Operational Plan: Northern Pike Eradication in the Cottonwood Creek Drainage – Phase 1

by Cody Jacobson Kristine Dunker and Dave Rutz

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Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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Weights and measures (metric)		General		Mathematics, statistics	
centimeter	cm	Alaska Administrative		all standard mathematical	
deciliter	dL	Code	AAC	signs, symbols and	
gram	g	all commonly accepted		abbreviations	
hectare	ha	abbreviations	e.g., Mr., Mrs.,	alternate hypothesis	H _A
kilogram	kg		AM, PM, etc.	base of natural logarithm	е
kilometer	km	all commonly accepted		catch per unit effort	CPUE
liter	L	professional titles	e.g., Dr., Ph.D.,	coefficient of variation	CV
meter	m		R.N., etc.	common test statistics	(F, t, χ^2 , etc.)
milliliter	mL	at	@	confidence interval	CI
millimeter	mm	compass directions:		correlation coefficient	
		east	E	(multiple)	R
Weights and measures (English)		north	Ν	correlation coefficient	
cubic feet per second	ft ³ /s	south	S	(simple)	r
foot	ft	west	W	covariance	cov
gallon	gal	copyright	©	degree (angular)	0
inch	in	corporate suffixes:		degrees of freedom	df
mile	mi	Company	Co.	expected value	Ε
nautical mile	nmi	Corporation	Corp.	greater than	>
ounce	OZ	Incorporated	Inc.	greater than or equal to	\geq
pound	lb	Limited	Ltd.	harvest per unit effort	HPUE
quart	qt	District of Columbia	D.C.	less than	<
yard	yd	et alii (and others)	et al.	less than or equal to	≤
-	-	et cetera (and so forth)	etc.	logarithm (natural)	ln
Time and temperature		exempli gratia		logarithm (base 10)	log
day	d	(for example)	e.g.	logarithm (specify base)	\log_{2} , etc.
degrees Celsius	°C	Federal Information		minute (angular)	,
degrees Fahrenheit	°F	Code	FIC	not significant	NS
degrees kelvin	Κ	id est (that is)	i.e.	null hypothesis	Ho
hour	h	latitude or longitude	lat or long	percent	%
minute	min	monetary symbols		probability	Р
second	S	(U.S.)	\$,¢	probability of a type I error	
		months (tables and		(rejection of the null	
Physics and chemistry		figures): first three		hypothesis when true)	α
all atomic symbols		letters	Jan,,Dec	probability of a type II error	
alternating current	AC	registered trademark	®	(acceptance of the null	
ampere	А	trademark	ТМ	hypothesis when false)	β
calorie	cal	United States		second (angular)	"
direct current	DC	(adjective)	U.S.	standard deviation	SD
hertz	Hz	United States of		standard error	SE
horsepower	hp	America (noun)	USA	variance	
hydrogen ion activity (negative log of)	рН	U.S.C.	United States Code	population sample	Var var
parts per million	ppm	U.S. state	use two-letter		
parts per thousand	ppt, ‰		abbreviations (e.g., AK, WA)		
volts	V				
watts	W				

REGIONAL OPERATIONAL PLAN SF.2A.2017.04

NORTHERN PIKE ERADICATION IN THE COTTONWOOD CREEK DRAINAGE – PHASE 1

by

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> Alaska Department of Fish and Game Division of Sport Fish

> > March 2017

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SIGNATURE PAGE

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ABSTRACT

This project will lay the groundwork for the eventual eradication of invasive northern pike from the Cottonwood Creek drainage by conducting biological assessments of 2 lakes known to contain invasive northern pike (Anderson and Kings lakes), surveying Little Niklason and Mud lakes for the presence of northern pike, collecting water quality data and bathymetric data from all drainage lakes, developing a preliminary pike eradication plan, and conducting public scoping to determine the level of support for eradication.

Key words: invasive species, northern pike, *Esox lucius*, Cottonwood Creek drainage, Anderson Lake, Kings Lake, Little Niklason Lake, Mud Lake, water quality, bathymetry

INTRODUCTION

Invasive northern pike *Esox lucius* pose a significant threat to salmon habitats in Southcentral Alaska (ADF&G 2007). Northern pike are native throughout much of the state but do not naturally occur south and east of the Alaska Range (Figure 1). Pike were introduced by anglers to the Yentna River drainage in the late 1950's and subsequently spread throughout the Susitna River basin through flood events and further illegal stockings (Mills 1986).



Figure 1.–Native and invasive ranges of northern pike; solid red delineations indicate the invasive range.

Northern pike are highly predatory and are responsible for the loss of several fisheries across their invasive range. The Cottonwood Creek drainage is an area of the Matanuska-Susitna Valley that has great potential to be impacted by invasive northern pike because of the optimal pike habitat conditions (i.e. shallow, vegetated waters) found throughout much of the drainage. Two drainage lakes, Anderson and Kings, are already known to harbor established populations of

northern pike. For several years, ADF&G has been blocking the outlets of these lakes with fyke nets to contain these northern pike populations. Since that time, the outlet of Anderson Lake has dewatered making it currently impassible for fish. Prior to northern pike establishment, both of these lakes contained robust populations of coho salmon, sockeye salmon and rainbow trout, but today few, if any, of these fish remain in either lake. Even with on-going attempts to prevent pike from spreading and the current status of Anderson Lake's outlet, there is potential for northern pike to escape during high water events or be moved from these lakes and colonize the entire Cottonwood Creek drainage. Further, northern pike in these lakes serve as a potential source population for proximate vulnerable drainages such as the Matanuska and Knik River drainages including Jim Creek, Wasilla Creek and other presently uninvaded waters in the Northern Cook Inlet region.

To prevent northern pike from spreading, Anderson and Kings lakes are high priorities for invasive northern pike eradication in the Northern Cook Inlet Region (ADF&G Strategic Planning Committee¹). There are nine connected lakes within the Cottonwood Creek drainage, and some of these lakes provide optimal habitat conditions for northern pike. The native fish assemblage in the drainage as a whole includes Chinook salmon, sockeye salmon, pink salmon, coho salmon, chum salmon, rainbow trout, Dolly Varden, threespine stickleback, slimy sculpins and longnose suckers. The salmon and trout in these lakes contribute substantially to sport fishing activities in the Matanuska Borough (Jennings et al. 2015). To prevent northern pike from spreading and affecting the drainage's native fish populations, efforts to remove northern pike from the Cottonwood Creek drainage will be conducted in 2 phases.

The first phase of this project will investigate the distribution of northern pike within the Cottonwood Creek drainage. As mentioned, our current understanding is that northern pike in the drainage are restricted to Anderson and Kings lakes. However, if their distribution is more widespread, it is prudent to learn this before subsequent steps are taken in the planning and implementation of northern pike eradication activities. In addition to assessing northern pike distribution in the drainage, we will conduct biotic and abiotic surveys in drainage lakes to prepare for eventual eradication activities. Finally, we'll develop a preliminary treatment plan for the affected areas of the drainage using the piscicide rotenone, the only proven invasive fish eradication method aside from draining a waterbody (Finlayson et al. 2010), and we'll conduct public scoping to gauge the level of public support for northern pike eradication in the drainage.

Phase 2 of this project will eventually complete the permitting processes, fine scale planning, and implementation of the northern pike eradication activities in the drainage. This operational plan focuses on the first phase of this project.

OBJECTIVES

This project will lay the groundwork for the eventual eradication of invasive northern pike from the Cottonwood Creek drainage by conducting biological assessments of 2 lakes known to contain invasive northern pike (Anderson and Kings lakes), surveying Little Niklason and Mud lakes for the presence of northern pike, collecting water quality data and bathymetric data from all drainage lakes, developing a preliminary pike eradication plan, and conducting public scoping to determine the level of support for eradication. The specific objectives of this project are:

¹ Alaska Department of Fish and Game. Unpublished 2010 memorandum. Region II Invasive Northern Pike Priorities. Alaska Department of Fish and Game, Division of Sport Fish. Anchorage.

- 1. Detect the presence of northern pike in Little Niklason and Mud lakes during the spring of 2017 such that the probability of detection is 0.99 given the population is at least 4 northern pike.
- 2. Estimate the mean CPUE of northern pike in Anderson and Kings lakes such that the estimate is within 3.5 fish of the true value 95% of the time in Anderson Lake and within 2.3 fish of the true value 95% of the time in Kings Lake.

Secondary Objectives:

- 1. Collect and analyze eDNA samples for all Cottonwood Creek drainage lakes where northern pike are not captured in gillnets to verify the absence of northern pike in these lakes.
- 2. Map Anderson, Kings, Mud, and Little Niklason lakes to verify surface acreage and volume.
- 3. Measure water quality (temperature, dissolved oxygen (DO), pH, specific conductance) monthly from Anderson, Kings, Mud, and Little Niklason lakes.
- 4. Calculate the mean catch per unit effort (CPUE) of juvenile salmonids and resident fishes in Anderson and Kings lakes.
- 5. Inventory dominant invertebrate taxa from Anderson and Kings lakes.
- 6. Document waterfowl usage of Anderson and Kings lakes.
- 7. Prepare a pike eradication plan and solicit public input.

METHODS

STUDY AREA

The Cottonwood Creek Drainage encompasses 70 mi.² between Palmer and Wasilla and contains the following interconnected lakes: Kings, Anderson, Dry, Niklason, Little Niklason, Cornelius, Cottonwood, Mud, and Wasilla lakes (Table 1; Figure 2). Of the drainage lakes, only Anderson and Kings are known to have established populations of northern pike. The drainage lakes vary considerably in size and degree of northern pike habitat. Known size and habitat information for the drainage lakes is summarized below in Table 1. Bathymetry for Anderson, Kings, Mud, and Little Niklason lakes will be updated during the fall of 2016 and spring of 2017.

	Pike	Littoral Area	Surface Area	Volume (Acre-	Max Depth	
Lake	Habitat	(Acres)	(Acres)	Ft)	(ft)	Notes
Kings	Good	123	154	1,139	23	East edge has good pike spawning habitat; known pike presence
Anderson	Good	101	135	1,404	28	East edge has good pike spawning habitat; known pike presence
Dry ^a	Good	20	20	120	6	Pike spawning habitat may be limited
Niklason	Marginal	36	72	11,309	57	Poor pike spawning habitat except the west side
Little Niklason ^a	Very Good	33	33	198	6	Potential strong-hold; Good pike habitat throughout
Cornelius	Poor	29	48	1,088	54	Good rainbow trout habitat
Cottonwood	Poor	131	262	2,835	39	Good rainbow trout habitat
Mud	Unknown	55	55	181	17	Primarily a duck pond
Wasilla	Poor	187	374	6,412	48	Rainbow trout and sockeye salmon fisheries; SWHS records of pike

Table 1.-Cottonwood Creek Drainage Lakes.

No previous bathymetric survey exists; sizes are estimated from Google Earth, but bathymetric surveys will be updated in the fall of 2016 and spring of 2017.

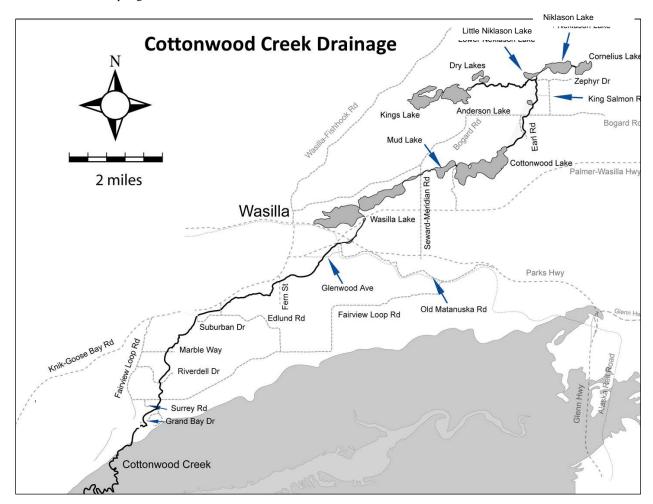


Figure 2.-Map of the Cottonwood Creek Drainage.

STUDY DESIGN – PRIMARY OBJECTIVES

Northern Pike Presence in Little Niklason and Mud lakes (Objective 1)

An important step in this project will be to learn if northern pike in the Cottonwood Creek drainage are actually restricted to Kings and Anderson lakes. This will involve intensive sampling of Little Niklason and Mud lakes with gillnets set in the vegetated littoral zone of each lake. Little Niklason Lake is the first lake directly downstream from Anderson Lake, and it probably contains some of the best northern pike habitat in the entire Cottonwood Creek drainage. If northern pike are detected in the netting effort on this lake, we will know definitively that northern pike have escaped the Kings/Anderson lakes complex and have spread further down the Cottonwood Creek system. This would then indicate the need for a broader eradication plan that encompasses more lakes in the system.

Mud Lake is located between the 2 largest lakes in the Cottonwood Creek drainage: Cottonwood Lake and Wasilla Lake. These 2 lakes account for some of the highest native rainbow trout production in the Matanuska-Susitna Valley. Cottonwood and Wasilla lakes are also large rearing lakes for wild sockeye and coho salmon. Mud Lake is the second to the last lake in the Cottonwood Creek system and the final lake to provide any decent northern pike habitat. Intensive netting in Mud Lake will allow us to avoid netting in Cottonwood and Wasilla lakes which would cause a great deal of unwanted bycatch. Because Mud Lake is located downstream of Cottonwood Lake it can be concluded that if northern pike are detected in Mud Lake, northern pike are likely also present in Cottonwood and Wasilla lakes. If northern pike are detected in Mud Lake, and, therefore, Cottonwood Creek drainage will probably not be implemented due to the size of the area and the native fish productivity of all of the lakes in the system.

Gillnets are frequently used for the detection and suppression of invasive northern pike in Alaska (Rutz and Dunker, *In Prep*, Glick and Willette 2016, Sepulveda 2013). Gillnets are most effective when fished in optimal habitat conditions for northern pike which typically include slow moving streams and sloughs and the shallow bays, embankments, and densely vegetated littoral zones of lakes (Inskip 1982).

Sampling Effort for Meeting Objective 1

To quantify the netting effort necessary to detect a northern pike population of at least 4 fish, we employed a method used following rotenone applications in Southcentral Alaska to evaluate treatment success in removing northern pike populations. In the spring and the fall of 2005, 2006 and 2007, northern pike were removed from Derks and Sevena lakes with gillnets and a subsequent removal estimate of abundance was calculated for each event per lake. The estimated northern pike catch rate for the final 24-hour day of netting (Table 2) provided the basis for assessing our ability to detect a small abundance of northern pike (4 individuals) in other lakes.

The capture rate in Derks Lake during the fall of 2005 (0.085) was one of the lowest observed during 3 years of northern pike removal efforts on the Kenai Peninsula (Table 2) and serves as a conservative surrogate to estimate northern pike detection probabilities in other lakes with small northern pike abundances.

		Sevena Lake (33.51 littoral zone surface acres)						Derks Lake	(18.89 littora	al zone surfa	ce acres)	
	200	2005 2006		6	2007		200	2005	2006		200	07
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Last day catch	5	27	7	3	2		6	15	1	12	10	32
Total seasonal catch	643	1,403	344	38	10		143	312	74	276	80	469
Population estimate +1 S.E.	680	1,435	359	44	11		153	474	75	394	86	1,444
Nets fished per day	12	12	12	12	24		12	12	12	12	24	24
Number of removal events	16	14	12	4	2		10	15	12	11	4	15
Population estimate at beginning of last sampling												
day	42	59	22	9	3		16	177	2	130	16	1,007
Capture rate on last day	0.118	0.454	0.318	0.333	0.727		0.377	0.085 ^a	0.500	0.092	0.625	0.032
Percent of available population caught per net on last day	0.010	0.038	0.027	0.028	0.030		0.031	0.007	0.042	0.008	0.026	0.001
Net density (nets/littoral surface acres)	0.358	0.358	0.358	0.358	0.716		0.635	0.635	0.635	0.635	1.271	1.271

Table 2.– Historical net effort, catch and removal population estimates for Derks and Sevena lakes during 2005 – 2007.

^a Although the catch rate on the last day of netting at Derks Lake during the fall of 2007 was the lowest estimated, it was disregarded because of suspected emigration of northern pike during the removal. The second lowest rate was estimated at Derks Lake in the fall of 2005 and emigration did not appear to be occurring during the removal period. As a result, the capture rate of 0.085 is used to estimate the probability of not detecting a northern pike for other lakes.

The probability of failing to detect any of 4 remaining northern pike (D_p) in a lake of known littoral zone surface acreage with a given amount of netting effort (net density and days of fishing) can be estimated as follows:

$$D_p = \hat{P}_f^{\ \ (T \times R)} \tag{1}$$

where

 \hat{P}_f = the estimated probability that none of 4 remaining fish are captured on the final day of netting at Derks Lake with 0.364 gillnets/surface acre

= 0.605,

T = number of days of gillnetting effort (24-hour day), and

R = relative net density

where

$$R = \frac{N/A}{0.365} \tag{2}$$

and

N = desired number of nets to set in lake to be evaluated,

A = littoral zone acreage of lake to be evaluated, and

0.365 = nets per littoral-zone-acre used at Derks Lake on last day of netting during the fall of 2005.

The 0.085 capture rate (most conservative from Table 2) can be viewed as the probability of capture per individual fish in a single 24-hour-day of gillnetting effort where gillnet density is equal to 0.365 nets per littoral-surface-acre. Therefore, the probability that an individual fish will not be captured under those conditions is equal to (1 minus 0.085) or 0.915. The probability of not capturing any of 4 fish given that 4 fish remain in a particular lake (\hat{P}_f) is 0.915⁴ or 0.701. A spreadsheet with these formulas was used to calculate the non-detection probabilities listed in Table 3 and used to calculate the netting effort needed to meet the objective criteria for Little Niklason and Mud lakes.

	Lak	e
	Little Niklason	Mud
Days	40 nets	40 nets
1	0.51	0.67
2	0.26	0.45
3	0.13	0.30
4	0.07	0.20
5	0.03	0.13
6	0.02	0.09
7	0.01	0.06
8	0.00	0.04
9	0.00	0.03
10	0.00	0.02
11	0.00	0.01
12	0.00	0.01
13	0.00	0.01
14	0.00	0.00

Table 3.–Probabilities of not detecting a small northern pike populations (N=4) in Little Niklason and Mud lakes for 1 to 14 days of netting with 0.365 nets per littoral surface acre.

For this project, 40 nets will be deployed in both Little Niklason and Mud lakes. To obtain a probability of non-detection of less than 0.01, Little Niklason Lake will need to be sampled for 8 days and Mud Lake will be sampled for 14 days (Table 3).

Gillnets will be deployed in the vegetated littoral zone of both lakes on a Monday morning and fished continuously through their sampling period. Staff will be present at both lakes every day to check the nets, collect and record data, and free any bycatch. GPS coordinates will be collected at all net locations. Ideally, nets will either remain or be redeployed in the same locations as they were initially set. However, net locations may be altered if bycatch rates are high.

If northern pike are detected in a particular lake, netting will be halted in that lake because northern pike presence will have been established and to prevent further bycatch.

CPUE of Northern Pike in Anderson and Kings lakes (Objective 2)

During early fall 2016, field crews will also deploy 18 gillnets and 20 minnow traps in the littoral zone of each lake to estimate the mean CPUE of pike and all other fish combined. All gillnets and minnow traps (baited with salmon roe) will be set for a 24-hour period in each lake. All net and trap locations will be numbered and GPS coordinates will be recorded for each set. All captured northern pike will be removed from the lakes and dissected to identify sex and to enumerate stomach contents to the lowest degree of taxonomic resolution possible. Netting and trapping efforts will be duplicated in the spring of 2017 to eliminate issues arising from any seasonal effects on fish movements.

Number of Salmpling Nets for Meeting Objective 2

Because all nets and traps will be set for 24 hours, CPUE will be treated as the mean catch per 24 hours of effort. The number of nets needed to obtain the objective criteria was calculated from the following:

$$n = \left(\frac{Z\alpha_{/2} s}{E}\right)^2 \tag{3}$$

where

 $Z\alpha_{/2}$ = probability from the standard normal distribution

= 1.96

s = estimated standard deviation

where

$$s = \frac{(\max \operatorname{catch} \operatorname{per} \operatorname{set} - \min \operatorname{catch} \operatorname{per} \operatorname{set})}{4}$$
(4)

where "max" and "min" come from the data collected by the project biologists and

E = Maximum error of the estimate.

The number of nets needed to meet the objective is listed in Table 4.

Table 4.–Number of nets needed to estimate the mean CPUE of northern pike within 3.5 fish of the true value 95% of the time in Anderson Lake and within 2.3 fish of the true value 95% of the time in Kings Lake.

Anderso	on Lake	Kings	Lake
	Sample		Sample
Error	Size	Error	Size
(number of	(number of	(number of	(number of
fish)	nets)	fish)	nets)
1	216	1	96
2	54	2	24
3	24	2.3	18
3.5	18	3	11
4	14	4	6
5	9	5	4

eDNA Assessment of Northern Pike Distribution

Assessment of environmental DNA (eDNA) is a relatively new technique where the presence of an aquatic species can be determined through quantitative polymerase chain reaction (qPCR) amplification of shed DNA material present in the environment such as lake water. This technique is thought to be more sensitive than traditional fisheries approaches for detecting aquatic taxa in low abundance (Ficetola 2008). ADF&G and the United States Fish and Wildlife Service (USFWS) recently partnered to develop and test eDNA markers for northern pike (Olsen et al. 2015), and ADF&G is now using eDNA to survey for northern pike and evaluate the success of current northern pike eradication projects (Dunker et al. 2016). For this project, we have sufficient funding to collect and analyze approximately 150 water samples, including control blanks, from lakes in the Cottonwood Creek drainage where northern pike are not presently known to occur. We'll distribute these samples proportional to the surface acreages of each lake (Table 5). Because eDNA is theoretically more sensitive than gillnetting, this assessment is meant to verify the results of the netting surveys. Recent northern pike eDNA surveys on the Kenai Peninsula resulted in an average positive detection rate of 82.4% (70 of 85 samples) from four lakes with known pike populations where northern pike abundance was depressed following long-term removal efforts (Dunker 2016). The sampling intensity (acres/sample) for the Kenai Peninsula eDNA survey approximated 4.6 surface acres/sample (Dunker 2016), the proposed sampling intensity for this study is similar and ranges from 5.5 to 6.2 acres/sample (Table 5).

If we find positive eDNA detections in lakes where northern pike are not captured in gillnets, this will indicate the need to collect more eDNA samples and conduct targeted netting in those locations (Dunker et al. 2016). Because Anderson and Kings lakes have confirmed northern pike populations, eDNA samples will not be collected from those two lakes. If gillnet surveys detect pike in Little Niklason or Mud lakes, no eDNA samples will be taken and instead, sampling will be allocated elsewhere in the drainage. Latent sources of northern pike eDNA (i.e. sources not from living fish but rather from sediment release, upstream sources, etc.) are potential sources of error when using this technique (Dunker et al. 2016). Some ambiguity in eDNA results may remain, but if there are any positive eDNA results in lakes where gillnets do not capture northern pike, then either follow-up netting, additional eDNA sampling, or both will be needed to definitively prove the presence or absence of northern pike in those lakes.

Lake	~ Surface	Proportion of	Sampling	# of eDNA Lake	# of eDNA
	Acreage	Overall Surface	Intensity	Water Samples to	Control Blank
		Acreage	(Acres/Sample)	Collect	Samples to Collect
Dry	20	2.3%	5.0	4	3 ^a
Niklason	72	8.3%	6.0	12	
Little Niklason	33	3.8%	5.5	6	
Cornelius	48	5.6%	6.0	8	
Cottonwood	262	30.3%	6.2	42	3 ^b
Mud	55	6.4%	6.1	9	
Wasilla	374	43.3%	6.2	60	3
Totals	864	100.0%		141	9

Table 5.–Number of eDNA samples that will be collected in proportion to total surface area of lakes in the Cottonwood Creek drainage given there are 150 samples.

^a These control blanks will apply to a group of lakes that will be sampled on the same day as follows: Dry Lake, Little Niklason Lake, Niklason Lake and Cornelius lake.

^b These control blanks will apply to a group of lakes the that will be sampled on the same day as follows: Cottonwood Lake and Mud Lake

Sample site locations in each lake will be determined after new bathymetric maps are generated. Samples will not be distributed randomly through each lake. Instead, because we have a limited number of samples we can collect, we will target favorable northern pike habitat to include littoral waters < 4m in depth and within or adjacent to dense standing aquatic vegetation beds

including submerged, emergent or inundated shoreline vegetation. After the new lake maps are generated (See Lake Mapping section), we will identify the best areas to sample within each lake and will partition samples as evenly as possible within the littoral zones of those areas. Site selection will be finalized during the winter of 2017.

Each eDNA sample will consist of a single surface water grab samples collected in a sterilized 1liter NalgeneTM bottle directly from a boat by a collector wearing a new pair of nitrile gloves prior to collecting each sample. Each day sampling occurs, we will collect several control blanks that will help identify whether eDNA contamination has occurred through our sample handling, transport, or filtering procedures. Control blanks will be collected in the same sample bottles and volume size as the lake water samples but will consist of filling the sample bottle with deionized water. One control, called a field blank, will be collected while in the field using the same handling protocol used for the lake samples. The field blank will help assess whether sample contamination is introduced during field collection activities. Another control, called a travel blank, will be collected in the lab prior to departure to the field and transported to and from the field in the same cooler used to transport the lake samples. The travel blank will help identify if sample contamination is introduced during transport. A lab blank will be collected in the same lab used to temporarily store and filter the lake water samples. The lab blank will serve to identify whether sample contamination is introduced during filtering processes or storage in the lab. Before taking a sample at each sampling site, the collector will wear new nitrile gloves. Samples will typically be collected from a boat. To reduce the risk of the boat hull and outboard motor introducing pike eDNA to a sample location, the boat hull (lower unit and trailer) will be disinfected with a 10% bleach solution spray and allowed to air dry before departure each day. Also, the samplers will collect the sample from the boat bow before the boat moves atop or beyond a sampling site. The samplers will systematically collect samples in in a sequential manner at each lake to avoid traveling past a sampling site before it is sampled. All samples collected in the field will immediately be sealed inside a Whirl-Pak[™] bag and chilled by packing them in ice inside an insulated cooler.

Within 2 days of sample collection, all samples will be filtered using a GeoTech series II peristaltic pump and 0.45 µm nitrocellulose membrane filters. After filtering, all membrane filters from unique samples will be stored in separate vials and placed into cold storage. If multiple filters are required to filter a single sample, all can be combined in a single vial. All field water sampling, equipment decontamination, sample filtering and storage are designed to follow established eDNA protocols (Wolt 2015). These decontamination procedures will include: 1) wearing new nitrile or latex gloves each time a new sample is handled, 2) using only tweezers to handle filters, and 3) sterilizing all tweezers and the filtering assemblies in a 50% bleach solution (50% deionized water, 50% household bleach containing 8.25% hypochlorite) bath for 10-15 minutes followed by two deionized water baths. Before filtering a new sample, we will spray the pump and associated work area with a 10% bleach solution or DNA AWAYTM and wipe the space dry. The filter assembly will be reassembled after sterilization and then rinsed by pumping 0.5-1.0 L of deionized water through as a final rinse.

Samples that are collected, filtered, and placed in cold storage will be transferred to the USFWS Conservation Genetics Lab in Anchorage for qPCR analysis. The USFWS will then inform us of all detection results.

Lake Mapping

Before rotenone treatments can be conducted in any of the drainage lakes where northern pike are confirmed, there is substantial field monitoring necessary for project planning and permitting. To begin, volume estimates must be verified for all drainage lakes, and updated bathymetric maps need to be generated. We will use a boat-mounted Lowrance HDS 7 survey unit and transducer to record depth and bottom structure data by driving a boat in concentric circles around each lake from the shoreline inward to the center. Transects will be spaced approximately 40 meters apart, and this can be gauged by watching the GPS track on the Lowrance unit's monitor. After concluding each survey, the depth and bottom structure data will be downloaded to a computer and submitted to ciBiobase Inc., a mapping company, that will run algorithms on the data and generates volume estimates and detailed bathymetric maps of each lake (Appendix A1).

Water Quality Monitoring

Water quality parameters including temperature, pH, turbidity, and dissolved oxygen will be collected monthly for 1 year in King, Anderson, Little Niklason, and Mud lakes in 1-meter increments from the deepest areas of each lake. GPS coordinates of all locations will be collected for repeatability. The water quality parameters will be collected using a portable YSI 556 unit, and a secchi disk will be used to measure turbidity. Samples will be collected from a boat during open water and by drilling through the ice during the winter. These seasonal data are important for eventual rotenone treatment planning and permitting.

Stream discharge information is also needed for estimating the necessary amounts of rotenone and potassium permanganate (used to neutralize rotenone) and for calculating the appropriate application rates of those chemicals. Stream discharge measurements will be collected from the outlets of Anderson and Kings lakes, if flowing, during water quality sampling in May 2017. Measuring techniques will comply with United States Geological Survey (USGS) specifications as described in Nolan and Shields (2000).

Biological Assessments of Kings and Anderson Lakes (CPUE of Salmonids, Invertebrate Taxa and Waterfowl Usage)

Pre-treatment biological assessments of Anderson and Kings lakes, the 2 lakes with the Cottonwood Creek drainage known to have northern pike, will also be conducted. Macroinvertebrate and plankton surveys will be collected in both Anderson and Kings lakes to document the dominant taxa and their relative abundance. In each lake, zooplankton evaluations will be made at 2 sites by replicate vertical tows using a 0.5 meter diameter Wisconsin net with 153 µm mesh at different locations near maximum lake depth. The Wisconsin net will be lowered to just above the lake bottom near maximum depth and then retrieved at a rate of 1 meter every 2 seconds. Zooplankton samples will be analyzed to a reasonable degree of taxonomic resolution and relative abundance. An Ekman dredge will be used to collect bottom sediment from two sites at both lakes; sediments will be screened to extract any invertebrates for later identification. Kick nets will be used to collect invertebrates along vegetated shorelines in 3 locations from both lakes. At both lakes, attempts will be made to visually locate and collect freshwater mussels and snails. All sample locations will be recorded with a GPS to ensure repeatability of site selections. All invertebrate specimens from all collection efforts will be preserved in 90% ethanol, labeled with the date, collector initials and site location, and archived for later evaluation.

In addition, all waterfowl, amphibians, and mammals observed during these sampling events will be noted.

Eradication Planning and Scoping

After conducting the biological and physical assessments of Anderson and Kings lakes and surveying the remainder of the drainage with eDNA samples and gillnets (in Little Niklason and Mud lakes), a northern pike eradication plan will be written. This plan will provide the details necessary to conduct rotenone treatments in these lakes and will serve as the basis for the public scoping process to determine the level of interest and support for removing northern pike from the affected lakes in the drainage.

This public scoping process will compose the final component of this project phase. A planning firm will be hired to facilitate public scoping with ADF&G on proposed northern pike eradication plans, conduct stakeholder interviews, and administer and evaluate public surveys on the topic. The results of the scoping process will be submitted to ADF&G in a report for determination on whether or not to proceed with the eradication activities. It is anticipated that there will be public support for this project. If that is the case, our next step will be to begin soliciting funds for the permitting, fine-scale planning, and eradication activities in the drainage.

DATA COLLECTION

Northern Pike Presence in Little Niklason and Mud lakes

All fish captured in gillnets will be counted and identified to species. Catch of all species other than northern pike will be recorded on the Bycatch Form (Appendix B1), and all fish other than northern pike will be released immediately. The fork lengths (FL; tip of nose of fork of tail) of all captured northern pike will be measured and recorded to the nearest millimeter. We will dispatch all captured pike on-site and will record their sex, maturity, and stomach contents (Appendix B2). Guidelines for completing the catch and sampling forms are found in Appendix B3.

CPUE of Northern Pike in Anderson and Kings lakes

For each net or trap set, all captured animals will be enumerated by species. The date, net or trap number, GPS location, net or trap set time and, net or trap pull time will also be recorded. All salmonid individuals and resident fish will be released alive, if possible. Data for each day's catch will be recorded in the bycatch and northern pike forms in Appendices B1 and B2.

eDNA Assessment of Northern Pike Distribution

Prior to collecting eDNA samples, approximate sample locations will be numbered and identified on a bathymetric map of each lake. Sample bottles will be labeled with the name of the lake, date, sampler initials, and sample number. An Excel spreadsheet of this information will be prepared prior to field sampling and printed to bring in the field. During sampling, the time each sample was collected will be recorded as well as notes on the day's weather conditions. These times will be entered in the Excel spreadsheet. During sample filtering, the time and date each sample was processed will also be recorded. Finally, while sampling in the field, GPS coordinates of all sample sites will be collected, and GIS maps will later be generated to illustrate sample locations in each lake.

Lake Mapping

All lake mapping data will be collected and stored digitally on a Lowrance HDS 7 GPS unit and stored on an SD card until it can be downloaded and submitted to ciBiobase. ciBiobase will then generate and supply bathymetric maps and volume estimates similar to that illustrated in Appendix A1.

Water Quality Monitoring

Monthly water quality data (temperature, dissolved oxygen, pH, and specific conductance) will be collected using a YSI 556 unit. All measurements will be recorded on data sheets in the field (Appendix B4) and entered into an Excel file to graph seasonal patterns.

Biological Assessments of Kings and Anderson Lakes (CPUE of Salmonids, Invertebrate Taxa and Waterfowl Usage)

All observed waterfowl taxa will be noted in a field notebook. During invertebrate surveys, invertebrates will be collected in the field and later identified down to the lowest known taxonomic level and entered into an Excel file in the lab.

DATA REDUCTION

Paper data forms completed by field crews for the gillnetting, water quality monitoring, and biological assessments of Anderson and Kings lakes will be entered into MicrosoftTM Excel data files.

DATA ANALYSIS

Northern Pike Presence in Little Niklason and Mud lakes

If northern pike are detected we will know that northern pike presence has been established.

If northern pike are not detected given the sample size determined above, we will conclude that either no northern pike are in the lake or that there is a population of less than 4 northern pike in the lake (probability of non-detection error < 0.01).

CPUE of Northern Pike in Anderson and Kings Lakes

Mean catch per 8-hour set will be calculated for each gear type and species following standard procedures for arithmetic mean and variance.

eDNA Assessment of Northern Pike Distribution

The United States Fish and Wildlife Service Conservation Genetics lab in Anchorage will perform all laboratory analyses of collected water samples and will provide either positive or negative results of eDNA detections for all samples.

Water Quality Monitoring

Water quality data for all drainage lakes will be summarized and presented in graphs to show seasonal patterns in each lake.

Lake Mapping

The mapping company ciBiobase will generate bathymetric maps and apply algorithms to our data to estimate lake size and volume. Bathymetric maps and data output files will be provided by ciBiobase to ADF&G within 2 weeks of data submission.

Biological Assessments of Kings and Anderson Lakes (CPUE of Salmonids, Invertebrate taxa and Waterfowl usage)

Invertebrate taxa will be ranked based on catch.

Presence of absence of waterfowl species observed during field excursions will be noted in our Excel data files.

SCHEDULE AND DELIVERABLES

2016		
	March	Begin monthly water quality monitoring in the Cottonwood Creek drainage lakes.
	April	Monthly water quality monitoring
	May	Monthly water quality monitoring
	June	Monthly water quality monitoring Subscribe to ciBiobase for lake mapping
	July	Monthly water quality monitoring
	August	Monthly water quality monitoring Purchase gillnets Begin mapping drainage lakes
	Aug. 29-Sept. 2	CPUE surveys in Anderson and Kings Lakes Biological Assessments of Anderson and Kings Lakes
	September	Monthly water quality monitoring Begin gillneting Little Niklason Lake to survey for northern pike
	October	Monthly water quality monitoring
	November	Monthly water quality monitoring
	December	Monthly water quality monitoring
2017	_	
	January	Monthly water quality monitoring
	February	Monthly water quality monitoring
	March	Monthly water quality monitoring
	May	eDNA survey of drainage lakes
		CPUE surveys in Anderson and Kings Lakes
		Gillnet Little Niklason Lake to survey for northern pike
		Gillnet Mid Lake to survey for northern pike
	May / June	Process eDNA samples
	June	Biological Assessment of Anderson and King's Lakes
		Complete mapping drainage lakes
	Summer	Draft Treatment Plan for pike eradication in the drainage
2018		
		Write Project Report
		Solicit funding and begin permitting for Phase 2

RESPONSIBILITIES

Personnel: Duties:	Cody Jacobson, Fishery Biologist I Primary project biologist; Coordinates all field logistics, purchasing, and project implementation; Enters and manages data; Prepare project reporting and presentations to the public.
Personnel: Duties:	Kristine Dunker, Fishery Biologist III, Provide oversight and make recommendations on study designs and project plans; assist with data analysis and project reporting; coordinate and assist with the completion of project deliverables.
Personnel: Duties:	Pat Hansen, Biometrician IV Provide guidance on study design; Review project operational plans and reports.
Personnel: Duties:	Dave Rutz, Fishery Biologist II Assists the project biologist with logistical planning, sampling, and provides input on study design.
Personnel: Duties:	FWT II Assists with field work and data collection

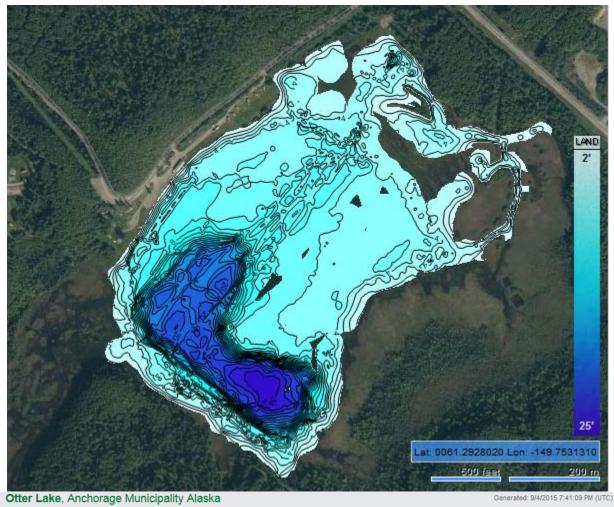
BUDGET SUMMARY

Line 100 Personnel	\$20,000
Line 200 Travel	\$ 400
Line 300 Contractual	\$22,000
Line 400 Supplies	\$ 9,700
Line 500 Equipment	-
Indirect	\$ 4,200
Total	\$56,300

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APPENDIX A: EXAMPLE OF BATHYMETRIC MAP



Waterbody Size: 60.14 ha (148.60 acres)

	Data Collector	Survey S	ize	Settings			
	Jay Baumer	Area:	48.02 ha (118.65 acres)	Track Buffer: Grid Cell Size:	25 m 5 m		
C Ser	Data Collection Date 8/13/2015 12:16:33 AM (UTC)	Percent: Volume:	79.84% of waterbody 1,089,284.80 cu. m (883.10 acre ft)	Min. BV Detect: Min. Veg Depth Dete	5%		
	Average Water Temperature			Quality Control			
	18.29° C (64.92° F)	Est. Wate	rbody Volume ?	Reviewer: Valley, Ray			
		1,364,443 (1,106.17	.10 cu. m acre ft)	Comments: See watervolume with new shapefile. Look at the thum			
	Start: 61.2854805, -149.73587036 End: 61.28646088, -149.73434448			This sh	new shapefile boundary. ould hopefully get you to the true volume		

report link

A S	Survey Summary													
	Type ?	PAC ?	Avg BVp ?	SD BVp ?	Avg BVw ?	SD BVw ?	Depth Range	Avg Depth	Distance	No. Points				
Full	Point	82.6%	29.9%	±23.4%	24.7%	±24.1%	0.5-7.59 m	2.06 m	34.72 km	11,620				
Survey	Grid	87.1%	29.4%	±22.6%	25.6%	±23.3%	0-7.63 m	2.26 m	-	15,556				

Appendix A1.–Example of Bathymetric Map produced by ciBiobase.

Note: All Cottonwood Creek drainage lakes listed in Table 1 will be remapped in this format.

APPENDIX B: SAMPLING FORMS

Appendix B1.–Bycatch form.

Lake	Gillnet #	Date	Time	Intial Set (Y or N)	Northern Pike	Arctic Grayling	Whitefish	Longnose Sucker	Rainbow trout	Burbot	Chinook Salmon	Coho Salmon	Sockeye Salmon	Dolly Varden	Pink Salmon	Chum Salmon	Muskrat	Beaver	Vole	Bird Bycatch	GPS Coordinates
																			<u> </u>		
													-					-	<u> </u>	-	
													-					-	┣─	-	
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Date:		Lake:	Gillnet #:									
Pike #	Fork Length (mm)	Reproductive Product (M, E, or A)	Maturity (M,I,or U)	Ses (M or F)	Stomach Contents	Comments						

Appendix B2.–Northern pike sampling form.

Appendix B3.–Def	inition of terms for catch and sampling forms (Appendices B1 and B2).
Gillnet #:	Assign each net a number in numerical order as they are set
GPS loc:	Shoreline start of net
Species:	Record everything caught, even birds and mammals
Mort:	"X" for mortality of bycatch only. Pike assumed to be killed.
Reproductive Prod	ucts: Before dissection of fish, squeeze to observe release of sex product
	M= milt
	E=eggs
	A= absent
Maturity: Dissect fi	sh.
	M = mature (Gonads enlarged)
	I = immature (Gonads not developed)
	U = unknown
Sex: Mark only if al	osolutely known after dissection of fish.
	$\mathbf{M} = \mathbf{male}$
	$\mathbf{F} = \mathbf{female}$

U = unknown

Stomach Contents: common abbreviations for species.

KS=king salmon; SS=silver salmon; RS=red salmon; CS=chum salmon; PS=pink salmon; WF=white fish; LS=long nose sucker; SB=stickleback; RT=rainbow; GR=grayling; NP=northern pike; BB=burbot; DV=dolly varden; SC=sculpin; PL=pacific lamprey.

Other catch could be: macro invertebrates, rodents, other mammals, birds, leeches, frogs...

Appendix B4.-Water quality data sheet.

Lake: _____ Sampler:

Time:

	Temperature	Specific Conductance	Dissolved Oxygen	Dissolved Oxygen	рН
	°C	S/cm	mg/L	%	
1 M					
2 M					
3 M					
4 M					
5 M					
6 M					
7 M					
8 M					
9 M					
10 M					
11 M					
12 M					
13 M					
14 M					
15 M					
16 M					
17 M					
18 M					

Visiblity (m):

Date:

Ice Thickness (In):

Comments: