cordillera Sedimentary Basin and Boreal forest ecoregion (Environment Yukon, 2011).

This territory is drained by six major watersheds; the Alsek, Yukon, Porcupine, Peel, Liard and North Slope. Southern Yukon River sub-watershed tributaries include the Teslin, Kusawa, Nisutlin and Yukon rivers. These rivers are described as low gradient (less than one percent grade) slow moving river systems. River bankful width in these rivers ranges from five meters (m) to 150m. Substrate in sampled tributaries (i.e., potential salmon spawning habitat) are dominated by gravel and cobble.

Yukon has a sub-arctic continental, dry climate with temperature ranges of between 36^{o}C and -60^{o}C. Due to its high latitude the region is subject to long periods of daylight during the summer months, and conversely, long periods of darkness in the winter. Average annual precipitation ranges from 20cm to 40cm. The western part of the study area is situated in a rain shadow and subject to droughts between April and July. Vegetation in this territory consists largely of boreal forest and tundra, where trees cover many plateaus and valleys in the south (Cody, 2000). Agriculture is limited to major river valleys such as the Yukon and Takhini (Energy, Mines and Resources, 2015).

The study area includes five First Nation traditional territories: the Teslin Tlingit Council, Carcross and Tagish First Nation, Ta'an Kwach'an Council, Champagne and Aishihik First Nations, and Kwanlin Dun First Nation.

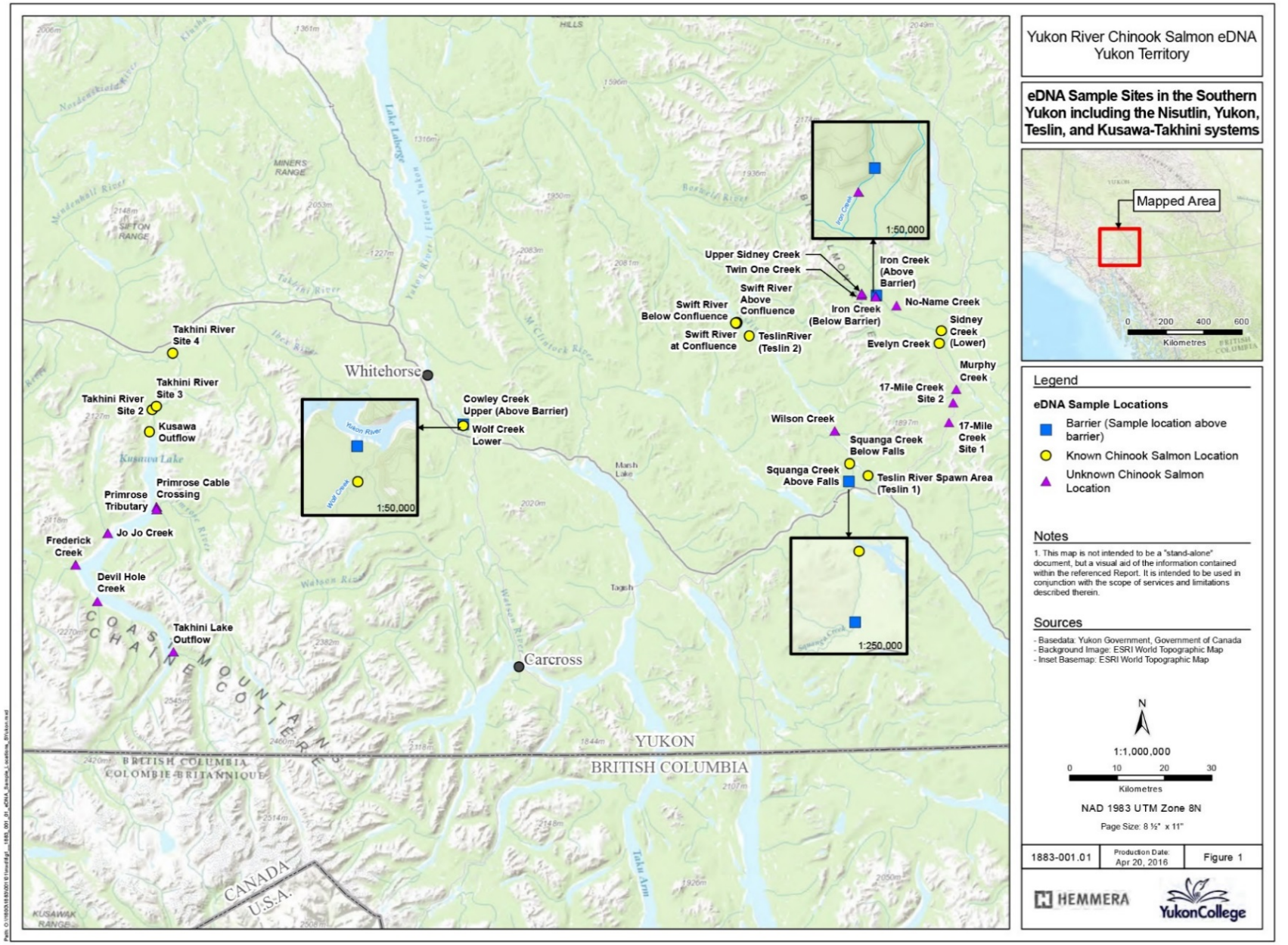

Figure 1: eDNA sample sites in southern Yukon including Nisutlin, Yukon, Teslin, and Kusawa-Takhini systems.

3. METHODS

3.1. SITE SELECTION

Inventory reports from 1997 and 1998 (Connor et al., 1997; Connor et al., 1999), discussions with a senior local salmon biologist (Al von Finster), and information from the YPWA (Energy Mines and Resources, 2011; Energy Mines and Resources, 2012) were used to inform selection of salmon spawning and/or habitat stream reaches. As a result, 30 locations within the Nisutlin, Yukon, Teslin and Kusawa-Takhini drainage basins were sampled to determine the distribution of Chinook populations in Southern Yukon aquatic environments (**Figure 1**). For the purpose of this study, sample locations (i.e., sites) designated as "known" indicate historical confirmed use by Chinook (as detected using conventional methods). Sample locations without previously known Chinook use are identified as "unknown" locations. Sites sampled in 2015 are described in the results section of this report (**Table 1**).

Natural and artificial barriers to salmon dispersal and movement were required to test hypotheses regarding methodological efficacy and primer effectiveness. These features were identified and used where they occurred within the study area. Three sites were sampled from systems (creeks and/or rivers) with identified salmon barriers (Cowley Creek, Squanga River and Iron Creek); at these sites there is no potential for Chinook upstream of the barrier. These sites served to test the potential for false positive detections (**Table 1**) within sites at which salmon were known to be excluded. Barriers at these sites include a perched culvert along the Alaska Highway at Cowley Creek; a natural waterfall at Squanga Creek; and a natural waterfall at Iron Creek (**Figure 1)**. The remaining 27 sites selected for sampling within this study all had potential for Chinook to occur based on current understanding of species distribution and dispersal capabilities. Thirteen sites were selected within known salmon spawning or rearing areas; 14 sites were selected within areas where salmon occurrence was unknown.

3.2. SAMPLE COLLECTION

Collection methods used in this study involved collection of duplicate or triplicate 1 L water samples at each sampling location. During sample collection, bottles were labelled using a permanent marker with the site name, location name, Universal Transverse Mercator (UTM) coordinate, collection time and name of collector. Where field technicians and biologists were required to enter the water to access the thalweg, samples were collected in areas with a strong current upstream of any potentially contaminated water (water may be contaminated by inadvertent introduction of Chinook DNA on collection equipment or personnel). Where a boat was used during sample collection, samples were collected from the prow of the boat, and upstream of any DNA that may have been inadvertently transported on the hull of the boat. During sample collection, field biologists wore clean nitrile gloves to avoid introduction of DNA from their hands. Sample bottles were also triple-rinsed, downstream of the collection site, with site water immediately prior to sample collection. Each bottle was then filled with water from the surface of the feature (creek, lake or river) distal to any areas where the water may have been contaminated by previous entry.

Immediately after sample collection, an YSI® brand water quality meter was used to collect water chemistry data at each location. The biologists recorded location UTM coordinates, water quality data and site photographs. Water chemistry parameters collected in the field included water temperature (°C), pH, dissolved oxygen content (mg/L) and conductivity (µS/cm).

Degradation of DNA in both the aquatic environment and in sample water may occur from exposure to elevated water temperatures, high or low pH levels and exposure to ambient ultraviolet rays (e.g., sunlight) (Herder et al., 2014; Hobbs & Goldberg, 2015). To avoid, or minimize, degradation of eDNA in collected samples, biologists placed sample bottles in an insulated cooler with ice packs during fieldwork until collection was complete (i.e., prior to off-site filtration and preservation) as per methods described by Hobbs et al. (2015).

Eighty-five surface water samples were collected from 30 locations; these are referred to as sites in this report. During field sample collection researchers followed standard eDNA surface water collection protocol as per Hobbs et al. (2015). At most sites (n=22; 73.4%) eDNA samples were collected in triplicate. Duplicate samples were collected at seven sites (n=7; 23.3%). Sample replication was reduced to minimize costs associated with filtration and analysis when there was a high degree of confidence that salmon were absent (i.e., above known barriers to salmon dispersal), as these sites were used only as controls and to provide confirmation of primer specificity. Five replicates were collected at one site (3.3%) (No Name creek) to examine differences above and below a hanging culvert (**Table 1**).

3.3. SAMPLE FILTRATION

Samples were stored in a refrigerator set to 4°C during holding for filtering, and were processed within 24 hours of collection in the same order as collected. During filtration, 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 alternating current Masterflex L/S Economy variable speed drive motor to create a vacuum. On completion of filtration, the filter was removed using sterilized forceps[2] and clean nitrile gloves.

The filter membrane was placed in a 2 mL sterile polypropylene cryogenic vial and filled with 95% molecular-grade ethanol. Vials were labelled and then placed inside similarly labelled whirl-pak storage bags for shipment to the lab. During filtration, data regarding filtered sample volume, identity of personnel responsible for filtration, and filter time were recorded in Microsoft ® Excel ® 2013 to provide insight regarding inhibition during subsequent analysis.

One control sample (i.e., distilled water) was processed, by each technician performing filtration, at the end of each filtering day using the same filtration protocol. This 'control' serves as a test against contamination for both the filtration and laboratory analysis processes, as required by provincial protocol (Hobbs et al., 2015). Preserved filter membranes were shipped, by courier, to Washington State University for subsequent extraction and analysis; distilled water samples (controls for contamination) were not identified to the lab prior to analysis.

[2] Forceps were sterilized in a 50% diluted household bleach solution.

3.4. DNA EXTRACTION AND QPCR

DNA was extracted from 85 preserved filter samples in a laboratory dedicated to the analysis of low-quantity DNA sources using a Qiashredder / DNeasy protocol (Goldberg et al., 2011). Each extract was run in triplicate using a species-specific qPCR assay that included positive and negative controls in each plate, as well as an internal control to detect polymerase chain reaction (PCR) inhibition. When PCR inhibition was detected, samples were run through a One-Step PCR Inhibitor Removal kit column (Zymo Research) and re-analyzed. When triplicate wells did not test consistently (i.e., one or two tested positive) the sample was rerun to confirm the result.

3.5. DATA ANALYSIS

The accuracy and efficacy of using an eDNA detection method for the assessment of Chinook presence in Yukon was assessed by analyzing the true positive, false positive and false negative detection rates. The true positive detection rate was calculated as the ratio of all positive replicates to all replicates collected from known locations (n=13). The false positive detection rate is the ratio of all positive replicates to all replicates collected at any site where salmon were not expected to occur due to barriers and to all replicates collected using distilled water (n=6). The false negative detection rate is the ratio of all negative replicates to all replicates collected from known locations (n=13). The reliability of eDNA detection methods is calculated by comparing Chinook distributions from each method using Fisher Exact and Pearson's Chi-squared with Yates' continuity correction tests (similar to methods described by Laramie et al., 2015).

The eDNA detection method is currently limited to determining species' presence or absence. Hence, there is an impetus in the scientific community to enhance the capability of the method and add to the growing knowledge-base exploring links between a measure of an aquatic species' DNA concentration and actual species abundance at the sample site location. Measurements of selected environmental variables were recorded at each collection site to facilitate subsequent calculation of system flow rate and volume. These variables were recorded to facilitate study of a potential correlation (strength and direction of a relationship) between salmon DNA concentrations and the surrounding environment[3]. Computations, calculations and analyses are completed using Microsoft ® Excel ® 2013 and MATLAB Version: 8.6.0.267246.

4. RESULTS

Fieldwork was conducted on August 18-20, 2015. Eighty-five samples were collected from 30 sites in four major, southern Yukon drainages (**Figure 1** and **Table 1**). Sample collection followed protocols for collection of surface water samples, for subsequent eDNA analysis, that have been demonstrated to be effective for eDNA field inventory for Chinook in Washington State (USA) as per Laramie et al. (2015). These protocols were summarized by Hobbs et al. (2015), however, the protocols have had limited previous field application in Yukon with only a single prior study conducted on Western toad (*Anaxyrus boreas*) and Columbia spotted frog (*Rana luteiventris)* (study conducted for Yukon Environment by B. Bennett, G. Rivest, J. Hobbs and C. Goldberg, 2015). The sample collection methods used during this study were consistent with British Columbia

[3] Prior to covariate analysis an analysis of variance (ANOVA) test was be performed to confirm in-equality of mean eDNA concentrations measured at independent sites.

(BC) provincially accepted eDNA standards authored by Hobbs et al. (2015). These methods have recently been accepted by the BC Ministry of Environment and are submitted as a Resource Inventory Standards Committee standard for application, by eDNA practitioners, in British Columbia (Hobbs et al., 2015).

Results from the lab are described as positive for the presence of Chinook eDNA if at least one replicate (of two or three replicate samples collected) at a site tested positive for the presence of Chinook DNA during qPCR (**Table 1**). Results are described as negative if all replicates collected at a site tested negative for the presence of Chinook eDNA during qPCR analysis. In total, 13 sites were classified as positive for detection of Chinook DNA (**Figure 2**) including one site where previous use by Chinook had not been detected using conventional methods. Seventeen sites were classified as negative for detection of Chinook DNA (**Table 1** and **Figure 2**).

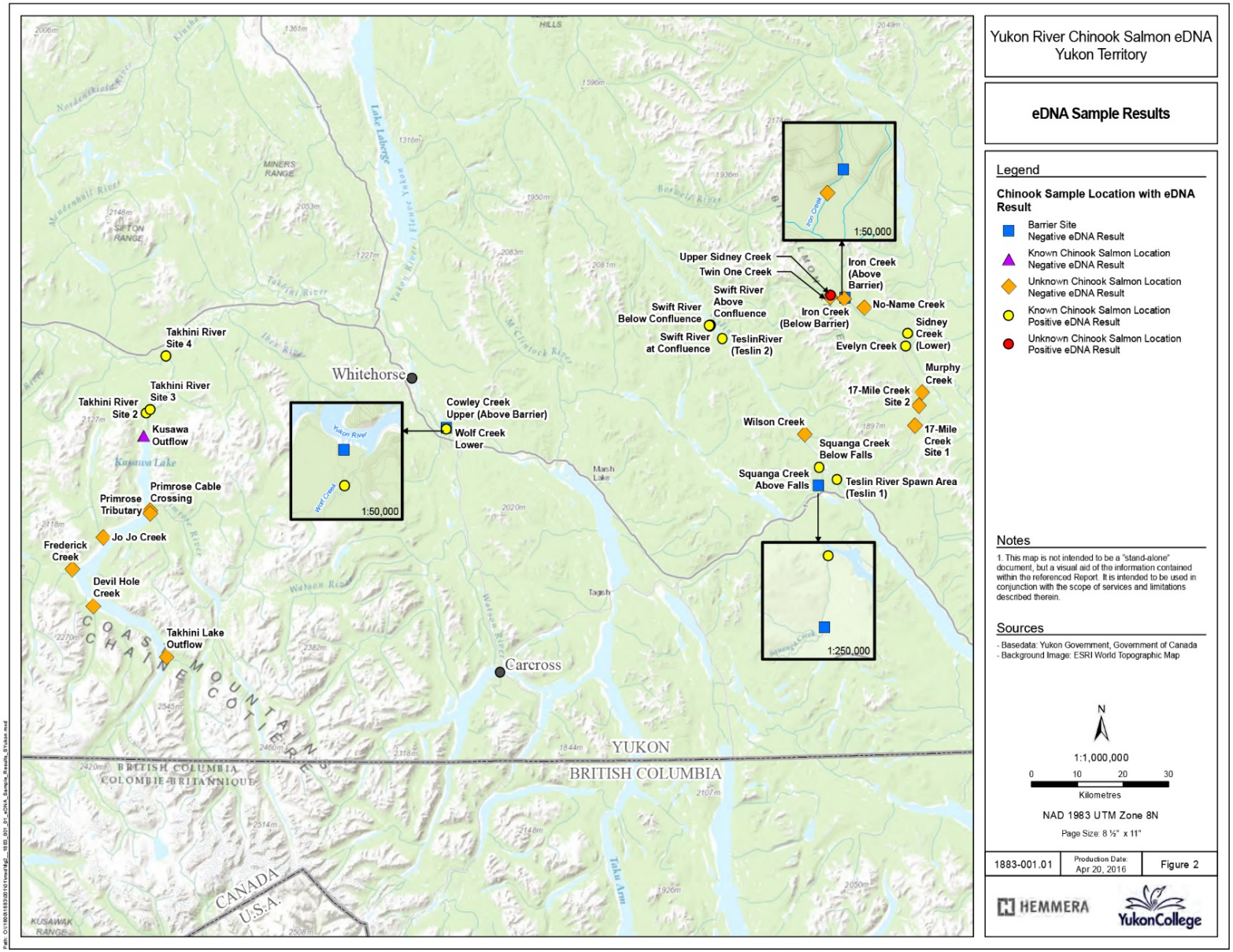

Figure 2: Sites where Chinook salmon presence was documented as known and unknown using conventional inventory methods; and, sites where Chinook DNA was detected using eDNA methods.

Table 1: Summary of sites sampled using eDNA methods for Chinook salmon in southern Yukon in 2015.

Site Name	Z	Easting	Northing	No. of samples	Barriers	Confirmed Chinook Site (Conventional Detection Method)	Confirmed Chinook (eDNA Detection Method)
17-Mile Creek Site 1	8	606948	6721439	3	No	Unknown	Negative
17-Mile Creek Site 2	8	607849	6725541	3	No	Unknown	Negative
Cowley Creek Upper (above barrier)	8	504501	6721027	2	Yes	No-Excluded	Negative
Devil Hole Creek	8	427283	6683856	3	No	Unknown	Negative
Evelyn Creek	8	604944	6737925	3	No	Known Positive	Positive
Frederick Creek	8	422707	6691491	3	No	Unknown	Negative
Iron Creek (above barrier)	8	591659	6748113	2	Yes	No-Excluded	Negative
Iron Creek (below barrier)	8	591485	6747866	2	No	Unknown	Negative
Jo Jo Creek	8	429548	6698124	3	No	Unknown	Negative
Kusawa Outflow	8	438294	6719412	3	No	Known Positive	Negative
Murphy Creek	8	608537	6728348	3	No	Unknown	Negative
No-name Creek	8	595865	6745993	5	No	Unknown	Negative
Primrose Cable Crossing	8	439791	6703704	3	No	Unknown	Negative
Primrose Tributary	8	439786	6703090	3	No	Unknown	Negative
Sidney Creek (lower)	8	605335	6740594	3	No	Known Positive	Positive
Squanga Creek above falls	8	585827	6709007	2	Yes	No-Excluded	Negative
Squanga Creek below falls	8	586035	6712721	3	No	Known Positive	Positive
Swift River above confluence	8	562262	6742357	3	No	Known Positive	Positive
Swift River at confluence	8	562161	6742147	3	No	Known Positive	Positive
Swift River below confluence	8	562031	6742216	3	No	Known Positive	Positive
Takhini Lake Outflow	8	443329	6673212	3	No	Unknown	Negative
Takhini River Site 2	8	438917	6723927	3	No	Known Positive	Positive
Takhini River Site 3	8	439753	6724733	3	No	Known Positive	Positive
Takhini River Site 4	8	443262	6735930	3	No	Known Positive	Positive
Teslin River Spawn Area (Teslin 1)	8	589933	6710184	3	No	Known Positive	Positive
Teslin River(Teslin 2)	8	564910	6739561	3	No	Known Positive	Positive
Twin One Creek	8	588403	6748154	2	Extralimital	Unknown	Negative
Upper Sidney Creek	8	588516	6748543	2	Extralimital	Unknown	Positive
Wilson Creek	8	582829	6719642	3	No	Unknown	Negative
Wolf Creek lower	8	504508	6720649	2	No	Known Positive	Positive

Table 2: Percentage of water samples that tested negative (0) and positive (1) for presence of Chinook salmon based on triplicate sampling at 22 sample sites.

Possible Detection Outcome for Triplicate Sampling	Number of Occurrences	% of Sites
000	11	50.0%
001,010,100	0	0.0%
110,101,011	2	9.1%
111	9	40.9%

Twelve sites at which Chinook were known to be present (i.e., all known extant occurrences) consistently tested positive using eDNA methods with 94.6% accuracy (true positive detection rate). One site (Upper Sidney Creek) where Chinook presence was unknown (i.e., no recent or previous confirmation of occurrence existed in available historic data) also tested positive using eDNA methods. Two replicates at known sites (Takhini River Site 2 and Takhini River Site 3) were classified as negative, resulting in a false negative detection rate of 5.4%; however, the overall site classification, based on interpretation of all three replicates, was assigned a value of positive consistent with interpretation methods used in other studies by the author and by Laramie et al. (2015).

Analysis of negative results indicates that all sites above barriers (n=3; 100%), where Chinook are known to not occur due to barriers to Chinook dispersal, tested negative resulting in a false positive detection rate of 0%. In addition, control samples (distilled water) also tested negative during qPCR analysis, confirming effective collection, filtration and qPCR methods. Results of the Fisher Exact and Pearson's Chi-squared with Yates' continuity correction analyses indicate that the effectiveness of the eDNA detection method to detect southern Yukon Chinook was not likely due to chance (p-value <0.0001, Chi Squared = 49.7478, df=1 and Fisher = $6x10^{-16} < 0.0099$). This provides further confidence in the efficacy of eDNA methods applied in this study.

The field crew measured and recorded environmental variables near each collection site, which included: channel (bankful) width, wetted width, average channel depth, gradient, water temperature, pH, and conductivity. Once dependent sample sites were removed from the data, four sites (ten sample locations) remained[4]. Sample size was too small to be analyzed using ANOVA and, as such, a correlation analysis could not be performed. Results were not adequate to merit investigation of a potential relationship between Chinook DNA concentrations and environmental parameters.

5. DISCUSSION

Results regarding distribution of Chinook from this study generally concur with previously known Chinook distribution (Connor et al., 1997; Connor et al., 1999; Al von Finster pers. obs.; Energy Mines and Resources, 2011; Energy Mines and Resources, 2012). With a detection rate of 94.6% and a false negative detection rate of 5.4%, eDNA detection methods appear to provide an accurate and effective method for fisheries managers, consultants, and researchers to detect Chinook salmon throughout Yukon and potentially within other northern Canadian watersheds.

eDNA methods have been demonstrated to provide an advantageous alternative to conventional inventory methods in many instances (Goldberg et al., 2015; Herder et al., 2015). eDNA methods can be used in conjunction with conventional methods to create efficiencies in more detailed monitoring studies (Hobbs et al., 2015). An understanding of the strengths and limitations of both eDNA methods and conventional methods can result in significant cost-savings during fisheries management.

It should be noted that at the single known extant site (Kusawa Outflow) that tested negative for Chinook eDNA sample water was collected well *above* (i.e., upstream of) the spawning area. River hazards

[4] Dependent sites were defined as sites with multiple potential contributing DNA sources (i.e. a dependent downstream site with more than one contributing upstream tributary).

(submerged rocks) prevented collection, by boat, immediately downstream of the known spawning location at the outflow of Kusawa Lake. This site was classified as a known positive site as salmon are known to enter Kusawa Lake above the spawning area, however, DNA is likely extremely dilute at the sample location. A negative qPCR result for this site does not contradict conclusions regarding methodological efficacy for eDNA studies on Chinook in Yukon as this result is reasonably ascribed to dilution associated with very high system volume at the sample site location.

As eDNA is a relatively new inventory method, it is appropriate to describe associated methodological limitations. As with conventional methods, eDNA is subject to type I (false positive) and type II (false negative) errors, as follows:

- A type I error, using eDNA, may occur if the sample is contaminated during collection, filtration or testing by eDNA from human introduction (i.e., inadvertently transported on clothing), osmotic diffusion and / or downstream transport from upstream source populations, or mislabeled or accidentally switched during qPCR laboratory testing. Rigorous adherence to standard operating procedures, specific to eDNA, mitigate the probability associated with these potential sources of bias.
- A type II error, using eDNA, may occur when the concentration of eDNA in the sample water occurs at trace concentrations below detection thresholds of qPCR analysis methods. High flow volume potentially dilutes the DNA concentration in a sample and/or reduces residence time of DNA in the system. Kusawa Lake and Takhini River are large volume waterways (**Figure 2**) which may explain inconsistent DNA detection results within these systems. Flow volume was highest at the Kusawa Outflow site, followed by the Takhini River Sites 2 and 3. At Kusawa Outflow all three replicates tested negative, and at Takhini River Site 2 and Site 3 only two of three replicates tested positive, despite known extant Chinook populations at all three sites.

It should be noted that these limitations are not unique to eDNA detection methods. These limitations may, in fact, be more prevalent in most conventional survey methods for aquatic species, as follows:

- A type I error (false positives) may occur through misidentification of the target taxa by inexperienced observers.
- A type II error (false negatives) may occur due to observability bias; target taxa are often cryptic, inconspicuous and may have discontinuous distributions. For rare species they also likely persist at low density. In addition, observer bias (different levels of skill and experience) strongly influence survey results.

In addition to the arguably greater potential for type I and type II errors that may occur using conventional methods, there are also additional concerns associated with the use of conventional methods. Conventional methods have a relatively greater risk to the target taxa as conventional methods are typically regarded as more invasive. Conventional methods may facilitate pathogen transfer, may result in method-induced mortality (trapping and subsequent mortality) and may have indirect negative effects on sensitive habitats (e.g., disturbing substrate during survey). Conventional methods also typically have more restrictive requirements for appropriate survey timing conditions, relative to eDNA detection methods, as they often require data collection during more limited phenological stages.

Potential for type I and type II errors persist regardless of methodology and as such eDNA qPCR analysis results should be interpreted with awareness and understanding of methodological limitations; however, there is emerging consensus in the literature (Goldberg et al., 2015; Herder et al., 2014) that eDNA methods are rapidly advancing toward becoming a preferred method for inventory of aquatic species. Traditional inventory methodologies for rare and elusive amphibian species are being surpassed by the application of eDNA methods for detection of aquatic organisms (Goldberg et al., 2015). Use of conventional methods for inventory of aquatic amphibians in Yukon will likely diminish as understanding and acceptance of eDNA methods evolve and relative limitations associated with traditional methods are increasingly recognized.

Measurement of DNA concentration collected using filtered eDNA samples does not provide a meaningful measure of specimen abundance in natural systems. Although it is feasible to measure eDNA concentrations in sample, against a standard curve produced by extracting known quantities of pure DNA for the target taxa, this only provides a relative concentration of all eDNA extracted from a sample against a known concentration of DNA from the target taxa as measured against a pre-developed standard-curve. Relating concentration of eDNA in a sample to abundance of a species in a natural system is not feasible or appropriate as eDNA concentration varies greatly, under different conditions, due to other factors that are impossible to quantify or estimate. These variables include:

- collection site location relative to eDNA source from target taxa,
- variable rate of output at source (eDNA is released at different rates during different phonological and life history phases for different species); and,
- variable DNA degradation rates in, and between, natural systems (degradation rates are influenced by variables such as temperature, exposure to ultra-violet radiation and pH).

As these variables are typically unknown and very difficult, if not impossible, to quantify in natural systems it is currently not appropriate to use eDNA concentrations as measured against a standard curve from eDNA extracted from a sample to estimate species abundance. There is consensus in the literature regarding the inapplicability of eDNA for derivation of abundance estimates for target taxa.

6. CONCLUSION

This study demonstrates the efficacy of eDNA methods to detect species presence in Yukon. Molecular detection methods described in this study (i.e., eDNA) can be used as either an exclusive or complementary tool, in parallel with conventional methods, to cost-effectively assess Chinook occurrence in natural aquatic systems, however, there are inherent methodological limitations. Results from this study were unable to demonstrate that a relationship exists between system-dependent eDNA concentrations when compared with selected environmental covariates.

More appropriate applications of eDNA methods include evaluation of species presence to prioritize areas for conservation and management. In these applications, results from eDNA can be used to identify areas where more intensive conventional methods can be applied to measure abundance once presence of the target taxa has been confirmed. eDNA methods can be used in Yukon to reduce the cost of inventories for aquatic taxa by replacing or complementing conventional inventory methods, where appropriate, to support conservation and management in Yukon.

If used appropriately, with clear recognition of both strengths and limitations, eDNA methods hold great promise. eDNA methods have significant potential to facilitate inventory and assessment of species presence for species that are difficult to detect (inconspicuous or secretive life-history) or species that occur with discontinuous distributions. eDNA provides a more (cost) effective, more efficient and far less invasive method than most conventional methods that are currently applied to assess presence of Chinook salmon in Yukon.

7. LITERATURE CITED

Cody, W. J. (2000). Flora of the Yukon Territory. NRC Research Press.

Connor M, Sparling P, Nordin K, Burns B, Withers SP. (1997). Chinook Assessment and Restoration/Enhancement Options for Selected Tributaries of the Teslin River, 1997. White Mountain Environmental Consulting, Teslin Tlingit Council, Laberge Environmental Services.

Connor M, Sparling P, Nordin K, Burns B, Withers SP. (1999). Chinook Assessment and Restoration/Enhancement Options for Selected Tributaries of the Nisutlin River and Teslin Lakes Drainages, 1998. White Mountain Environmental Consulting, Teslin Tlingit Council, Laberge Environmental Services. Project # RE-20-98.

Darling, J. A., & Mahon, A. R. (2011). From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research*, *111*(7), 978- 988.

Department of Fisheries and Oceans. (2014). Chinook. http://www.pac.dfo-mpo.gc.ca/fm-gp/rec/species-especes/chinook-quinnat-eng.html. Updated: April 2, 2014.

Eiler, J. H., Schreck, C. B., & Evans, A. N. (2013). Migratory Patterns of Wild Chinook Salmon Returning to a Large, Free-flowing River Basin. *Distribution and Movements of Chinook Salmon, Oncorhynchus tshawytscha, Returning to the Yukon River Basin. Abstract approved: _*, 123.

Energy, Mines and Resources. (2015). Government of Yukon 2015
http://www.emr.gov.yk.ca/agriculture/soils_geography.html
http://www.emr.gov.yk.ca/agriculture/yukon_climate.html

Energy Mines and Resources. (2011). Yukon Placer Secretariat.
http://www.yukonplacersecretariat.ca/history.html. Accessed March 31, 2016.

Energy Mines and Resources. (2012). Yukon Placer Secretariat: Placer Atlas.
http://www.yukonplacersecretariat.ca/placer_atlas.html. Accessed March 31, 2016.

Environment Yukon. (2011). Yukon Water; an Assessment of Climate Change Vulnerabilities. Government of Yukon. pp 98. ISBN 978-1-55362-517-9

Goldberg, C and K. Strickler. 2013. eDNA protocol sample collection with cellulose nitrate filters. University of Idaho.

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, *183*, 1-3.

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

Hobbs, J and C. Goldberg. (2015). Western Toad and Columbia Spotted Frog eDNA inventory and method assessment. Prepared for Yukon Conservation Data Centre.

Hobbs, J., E. Vincer and C. Goldberg. (2015). Standard Operating Procedure. *Environmental DNA Protocol for Freshwater Aquatic Ecosystems*. Prepared for B.C. Ministry of Environment. 1-25.

Janosik, A. M., & Johnston, C. E. (2015). Environmental DNA as an effective tool for detection of imperiled fishes. *Environmental Biology of Fishes*, *98*(8), 1889-1893.

Kilduff, D. P., Botsford, L. W., & Teo, S. L. (2014). Spatial and temporal covariability in early ocean survival of Chinook salmon (Oncorhynchus tshawytscha) along the west coast of North America. ICES Journal of Marine Science: Journal du Conseil, 71(7), 1671-1682.

Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biological Conservation, 183, 29-37.

National Oceanic and Atmospheric Administration. (2015). NOAA Fisheries Chinook. http://www.nmfs.noaa.gov/pr/species/fish/chinook-salmon.html. Updated: Feb 10, 2016.

Stram, D. L., & Ianelli, J. N. (2009). Eastern Bering Sea pollock trawl fisheries: variation in salmon bycatch over time and space. In *American Fisheries Society Symposium* (Vol. 70, pp. 827-850).

Zuray, S., Kocan, R., & Hershberger, P. (2012). Synchronous cycling of Ichthyophoniasis with Chinook density revealed during the annual Yukon River spawning migration. *Transactions of the American Fisheries Society*, 141(3), 615-623.