Regional Operational Plan SF.2A.2020.18

Operational Plan: Kenai Peninsula Nonnative Fish Control, Monitoring, and Native Fish Restoration

by

Robert Massengill

Robert N. Begich

and

Kristine Dunker

September 2020

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

REGIONAL OPERATIONAL PLAN SF.2A.2020.18

OPERATIONAL PLAN: KENAI PENINSULA NONNATIVE FISH CONTROL, MONITORING, AND NATIVE FISH RESTORATION

by
Robert Massengill
Alaska Department of Fish and Game, Division of Sport Fish, Soldotna
Robert N. Begich
Alaska Department of Fish and Game, Division of Sport Fish, Soldotna
and
Kristine Dunker
Alaska Department of Fish and Game, Division of Sport Fish, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

September 2020

The Regional Operational Plan Series was established in 2012 to archive and provide public access to operational plans for fisheries projects of the Divisions of Commercial Fisheries and Sport Fish, as per joint-divisional Operational Planning Policy. Documents in this series are planning documents that may contain raw data, preliminary data analyses and results, and describe operational aspects of fisheries projects that may not actually be implemented. All documents in this series are subject to a technical review process and receive varying degrees of regional, divisional, and biometric approval, but do not generally receive editorial review. Results from the implementation of the operational plan described in this series may be subsequently finalized and published in a different department reporting series or in the formal literature. Please contact the author if you have any questions regarding the information provided in this plan. Regional Operational Plans are available on the Internet at: http://www.adfg.alaska.gov/sf/publications/

Robert Massengill,
Alaska Department of Fish and Game, Division of Sport Fish,
43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669, USA

Robert N. Begich
Alaska Department of Fish and Game, Division of Sport Fish,
43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669, USA

and

Kristine Dunker,
Alaska Department of Fish and Game, Division of Sport Fish,
333 Raspberry Road, Anchorage, AK 99518, USA

This document should be cited as follows:
Massengill, R., R. N. Begich, and K. Dunker. 2020. Operational Plan: Kenai Peninsula nonnative fish control, monitoring, and native fish restoration. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.2A.2020.18, Anchorage.

SIGNATURE/TITLE PAGE

Project Title:	Kenai Peninsula Nonnative Fish Control, Monitoring, and Native Fish Restoration
Project leader(s):	Robert Massengill, Robert Begich, and Kristine Dunker
Division, Region, and Area	Division of Sport Fish, Region II, Soldotna Office
Project Nomenclature:	F-10-36; R-2-15
Period Covered	July 2020–June 2022
Field Dates:	1 July 2020–31 October 2020, 1 April 2021–30 June 2022 1July 2020–31 October 2020, 1 April 2021–30 June 2022
Plan Type:	Category II

Approval

Title	Name	Signature	Date
Project leader	Rob Massengill		
Project leader	Robert Begich		
Project leader	Kristine Dunker		
Biometrician	Benjamin Buzzee		
Research Coordinator	Tim McKinley		

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

This project will investigate whether invasive northern pike (*Esox lucius*) and other nonnative fish are present in the Northern Kenai Peninsula Management Area (NKPMA) and evaluate the success of eradication efforts for these populations. Where northern pike have already been successfully eradicated, this project will supplement restoration and monitoring of native fish populations. Nonnative fish detection will be accomplished by gillnet surveys using a standardized protocol that adjusts netting effort to lake size. The prioritization of waterbodies selected for surveys will be founded on a risk assessment evaluation. At select waterbodies, gillnet surveys may be undesirable, and environmental DNA (eDNA) detection methods may be used alone or in tandem with reduced gillnetting efforts. When an invasive fish species is detected in a waterbody, this project will initiate the collection of baseline environmental and biological data necessary for informing a response action plan. Native fish populations will be restored, as applicable, to waters where nonnative fish have been removed. This is typically accomplished by collecting wild fish from a nearby source and stocking them in the affected waters, particularly where natural recolonization of native fish populations is unlikely to occur rapidly. Restored native fish populations will be assessed periodically using gillnet and minnow trap catch per unit effort (CPUE) and length frequency distributions.

Key words: Northern pike, *Esox lucius*, restoration, CPUE, invasive, rotenone, eDNA

INTRODUCTION

PURPOSE

This project will provide information to fishery managers about the presence and distribution of invasive northern pike (*Esox lucius*) and other nonnative fish, evaluate the status of restored native fisheries in former pike-invaded waters, collect wild native fish for restoration-related purposes, and collect baseline environmental and biological data from waters where new nonnative fish populations are detected.

BACKGROUND

Nonnative freshwater fish reported to or found by the Alaska Department of Fish and Game in southcentral Alaska include northern pike, goldfish (*Carassius auratus*), yellow perch (*Perca flavescens*), fathead minnow (*Pimephales promelas*), muskellunge (*Esox masquinongy*), largemouth bass (*Micropterus salmoides*), blackfish (*Dallia pectoralis*), signal crayfish (*Pacifastacus leniusculus*), and red swamp crayfish (*Procambarus clarkia*) (Fay 2002; K. Dunker, Sport Fish Biologist, ADF&G, Anchorage, personal communication). There has also been an unverified report of Plecostomous catfish (*Hypostomus plecostomus*) (K. Dunker, Sport Fish Biologist, ADF&G, Anchorage, personal communication).

The most widespread nonnative fish species in southcentral Alaska is northern pike, which is native to Alaska north and west of the Alaska Range (Figure 1). Northern pike are implicated in the decline of native fisheries throughout the southcentral region (Rutz 1999; Patankar et al. 2006; Sepulveda et al. 2013; Sepulveda et al. 2015; Glick and Willette 2016; Dunker et al. 2018). There is strong evidence that northern pike prefer soft-finned juvenile salmonids over other available prey species in southcentral Alaska (Patankar et al. 2006; Sepulveda et al. 2013). Consumption of juvenile salmonids by introduced northern pike has been observed elsewhere in the northwestern United States (Rich 1992; McMahon and Bennett 1996; Schmetterling 2001; Muhlfeld et al. 2008). In Southcentral Alaska, prey of northern pike may be particularly vulnerable because they evolved in the absence of these predators whereas in interior Alaska, native northern pike share an evolutionary history with their prey, which appear to have evolved adaptations for predator avoidance (Oswood et al. 2000). Also, prevalent shallow lake morphology and slow stream velocities throughout much of southcentral Alaska offers prey

limited deepwater refugia from northern pike, which typically occupy shallow, vegetated habitat (Inskip 1982; Cook and Bergersen 1988; Dunker et al. 2018).

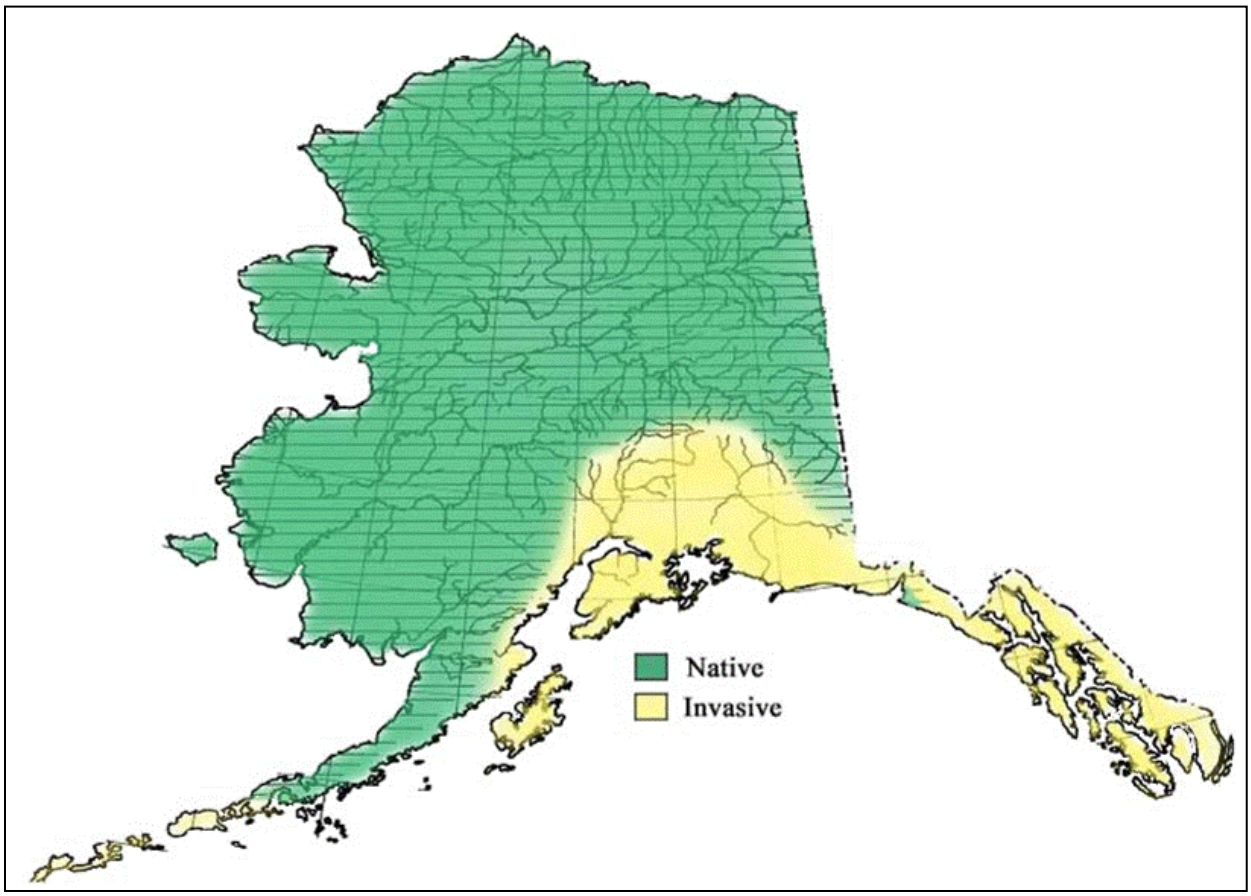

Figure 1.–Map of the native and invasive range of northern pike in Alaska

Introduced northern pike were first documented on the Kenai Peninsula in the Soldotna Creek drainage in the 1970s (ADF&G, Division of Sport Fish, Soldotna, unpublished). Subsequent dispersal and more illegal introductions have resulted in northern pike occurring in at least 25 Kenai Peninsula waterbodies (Figure 2)[1]. Northern pike were discovered in 15 of these waterbodies since 2000; however, the dates of the introductions are unknown. Kenai Peninsula northern pike have reduced or eliminated wild and hatchery-produced fish populations from some lakes (Begich 2010; Begich and McKinley 2005; McKinley 2013; Massengill 2014a,b, 2017, *In prep*). Beginning in 2008, the Alaska Department of Fish and Game (ADF&G) initiated a program to eradicate northern pike from the entire Kenai Peninsula. Initial efforts focused on eradicating northern pike from landlocked lakes (Massengill 2014a,b) followed by eradication in progressively more complex and open waterbodies within the Swanson River and Soldotna Creek drainages. The Tote Road "pike lakes" (TRPL) contained the last known northern pike populations on the Kenai Peninsula, and that population was eradicated in the fall of 2018.

Later in 2018, a new report of northern pike occurred in the Miller Creek drainage which is located near the northern tip of the Kenai Peninsula, and the majority of the drainage resides

[1] A lake referred to as G Lake in the Tote Road lake complex located south of Soldotna had illegally introduced muskellunge. Muskellunge are a member of the Esocidae (pike) family and are similar in appearance to northern pike.

within the boundaries of Kenai National Wildlife Refuge (KNWR). Intensive gillnet surveys throughout the drainage in 2019 determined the northern pike population was probably confined to Miller Creek, Vogel Lake, and North Vogel Lake (Figure 3). A partnership between ADF&G, United States Fish and Wildlife Service (USFWS), and the Kenai Watershed Forum (KWF) has formed to develop a response action to address the northern pike issue in the Miller Creek drainage.

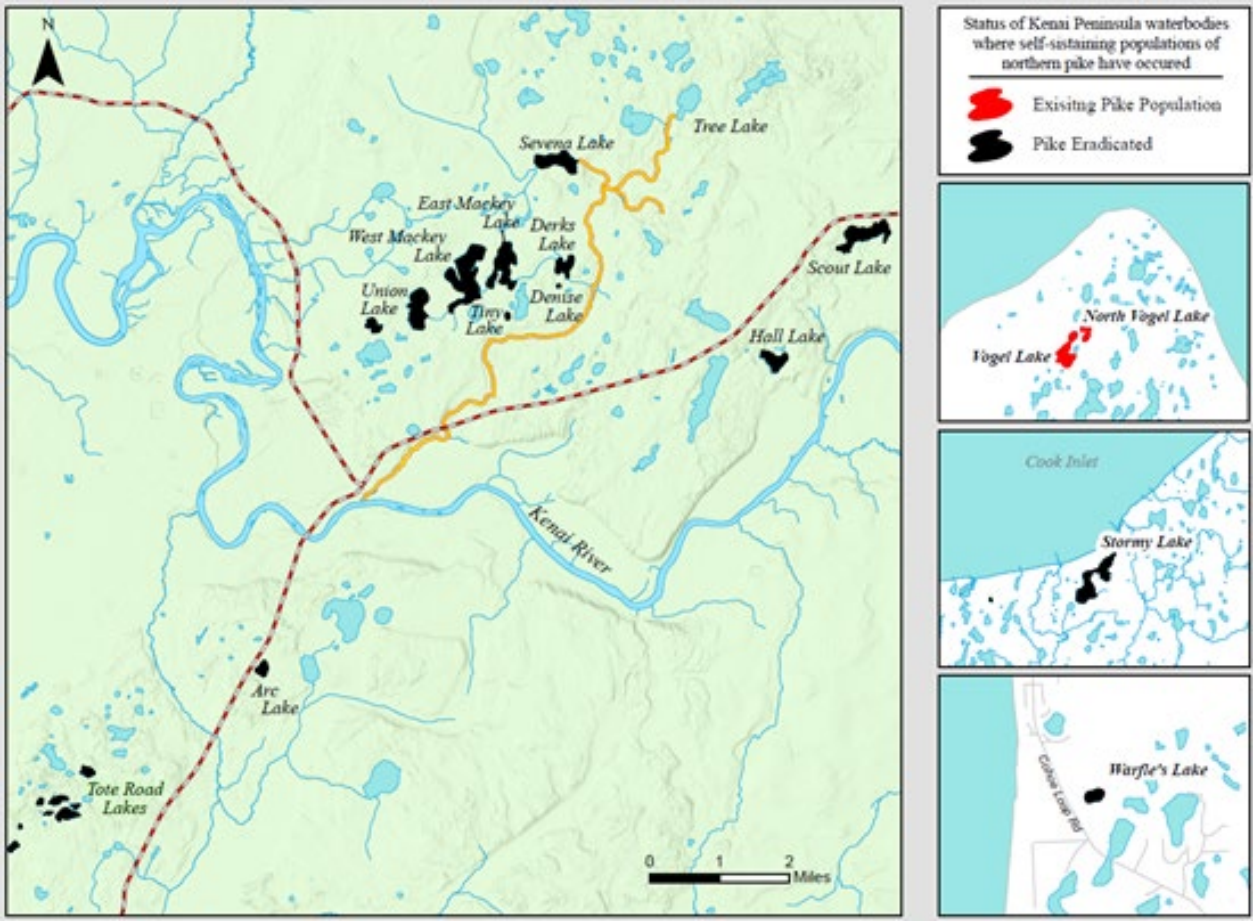

Figure 2.–Map showing the status of Kenai Peninsula waters associated with northern pike.

Other nonnative fish detected on the Kenai Peninsula include blackfish, yellow perch, red swamp crayfish, and goldfish. Of these, the only known yellow perch population was in a small lake in Nikiski and this was eradicated with a rotenone treatment in 2000 by ADF&G shortly after its discovery. Red swamp crayfish, twice found in the Kenai River drainage since 2000, never established sustaining populations and are no longer believed to be present. Goldfish were detected in Loon Lake (Soldotna Creek drainage) in 2017 and in a flooded gravel pit near Funny River Road (near Soldotna) in 2007. Both populations of goldfish were eradicated by ADF&G. The population near Funny River Road was removed by draining the pond, and the Loon Lake population was removed via rotenone treatment in 2017 done in conjunction with a northern pike removal effort. Blackfish, found in 2 small Kenai River tributaries near Kenai, have been present for decades and remain today. A small reproducing population muskellunge was detected in 2017 in a 20-acre lake near Soldotna and removed with a rotenone treatment in the fall of 2018 in conjunction with a multi-lake northern pike eradication effort. Genetic analysis of the

muskellunge indicated they were probably imported from Wisconsin. Fathead minnows were introduced to a 1-acre manmade pond in the City of Kenai and were first detected in 2018 and subsequently eradicated with a rotenone treatment in 2019 by ADF&G.

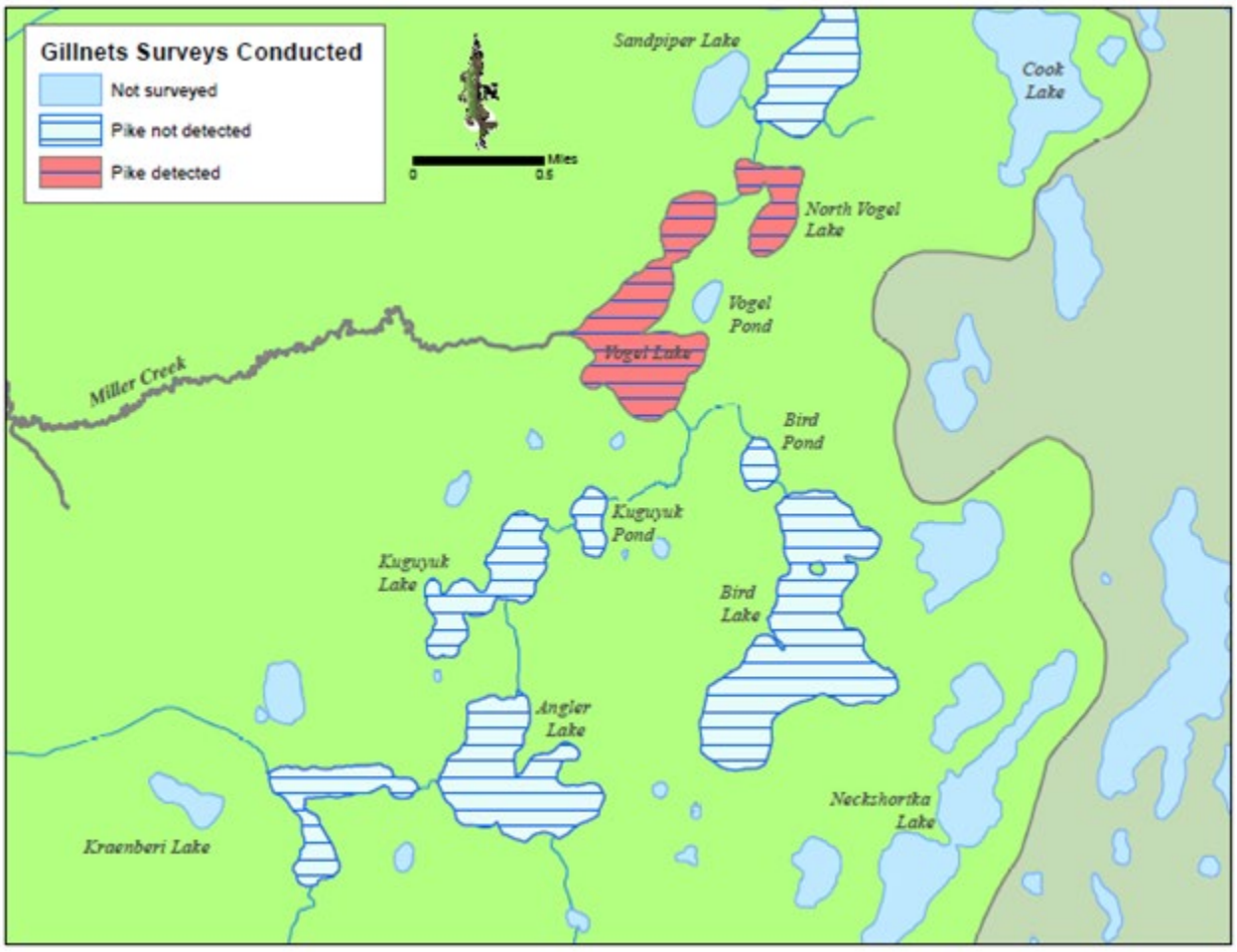

Figure 3.–Map of the Miller Creek drainage and the lakes surveyed for invasive northern pike.

OBJECTIVES

PRIMARY OBJECTIVES

1) Conduct gillnet surveys between 1 July 2020 and 30 June 2022 and within 6 months of completing nonnative removal efforts to evaluate the success of northern pike (or other nonnative fish) removal such that the probability of detection of nonnative fish is 0.80 assuming the nonnative population is at least 20 fish greater than 300 mm fork length.
2) Conduct gillnet surveys between 1 July 2020 and 30 June 2022 to detect the presence of northern pike (or other nonnative fish) in all high or medium threat waters that have native salmonids present such that the minimum probability of detection is 0.50 given the nonnative population is at least 20 fish greater than 300 mm fork length[2].

[2] Effort may be increased to attain a higher probability of detection as dictated by site-specific concerns or if no salmonids are present.

SECONDARY OBJECTIVES

1) Collect and analyze eDNA samples for northern pike in all waters where gillnet surveys to detect suspected northern pike populations are undesirable or insufficient to meet precision criteria for Objectives 1 or 2.
2) Map all waters where new nonnative fish discoveries are made to estimate surface acreage, volume, and create bathymetric maps.
3) Measure water quality (temperature, dissolved oxygen, pH, specific conductance and stream discharge) monthly when feasible for 1 calendar year from all waters where new northern pike discoveries are made.
4) Inventory invertebrate taxa at waters where new nonnative fish discoveries are made and conduct minnow trapping to detect the presence of small or juvenile fish.
5) Prepare a nonnative fish eradication or control plan for all waters where new nonnative fish discoveries are made.
6) When feasible, implement a quick-response control or eradication plan as soon as practical when new nonnative fish populations are detected.
7) Collect wild native fish and release them into waters where restoration of the native fish assemblage is appropriate following the removal of nonnative fish.
8) In every waterbody where nonnative fish have been removed and salmonids have been restored, calculate the mean catch per unit effort (CPUE) for all salmonid species by gear type, with gillnets fished for up to 96 hours of effort and minnow traps fished up to 120 hours of effort, at least once every 3 years for a 9-year period following the removal effort.
9) In every waterbody where northern pike have been removed and salmonids have been restored, collect fork length (FL) for salmonid species collected in gillnets and minnow traps at least once every 3 years for a 9-year period following the removal effort.
10) Create fork length (FL) histograms using 50-millimeter fork length classes for all fish species caught in gillnets by species, lake, and year during FY21 and FY22

METHODS

STUDY DESIGN

The study area encompasses the entire northern Kenai Peninsula management area (NKPMA).

Gillnet Surveys for Northern Pike

The goal for Primary Objectives 1 and 2 is to assess the presence or absence of northern pike or other nonnative fish in suspect NKPMA waters, primarily with gillnet surveys. Gillnets are frequently used for the detection and suppression of invasive northern pike in Alaska (Massengill 2010; Sepulveda et al. 2013; Dunker and Rutz. 2014; Glick and Willette 2016; Bradley et al. 2020). Northern pike are most susceptible to capture when gillnets are fished in their preferred habitat, which typically includes low flow or lentic waters, side sloughs, embankments, and densely vegetated littoral zones (Inskip 1982).

The response time to survey suspect waters will be based on a threat classification system (i.e., high, medium, and low risk; Figure 4). Netting effort will be based on a lake category assignment that considers the lake's fish assemblage and invasive fish management history. Lake category definitions are listed below.

1) Restored (R): A restored waterbody is one where northern pike or other nonnative fish populations have been eradicated. These "restored" waters will undergo an assessment to evaluate the success of the eradication effort (i.e., an intensive gillnet survey satisfying precision criteria for Primary Objective 1) within 6 months of the eradication effort. If no undesirable fish are detected during the survey, additional gillnet and minnow trap surveys will occur at least once every 3 years thereafter for 9 years with enough netting effort each time to satisfy Secondary Objective 4 requirements. These subsequent surveys are primarily designed to monitor restored native fish populations and secondarily to provide an opportunity to detect a new nonnative fish introduction.
2) Unrestored, salmonids present (USP): In a waterbody where nonnative fish presence is suspected and the waterbody is known to support a salmonid population, the netting effort should satisfy Primary Objective 2 precision criteria.
3) Unrestored, salmonids absent (USA): In a waterbody where nonnative fish presence is suspected and the waterbody is not known to support a salmonid population, netting effort will satisfy precision criteria for either Primary Objective 1 or Primary Objective 2 depending on the preference of the project leader.

The purpose of the lake category assignment is to limit bycatch of sport fish, specifically salmonids where present, while allowing for moderate gillnetting effort that provides a reasonable detection probability for a nonnative fish population at low abundance.

Instances where a waterbody meets criteria for 2 different threat classifications, the waterbody will be assigned the greater threat class. For example, if a lake satisfies criteria for both a medium and high threat waterbody, it will be assigned as a high threat and surveyed as soon as possible. Threat ranking criteria are provided in a flowchart (Figure 4) which prescribes the maximum time that can elapse before a fish survey occurs. Appendix A1 provides a current list of the NKPMA waterbodies where suspicion exists for nonnative fish presence including lake category assignment (R, USP, and USA) and threat classification (high, medium, and low) for each and the year when a survey is scheduled.

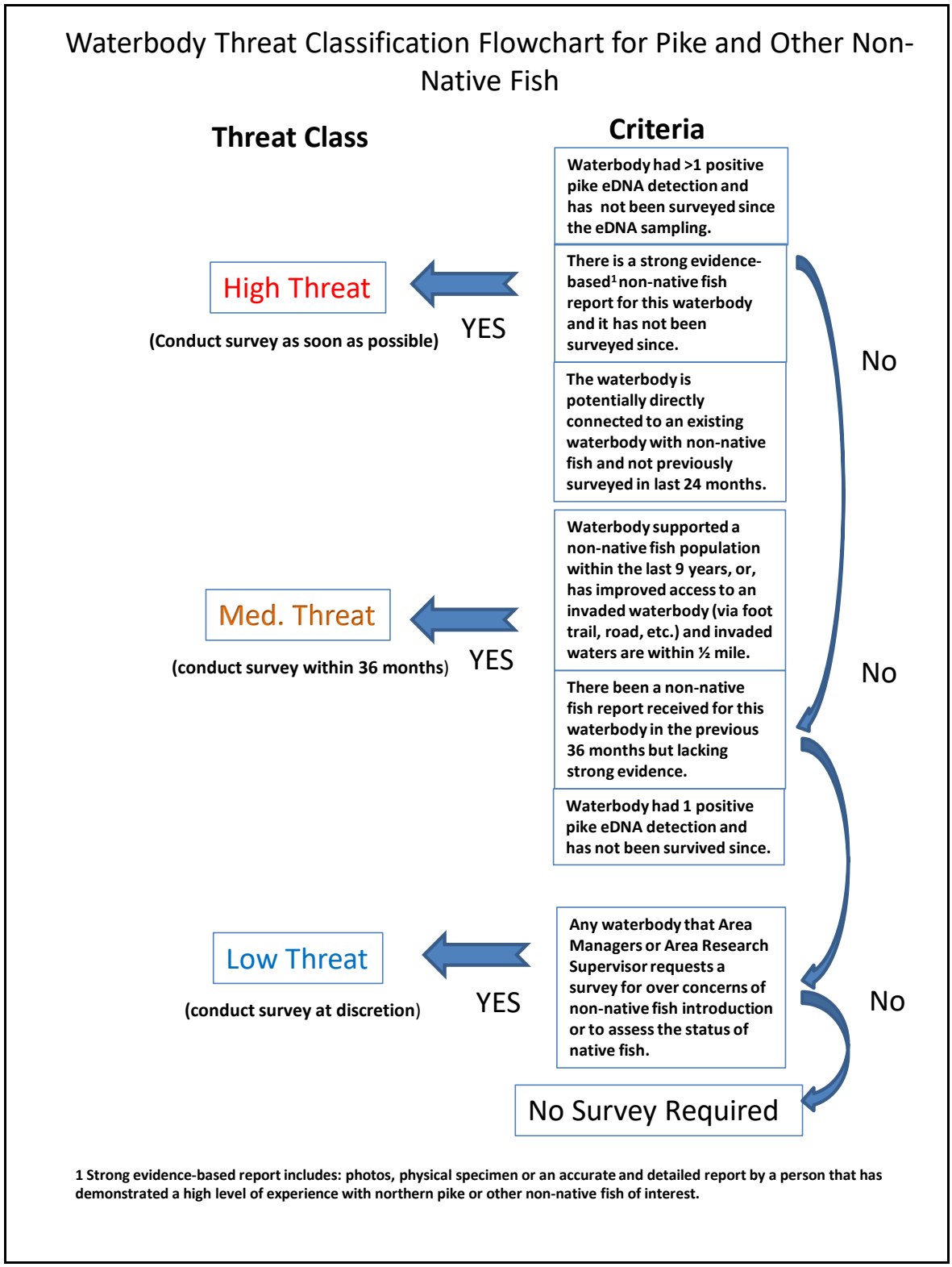

Figure 4.–Flowchart for assessing the invasive fish threat to a waterbody and timeframe for conducting a gillnet detection survey.

Sampling Effort

Gillnet surveys designed to detect the presence of northern pike or other nonnative fish will be conducted with enough effort to satisfy precision criteria for Objectives 1 or 2 based on lake category assignment (i.e., R, USP, or USA). To quantify the netting effort necessary to detect a northern pike population of at least 20 fish with an estimated probability of detection of 80% and 50% for Objectives 1 and 2, respectively, we utilized data from past northern pike removal experiments that used gillnets.

Between 2005 and 2010, ADF&G conducted 12 northern pike removal experiments on the Kenai Peninsula. Data collected from these experiments included catch C_{ij} and effort E_{ij} (in units of net-hours per surface acre) for sample i (where $i = 1, \ldots, s$) and experiment j (where $j = 1, \ldots, 12$). Populations were assumed to be closed except for captured fish and fishing was assumed to represent a Poisson process with a constant probability of capture for all individuals. Data were analyzed using a hierarchical version of Leslie's regression method (Seber 1982):

$$CPUE_{ij} = K_j N_j - K_j C_{ij}^{*} \quad (1)$$

where N_j is the initial population size in experiment j, K_j is the average probability that a northern pike of any size is captured with 1 unit of effort during experiment j,

$$CPUE_{ij} = {}^{C_{ij}}\!/_{E_{ij}} \quad (2)$$

and

$$C_{ij}^{*} = \sum_{k=1}^{i-1} C_{kj} \quad (3)$$

For i in 2, …, ($s + 1$) with $C_{1j}^{*} = 0$.

The probabilities of capture for each experiment are assumed to come from a common distribution: $K_j \sim$ beta(a, b). The analysis was conducted using the RJAGS package (Plummer 2013) within R[3]. Noninformative priors were used for all parameters. Although Leslie's method is typically used to estimate the initial population size, our interest was in the posterior and predictive distributions of K for estimating the probability of detecting small northern pike populations. Percentiles from the predictive distribution for the value of K in a new removal experiment are shown in Table 1 and the predictive distribution is shown in Figure 5.

Table 1.–Percentiles from the predictive distribution of *K*.

Percentile	Predicted *K*
5%	0.001
10%	0.003
50%	0.019
90%	0.055
95%	0.073

[3] R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

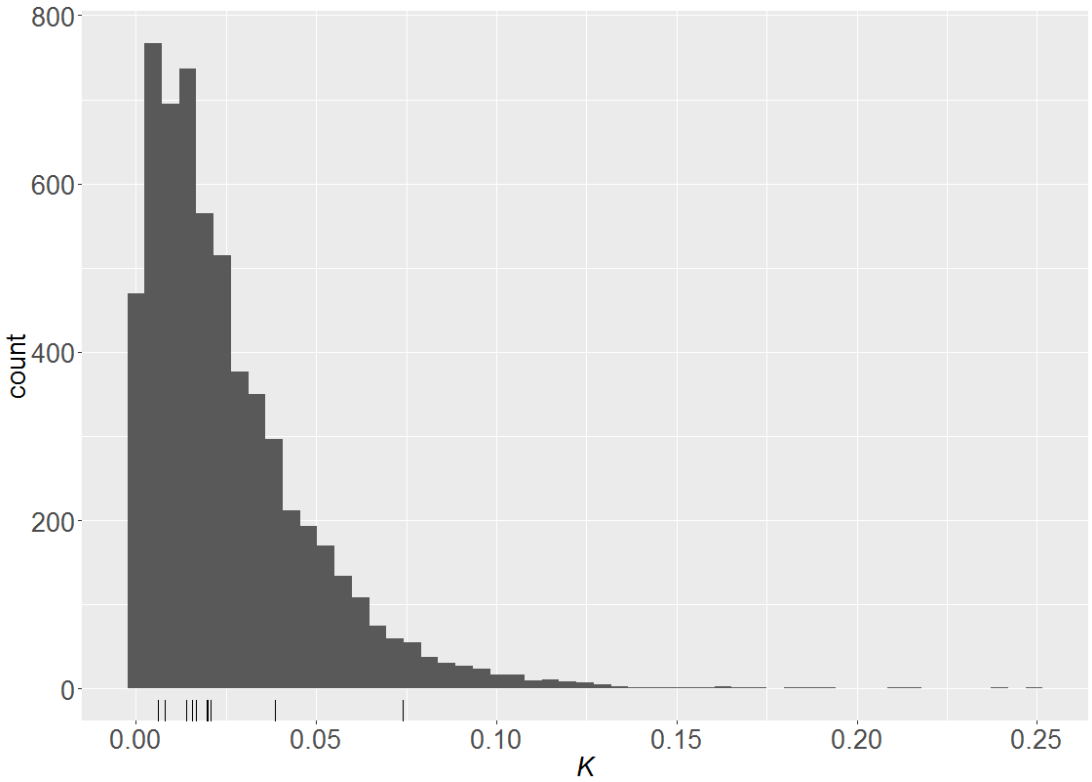

Figure 5.–Prediction distribution for K, the average probability a fish is captured in a new removal experiment with 1 unit of effort.

Note: Tick marks along the *x*-axis show the median values for *Kj*, the average probability a fish is captured with 1 unit of effort in each of the previous removal experiments.

Under the assumption that fishing represents a Poisson counting process, the probability of failing to detect a population of northern pike of size N as a function of net-hours per acre (E) is as follows:

$$D_p = \exp\left(-KE\right)^N \tag{4}$$

We will use the median value of K from Table 1 to calculate probabilities listed in Tables 2 and 3. The netting effort and associated probabilities found in Table 3 will be used to satisfy the precision criteria found in Objectives 1 and 2. Table 2 is provided for rare occasions when additional netting effort is needed to detect a very small northern pike population (4 individuals) and only done when ADF&G or area staff determine the bycatch risk associated with increased netting effort is outweighed by the concern over potential northern pike presence.

Table 2.–Probability of failing to detect a population of 4 northern pike with various levels of net density (nets per surface acre [sa]) and net hours.

	Net densities					
Netting hours	0.1nets/sa	0.25nets/sa	0.5nets/sa	0.75nets/sa	1nets/sa	2nets/sa
24 hours	0.829	0.626	0.392	0.246	0.154	0.024
48 hours	0.688	0.392	0.154	0.06	0.024	0.001
72 hours	0.57	0.246	0.06	0.015	0.004	0
96 hours	0.473	0.154	0.024	0.004	0.001	0

Table 3.–Probability of failing to detect a population of 20 northern pike.

	Net densities			
Netting hours	0.1nets/sa	0.25nets/sa	0.5nets/sa	0.75nets/sa
24 hours	0.391	0.096	0.009	0.001
48 hours	0.153	0.009	0	0
72 hours	0.06	0.001	0	0

Gillnetting Methods

In summary, the gillnetting effort required for a specific waterbody will be based on the following considerations:

1) the lake category assignment (R, USP, or USA)
2) lake surface acreage
3) applicable objective (Primary Objective 1, 2 or Secondary Objective 8)

The gillnets we will use are manufactured by Duluth Nets[4] and made of single-strand monofilament mesh hung from a polypropylene floating line with the net bottom attached to 30 lb lead line. Each net is 120 ft long, 6 ft deep, with six 20 ft wide panels of differing-size mesh (1 each of sequentially attached 0.5-inch, 0.625-inch, 0.75-inch, 1.0-inch, 1.5-inch, and 2.0-inch stretched mesh) all tied with #9 twine. Gillnets will be deployed in vegetated littoral areas and fished continuously as practical. When continuous intensive gillnetting effort is unsafe or logistically impractical, separate netting efforts will be repeated until the sum of netting effort achieves the effort goal. As practical, staff will be present to continuously tend the nets, and at a minimum, nets will be tended daily. If a northern pike or other nonnative fish is captured in a waterbody where the sole purpose of the survey was to determine their presence, the netting will be halted if bycatch becomes a concern; otherwise, gillnetting will be allowed to continue at the discretion of the project leader to collect biological data from the invasive fish population.

eDNA Sampling

Background

Environmental DNA (eDNA) is the DNA from an organism shed into the environment. Organisms shed their DNA continuously from cell sloughing, waste production, carcass deposition, gamete expression, and other mechanisms. Sampling for eDNA is potentially more sensitive than traditional fisheries approaches for detecting aquatic taxa in low abundance (Ficetola et al. 2008). For aquatic species detection, eDNA is commonly collected within water samples (i.e., ~1000 ml bottle) concentrated by filtration, and the filtrate is processed further for quantitative polymerase chain reaction (qPCR) amplification.

In circumstances when gillnetting may be an undesirable method for detecting northern pike (i.e., logistical, safety, or bycatch concerns), eDNA detection methods may be used to supplement a reduced netting effort or as a complete substitute for gillnetting. In those situations, an effort will be made to achieve similar precision criteria listed for Objectives 1 and 2. Currently, ADF&G is equipped to rapidly detect northern pike using eDNA methods.

[4] Product names and companies used in this publication are provided for completeness and do not constitute endorsement.

ADF&G and the USFWS developed and tested several genetic markers for use in detecting northern pike eDNA that resulted in the selection of a preferred marker (*EluCOI*) located in the cytochrome oxidase 1 gene of mitochondrial DNA (Olsen et al. 2015). Since 2014, ADF&G has used this marker to assess northern pike distribution and evaluate the success of northern pike eradication projects (Dunker et al. 2016; ADF&G unpublished[5]). Processing of the eDNA samples was done by USFWS Conservation Genetics Lab in Anchorage using a benchtop laboratory method called quantitative polymerase chain reaction (qPCR).

A portable device called Biomeme Two3 is a qPCR thermocycler that provides onsite real-time eDNA processing capability. The Biomeme Two3 can process 3 eDNA samples simultaneously in about 1 hour compared to traditional benchtop processing methods which can take weeks or months for results depending on lab scheduling and turn-around time. Performance testing of the Biomeme Two3 against traditional benchtop qPCR processing suggests the Biomeme Two3 produces a lower probability of detection than traditional benchtop processing and requires the processing of about 1.9 samples for every sample processed by traditional benchtop qPCR methods for a positive detection (Sepulveda et al. 2018).

eDNA Sampling Protocol

We will adopt many of the eDNA collection and handling methods described by the United States Fish and Wildlife Service (USFWS), United States Geological Survey (USGS), and the United States Forest Service (USFS) to improve quality control that reduces the risk of contaminating or degrading eDNA samples (Carim et al. 2015; Laramie et al. 2015; USFWS[6]). Many factors can affect the detection and persistence of eDNA. False positive results can be caused by contamination during sample handling or processing, persistence of eDNA in the environment after the organisms are gone, or the transport of eDNA. Likewise, false negative results can be caused by insufficient assay sensitivity, a method failure during sample processing (i.e., inhibition of DNA amplification), a lack of target DNA in the sample, or degradation of the eDNA in the sample prior to processing (Evans et al. 2017).

eDNA samples will be collected from suspect waters either by foot along the shoreline or from a boat. Great care will be taken to ensure outer gear worn by collectors (e.g., waders, life jackets) and collection equipment (e.g., swing sampler, transport coolers) are decontaminated with a 10% bleach solution allowing for a 10-minute soak before rinsing with tap water. All sample containers will be purchased sterilized or will be sterilized by samplers using a 20% bleach solution soak for 10 seconds of contact time followed by a deionized water or distilled water triple rinse. All decontaminated sample containers will be stored inside a clean plastic bag until they are used for sampling.

When sampling from a boat, to reduce the risk that the boat could transfer northern pike eDNA, the boat hull, lower unit, and trailer will be first cleaned of debris with a high pressure wash followed by a 10% bleach solution spray allowing for a 10-minute contact time prior to sample collection[6]. When samplers are collecting from a boat, they will collect the sample from the bow of the boat before the boat travels atop or beyond a sample site. Whether sampling from a boat or

[5] Tote Road Pike Lakes Restoration: Invasive Northern Pike Eradication Treatment Plan. 2017. Unpublished and located at the ADF&G Soldotna Office.

[6] USFWS. 2019. Quality Assurance Project Plan: eDNA Monitoring of Bighead and Silver Carps.. https://www.fws.gov/midwest/fisheries/eDNA/documents/QAPP.pdf.

by foot, samplers will systematically collect samples in a sequential manner at each waterbody to avoid traveling past a sample site prior to it being sampled.

Before collecting a sample, the collector will don nonpowdered nitrile gloves. Water samples will be collected in duplicate 1000 ml surface water grab samples collected in either a sterilized 1-liter Nalgene bottle or Whirl-Pak bag. Duplicate 1000 ml samples will be collected at each sample location if sample processing will be done using a Biomeme Two3 thermocycler. This is to compensate for its lower detection efficiency when compared to traditional benchtop qPCR processing. All sample containers will be labeled with a location code, unique sample code, and collection date, and then placed inside a secondary Whirl-Pak bag and chilled by placing it on ice inside a disinfected insulated cooler until filtered. All sample locations will be recorded with a handheld global positioning system (GPS).

Each day sampling occurs, crews will collect several control blanks that will help identify whether eDNA contamination has occurred during handling or transport of the samples. Control blanks will be collected in the same sample containers and volume size as the actual lake water samples, but the sample itself will consist of filling the container with deionized water. One control, called a field blank, will be filled with deionized water as well. The field blank will help assess whether sample contamination was introduced during field collection activities. Another control, called a travel blank, will be collected at the Soldotna Field Office prior to departure to the field and will be 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 was introduced during transport. A lab blank will be collected in the same lab room where sample filtering occurs. The lab blank will serve to identify whether sample contamination was introduced during the filtering processes.

Within 2 days of sample collection, all samples will be filtered using a GeoTech series II peristaltic pump and approximately 1.0 µm Whatman glass filters. After filtering, all filters from each unique sample will be stored together in a Whirlpak bag and placed into cold storage. Each bag will be given a unique sample ID. All field water sampling, equipment decontamination, sample filtering, and storage will follow established eDNA collection protocols (Laramie et al. 2015). Decontamination procedures will include wearing new nitrile or latex gloves each time a new sample is handled, using only sterilized tweezers to handle filters, and sterilizing all filtering assemblies prior to use in a 50% bleach solution (50% deionized water and 50% household bleach containing 8.25% hypochlorite) bath for 10–15 minutes followed by 2 deionized water baths. The filter assemblies will be reassembled after sterilization and then rinsed again by pumping 0.5–1.0 L of deionized water through the assembly. Before filtering a new sample, work areas and the pump will be sprayed with a 10% bleach solution or DNA AWAY and then wiped dry with a sterilized tissue. Filtered samples will be placed on ice until processed by the Biomeme Two3 or forwarded to an offsite lab for traditional benchtop qPCR processing. Samples that are collected and filtered for processing using the Biomeme Two3 method will be entirely processed at the Soldotna ADF&G office. Filtrate intended to be processed by traditional benchtop processing will be refrigerated and expressed shipped to the laboratory.

Sample filtrate for Biomeme Two3 processing will be extracted and analyzed using methods described by Sepulveda et al. (2018) Filtrate extraction will be done with a Biomeme Field Test Kit which is designed for use only with mixed cellulose ester (MCE) filters. The Biomeme kit utilizes a filtration-based method in which DNA selectively binds to the silica membrane inside Biomeme's proprietary sample column. Subsequent washes through a sequence of specially

formulated buffers produce purified DNA upon elution. Biomeme's 6-step protocol takes about 5 minutes (Appendix B1). The purified DNA is then stored in the elution buffer until qPCR.

To analyze DNA extract for presence of northern pike DNA, a Biomeme Two3 portable real-time thermocycler will be used. The Biomeme Two3 has 2 channels, one where the northern pike marker fluorescence occurs (FAM) and another that is an internal positive control (IPC), and 3 wells so duplicate reactions can be run for 3 samples simultaneously.

Purified DNA will be pipetted into each well, which is prefilled with a lyophilized assay that includes the EluCOI marker specific to northern pike DNA (Olsen et al. 2015). The Biomeme's recommended thermocycler protocol for this assay is found in Appendix B1.

Output of the Biomeme Two3 thermocycler is provided via a smartphone interface and includes amplification curves and the cycle number at which fluorescence increased above background values (Cq) for the northern pike marker (FAM channel) and for the IPC. Samples that are positive for northern pike DNA will be those that amplified. Samples which fail to amplify will be considered nonpositive.

After processing, if multiple positive eDNA detections occur from waters where northern pike have not been physically confirmed before, and all eDNA control blank samples test negative for northern pike eDNA (no contamination suspected), this will indicate the need to conduct gillnet surveys and ground-truth the eDNA results. A single positive eDNA detection alone will not signal the need to conduct a gillnet survey. This is because ADF&G has yet to confirm northern pike presence via gillnetting when only a single eDNA sample was positive (authors' personal observations). Other states are currently developing guidelines on what conditions must be met before scoring an eDNA sample as a positive detection. Such criteria may include requiring that multiple markers located on different genomic regions amplify and that the results are reproducible in multiple labs. For this project, multiple positive eDNA detections will indicate the need to ground-truth results with a gillnet survey. Only when a northern pike is physically collected will a conclusion be made that northern pike are present in a waterbody.

Prior to collecting eDNA samples, approximate sample locations will be identified and numbered on a bathymetric map of each lake. Sample containers or bags will be labeled with the name of the lake, date, sampler initials, and unique sample ID.

eDNA Sampling Effort

To develop an eDNA sampling effort that is sufficiently robust to detect northern pike populations with low abundance, the estimated mean detection probabilities of northern pike eDNA reported in Dunker et al. (2016) was utilized. The detection probabilities were estimated from results using replicate 1-liter samples collected at 1, 10, and 40 meters from a single, caged, live northern pike and were estimated to be 0.89, 0.57, and 0.27 respectively. For this project, 1-liter samples will be collected in duplicate to account for the lower detection probabilities using the Biomeme Two3 device.

The following calculations will be used to estimate how many eDNA samples are needed to detect a small northern pike population ($N = 20$) with a desired probability of detection provided the lake acreage is known and no gillnet sampling occurs. Calculations will be based on 3 assumptions: 1) fish are randomly distributed throughout the sampling area, 2) there are no false detections, and 3) the probability of detection beyond 40 m is zero, because no estimates are available for this range.

To account for differences in the probability of detection due to the distance between a possible northern pike and the sample site, a 40-meter circle around each sample site will be divided into 3 distinct subregions centered around the sample site. These subregions will be the circular area less than 1 meter from the center (the sample site), the area between 1 and 10 meters from the center, and between 10 and 40 meters from the center, which will be labeled subregions 1, 2, and 3 respectively. Because Dunker et al. (2016) estimated the probability of detection at 1, 10, and 40 meters, their estimates will be used as conservative proxies for the probability of detection over the entire respective subregions. If P represents the probability of detecting a northern pike, D represents the event a northern pike is detected, and R_i represents the event that a single northern pike is present in subregion i for i = 1, 2, or 3, then by the law of total probability and the definition of conditional probabilities:

$$\mathrm{P(D)} = \mathrm{P(D} \mid R_1) \times \mathrm{P}(R_1) + \mathrm{P(D} \mid R_2) \times \mathrm{P}(R_2) + \mathrm{P(D} \mid R_3) \times \mathrm{P}(R_3) \tag{5}$$

Thus, the probability a northern pike is detected is equivalent to the probability a northern pike is detected given it is in a particular region times the probability it is in the region summed over all regions. The probabilities of detection given a northern pike is present in the region ($\mathrm{P(D} \mid R_i$)) are taken as the estimates from Dunker et al. (2016). Under the assumption that northern pike are randomly distributed, the probability a northern pike is present in a region is the proportion of total area represented by that region or

$$\mathrm{P}(R_i) = \frac{area\ of\ region\ i}{total\ area\ of lake} \tag{6}$$

which is computed by dividing the fixed area of each circular region by the known surface area.

Finally, assuming sample sites are identical and there are no false positives, it can be shown that the probability of detection given the northern pike is at 1 sample site is equal to the probability of detection given the pike is at 1 of S sample sites for S = 1, 2, …, n. Thus, the only change in the probability calculation for S sites is the proportion of area represented by each subregion is now $S \times \mathrm{P}(R_i)$. By another application of the law of total probability and definition of conditional probabilities:

$$\mathrm{P(D\ at\ S\ sites)} = \mathrm{P(D} \mid R_1) \times \mathrm{S} \times \mathrm{P}(R_1) + \mathrm{P(D} \mid R_2) \times \mathrm{S} \times \mathrm{P}(R_2) + \mathrm{P(D} \mid R_3) \times \mathrm{S} \times \mathrm{P}(R_3) = \mathrm{S} \times \mathrm{P(D)} \tag{8}$$

Because the N northern pike are assumed randomly distributed (which is a conservative assumption because nets are fished in the best northern pike habitat), the number of northern pike that are assumed successfully detected follows a Bin(N, S*P(D)) distribution. The probability of at least 1 detection at S sites is $1 - (1 - \mathrm{S} * \mathrm{P(D)})^N$. This equation can then be set equal to the desired probability of detection and solved for S. Table 4 displays calculated eDNA sampling requirements for a variety of desired probabilities of detection and acreages assuming a population of 20 northern pike.

Table 4.–Number of samples required to achieve the desired probability of detection for a population of 20 northern pike.

	Acres					
Probability of detection	10	25	50	75	100	200
0.50	1	3	5	8	10	19
0.75	2	5	10	14	19	38
0.80	3	6	11	17	22	44
0.85	3	7	13	19	26	51
0.90	4	8	16	23	31	61
0.95	4	10	20	30	39	78

Protocol for New Northern Pike Discoveries

If northern pike are discovered in a waterbody, a variety of site-based data will be collected to aid in planning a control or eradication action and to better assess the ecological threat posed by the northern pike population. Data collection will focus on documenting baseline environmental and biological conditions and containment options.

Lake Mapping

In waterbodies where invasive northern pike are newly detected, lake bathymetry data will be collected to produce volume estimates and a bathymetric map useful for planning northern pike control or eradication efforts. To collect bathymetry data, a boat-mounted Lowrance HDS chartplotter and transducer will be used to record *x*, *y*, *z* coordinate mapping data. The lake perimeter will be mapped first as near to shore as feasible, and then mapping will continue repeating the perimeter circuit from about 20 m farther offshore. After these two complete lake perimeter circuits are completed, the rest of the lake will be mapped by sequential line transects, typically orientated along the greatest length of lake. On lakes with distinct bays or an irregular shape, transects will be run by section. Typically, transects lines are less than 40 m apart; this can be gauged by watching the GPS track on the Lowrance unit's monitor. Details regarding specific Lowrance HDS settings and mapping options can be found at BioBase's support resources web site[7].

Water Quality Monitoring and Stream Discharge

Water quality data will be collected monthly for 1 year at waters where northern pike have been discovered. Water quality data will be collected using a portable Quanta Hydrolab to record temperature, pH, specific conductivity, and dissolved oxygen concentration. Collection of water quality data will be in 1-meter increments starting near the deepest area of each lake and thereafter in 1-meter increments upward to include just below the lake surface. All sampling locations will be recorded with a handheld GPS. A secchi disk will be used to measure turbidity to the nearest 0.1 m. Measurements will be collected from a boat during open water season and by drilling through the ice during the winter.

If the waterbody containing northern pike includes water inlets and (or) outlets, stream discharge measurements will be collected at those sites monthly for at least 1 year. In addition, monthly discharge will be measured at streams linking the infested waterbody to other waterbodies, from

[7] https://www.biobasemaps.com/SupportResources (accessed 6/18/2020)
or specifically https://s3.amazonaws.com/downloads.digitalmarine.com/BioBaseQuickReferenceSOPHDSEliteTi2V1.6.pdf

headwaters to the drainage's terminus at a mainstem river. Stream discharge measurements will be collected with a Price Pygmy current meter (magnetic head) attached to a Scientific Instruments wading rod with an attached electronic AquaCount display screen. Stream discharge will be collected in accordance with principals provided by the ADF&G Statewide Aquatic Resources Coordination Unit training course titled "How to Measure Stream Discharge" that comply with United States Geological Survey (USGS) specifications as described in Nolan and Shields (2000).

Invertebrate Surveys and Minnow Trapping

Beyond biological data obtained for fish by the initial gillnet survey, macroinvertebrate and plankton surveys will be collected in waters where northern pike are discovered to document dominant taxa present. For each lake, zooplankton evaluations will be made at 2 sites by replicate vertical tows using a 0.5 m 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 2 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 5 locations. Opportunistic 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 will be preserved in 90% ethanol, labeled with the date, collector initials, and site location, and archived for later evaluation at the ADF&G Soldotna office.

At each waterbody where northern pike are discovered, 5 minnow traps baited with salmon eggs will be fished continuously for at least 24 hours to detect the presence of small or juvenile fish. Minnow traps will be fished near shoreline weed beds and in or near lake tributaries. Minnow trap set locations will target protective cover habitat and spacing between traps will be greater than 50 m to ensure adequate coverage.

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

Control Options

Land Status Determination

Landownership status will be identified for all lands surrounding waterbodies discovered with northern pike including lands surrounding other waters linked to the northern pike waterbody that could potentially be within a "treatment area" for a pesticide application. Land ownership can be identified using the Kenai Peninsula Borough's online GIS mobile viewer application found at: http://www.kpb.us/gis-dept.

Control Plan and Implementation

Based on gillnet survey results and an assessment of connectivity to other waters, the physical detection of a northern pike population will likely require an appropriate control action. When feasible, an appropriate initial response to an invasive fish detection is to immediately contain the population whenever feasible. This response aligns with universal early detection rapid response (EDRR) protocols for the control of invasive species as found online at

https://www.invasive.org/edrr/index.cfm. In most instances, containment of invasive fish in an open waterbody will involve installing fish passage barriers at all inlets and outlets.

Typically, containment strategies will have site-specific challenges, but successful approaches used for blocking northern pike passage in small northern Kenai Peninsula streams have included installation of fyke nets or stainless-steel screen panels with 1/4-inch to 1/8-inch mesh. Fabric mesh fyke nets will be shrouded in plastic-coated wire poultry fencing to reduce animal damage that could compromise the barrier. If abrupt stream elevation drops are present near lake inlets or outlets (e.g., beaver dams, spillways, perched culverts), sometimes a relatively simple modification (e.g., sandbag layer, wooden chute installation) can create a more abrupt and defined vertical drop to reduce successful upstream passage of northern pike. Little information is available that quantifies the jumping ability or physiological limits of northern pike, but anecdotal information suggests vertical drops greater than 0.3 feet can be effective to reduce upstream pike movement[8].

Eradication

The decision whether to implement a control or eradication action must weigh the consequences an invasive fish population poses to ecological and economic concerns. When agency resources are sufficient to act quickly, a rapid response plan to eradicate with rotenone (a plant based piscicide) is a suitable option if permitting can be expedited or given emergency exemption. In small closed lakes (<40 acres) intensive under-ice gillnetting has also proven to be an effective eradication alternative (unpublished data, Soldotna ADF&G office), but only when the northern pike population is small (<30 individuals) and reproductive success is low as noted by the lack of a wide range in age-classes or the presence of juvenile northern pike during sampling efforts. Successful northern pike eradications using only gillnets has usually involved fishing gillnets continuously from fall ice-up until spring ice-out with gillnet densities of 0.5 to 2.0 nets/acre (ADF&G unpublished data).

For infestations where a quick-response action is not possible, a formal restoration plan will be drafted to facilitate the scoping, permitting, and eradication or control options available.

Native Fish Restoration and Monitoring

Overview

The goal of the native fish restoration component for this project is to reestablish self-sustaining native fish populations that were historically present in a waterbody but lost or severely reduced by nonnative fish impacts or by the management action used to remove the invader. In some cases, native fish will be introduced solely to provide a replacement fishery to that provided by the nonnative fish, even if a sport fishery for native fish was never historically present. For waters that are sufficiently open to allow natural recolonization of native fish via migration, planned releases of native fish may not be necessary for fish populations to recover following removal of the nonnative fish population. In waters where natural recolonization is unlikely to occur rapidly, transplanting native fish may be required to successfully restore a fishery.

[8] Diebel, M. W. 2013. Priorities for barrier removal to improve access to northern pike spawning habitat in Green Bay tributaries. Project completion report to The Nature Conservancy.

Restoration

Recent ADF&G practices to accomplish wild native fish restoration have generally been accomplished by 2 methods. The first is by collecting native fish from the invaded waters, if they are still present in suitable numbers, and temporarily holding them offsite in a safe area (net pen or small closed pond) until reintroduction can occur after eradication of northern pike (Massengill 2017b). The second method is by collecting representative native fish from a separate waterbody, ideally within the same drainage, and releasing them into the restored waters following the removal of the invading fish population (Massengill *In prep*[9]). In rare circumstances, native fish broodstock may be collected from the invaded waters prior to the eradication effort. The broodstock can be used for producing hatchery-reared offspring that are used for reintroduction. This latter method is suggested for circumstances where the native fish population is very scarce, possibly genetically unique, and collecting enough individuals for successful reintroduction is impractical (Massengill 2017b).

Rainbow trout (*Oncorhynchus mykiss*), Dolly Varden (*Salvelinus malma*), juvenile coho salmon (*Oncorhynchus kisutch*), and threespine stickleback (*Gasterosteus aculeatus*) are the most common native species impacted by invasive northern pike on the Kenai Peninsula due to their propensity to share similar habitat with northern pike. Past native fish restoration efforts have focused mostly on collecting these species for reintroduction (ADF&G Soldotna office, unpublished data). Typically, the stocking densities of juvenile salmonids is based on recommended ADF&G stocking density guidelines for hatchery-stocked rainbow trout fry of about 100 fish/acre (Havens and Sonnichsen 1992). The frequency of salmonid stocking (a single year event vs. repeated annual or biannual events) will depend on management goals and fish population structure determined from poststocking fish surveys. Threespine stickleback reintroductions, following northern pike removal, have been successful on the Kenai Peninsula. Stocking goals for stickleback have varied and are usually in the range of about 35 to 75 fish/surface acre (Bell et al. 2016; Massengill *In prep*[10]).

Minnow trapping in streams or lakes has proven to be an efficient method for collecting most juvenile native fish species and is usually recommended over other methods (i.e., backpack electrofishing, fyke net traps, and hand-dipnetting; ADF&G unpublished data).

This project will provide the resources and support for native fish restoration efforts to restore waters as needed. Native fish restoration efforts will typically be planned and described in a "treatment plan" that is developed specifically for each restoration project.

Currently, there is an actively utilized treatment plan for eradicating northern pike and stocking native fish species in the Tote Road lake complex south of Soldotna (*Tote Road Pike Lakes Restoration*, archived in the Soldotna ADF&G office). Under the guidance of this plan, 8 lakes in the Tote Road area were treated with rotenone in the fall of 2018 to remove invasive northern pike and muskellunge. Beginning in the summer of 2019, native fish collected from Kenai River tributaries (e.g., Soldotna Creek) were released into the lake complex (Table 5). In 2020–2023, similar annual stocking rates for rainbow trout and coho salmon will continue.

[9] Massengill, R. *In Prep*. Soldotna Creek Restoration: Invasive northern pike eradication, 2014–2017. Alaska Department of Fish and Game, Special Publication, Anchorage.

Table 5.–Native fish stocking goals and achieved number stocked in the Tote Road lakes complex, 2019.

		Coho salmon		Rainbow trout	
Lake name	Acerage	Stocking goal	Number released	Stocking goal	Number released
CC Lake	4.4	344	443	96	97
Crystal Lake	16.7	1,306	1,389	364	331
Freds Lake	6.1	477	552	133	58
G Lake	17.3	1,353	1,380	377	358
Hope Lake	26.9	2,104	2,104	587	591
Leisure 1	11.1	868	868	242	219
Leisure Pond	1.5	117	120	33	33
Ranchero Lake	7.7	602	606	168	157
Total	91.7	7,171	7,462	2,000	1,844

Coho salmon, which are far more prevalent and easier to obtain in the wild, will help provide a sport fishery in these lakes within 1 year. It is hoped the rainbow trout population will eventually become self-sustaining because there is some anecdotal evidence that introduced rainbow trout in some of the lakes during the 1970s and 1980s were spawning in lake outlets, but is unclear if the progeny were successful. Threespine stickleback, historically native to these lakes, were restored during 2019 by a collaborative effort between various universities that was led by Dr. Hendry of McGill University (Quebec). Beginning in 2022, native fish surveys will be done in the Tote Road lakes to assess whether the introduced rainbow trout are successfully reproducing. If no reproduction is evident, ADF&G will consider the following options to provide for a sustainable long-term native fish fishery in this lake complex:

1) continue with annual or semi-annual wild salmonid releases
2) improve habitat to promote rainbow trout spawning success
3) stock some lakes with juvenile coho salmon produced in area schools under the ADF&G *Salmon in the Classroom* program
4) consider requests from the public to stock hatchery-reared fish in some of the lakes

Monitoring Restored Waters

In every waterbody where invasive fish have been removed and native fish populations restored, at least once every 3 years, for a 9-year period following the removal of the invasive fish, this project will conduct gillnet and minnow trap surveys to monitor native fish populations based on catch per unit effort (CPUE). To avoid excessive impacts to restored native fish populations, the amount of gillnetting effort will be at the discretion of the project leader. In most instances, not more 96 hours of cumulative gillnetting will be applied to a lake and typically the effort will be about 24 hours. Actual effort will be based on observed catch rates and site-specific safety and bycatch concerns. Minnow trap surveys will be conducted such that 5 minnow traps (18-inch long galvanized mesh screen traps with funnel entrances at both ends) baited with salmon eggs are fished continuously for at least 1 hour each in nearshore locations offering protective cover such as weed beds, snags, or tributary mouths.

Fork Length Histograms

For all fish species caught by gillnet in both suspect and restored water bodies, fork length (FL) will be measured and used to create FL histograms, using 50 millimeter length classes, for each species by waterbody and year during 2020–2022.

DATA COLLECTION AND REDUCTION

Gillnet Surveys and Minnow Trapping

Northern pike captured in gillnets will be sacrificed, counted, and measured for fork length (FL; tip of nose to fork of tail) to the nearest 1 millimeter. In addition, all northern pike will be examined in the field to identify sex, maturity (i.e., immature, mature, ripe, or spent), and stomach contents (prey taxa presence). Cleithra and otolith bones, and a clipped fin as a genetic sample will be collected for archival purposes. All live resident fish will be identified to species, counted, and if possible without significantly increasing handling stress, measured for FL to the nearest millimeter. Resident species mortalities will also be examined to identify sex and to collect scale samples for archival purposes. Fish from all minnow trap catches will be identified to species, counted, and measured to the nearest 1 millimeter prior to release. All catch data will be recorded on Rite-in-the-Rain notebooks and later transcribed into an Excel file. Site location and the date and time of sets and pulls will be recorded for all gillnet and minnow trap sets. All set locations will be recorded on a handheld GPS and labeled with a unique identifier name.

eDNA Sampling

Each eDNA sampling location will be recorded with a handheld GPS and given a unique identifier name. Control blank samples will be similarly labeled. Each duplicate water sample collected will be given a unique identifier name in addition to labeling the waterbody name and collection date. During sample filtration, sample data will be recorded in an Excel file on a laptop computer. These data will include the sample collection and filtering date, filtering time, numbers of filters used per sample, waterbody name, unique sample identifier, initials of the collector and the person doing the filtering, collection site location (lat/long) and any comments. Original GPS location data will be downloaded to Garmin Basecamp software on a PC. Sample site location data will be converted in Basecamp to Excel format and copied into the same Excel file holding the sample collection and filtering data.

Lake Mapping

After collecting lake mapping data stored by the Lowrance chartplotter as an .sl2 file on an external memory SD card, the data will be downloaded to a computer then uploaded to a cloud-based subscription service (BioBase). BioBase will run algorithms on the data and generate a downloadable product that includes a lake report containing the lake volume and surface area estimates and a bathymetric map.

Water Quality Monitoring and Stream Discharge

All water quality and stream discharge data will be recorded on data sheets in the field (Appendices C1 and C2, respectively) and later entered into an Excel file.

Invertebrate Surveys

During invertebrate surveys, invertebrates will be collected in the field and later identified to the lowest possible taxonomic level, usually to Order or Family, using taxonomic keys found in Koenings et al. (1987), Bachmann (1973) and Pennak (1989), and recorded in an Excel file. Set location, date, time, and collector initials will be recorded on a Rite-in-the-Rain notepad and later transcribed to an Excel file. Original GPS location data will be recorded with a handheld GPS and downloaded to Garmin Basecamp software on a PC. Sample site location data will be converted in Basecamp to Excel format and copied into the same Excel file holding the sample collection and identification data.

DATA ANALYSIS

Northern Pike Surveys

Gillnet Sampling

The capture of a northern pike during a gillnet survey will confirm presence. If no northern pike are caught, it will be concluded that either no northern pike are present or that the population is less than 20 individuals. For lakes surveyed with gillnet effort under Objective 1 precision criteria, the probability of failing to detect a population of 20 individuals will be less than 0.20. For lakes surveyed with gillnet effort under Objective 2 precision criteria, the probability of failing to detect a population of 20 individuals will be less than 0.50.

eDNA Sampling

Interpreting eDNA detection results requires discretion and an understanding that nonliving sources of DNA and sample contamination can occasionally confound results. Local experience with eDNA sampling has indicated that positive eDNA detections are not always associated with the presence of a live northern pike population. On the Kenai Peninsula, northern pike eDNA surveys at lakes where only a single sample tested positive ($N = 7$) have yet to be associated with a live northern pike population after follow-up gillnet surveys were completed. In light of this, only eDNA surveys yielding greater than 1 positive eDNA detection will trigger the need for a follow-up gillnet survey. For instances when there are no eDNA detections in a survey, it will be concluded that the probability of failing to detect a northern pike population of 20 individuals is less than 0.20 and no further sampling is necessary unless new information or reports are garnered that raise suspicion that invasive fish may be present.

Lake Mapping

The mapping company ciBiobase will generate bathymetric maps and apply algorithms to depth 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.

Water Quality Monitoring and Stream Discharge

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

Restoration Monitoring via CPUE

Gillnet and minnow trapping CPUE will be calculated for each gear type using standard procedures for arithmetic mean and variance for each species captured at a surveyed waterbody.

Invertebrate Surveys

After identification of taxa in the invertebrate samples, a list of invertebrate taxa presence will be produced. The list may be used for comparison of taxa presence should the waterbody be subject to a rotenone treatment and resurveyed for invertebrates.

Fork Length Histograms

When sample sizes are sufficiently large, then for each species, the fraction p_k of fish in length group *k* will be estimated as follows:

$$\hat{p}_k = \frac{n_k}{n} \tag{9}$$

Where n_k is the number of fish in length group *k* and *n* is the total number of fish of that species sampled. The estimated variance of $\hat{p}_k$ is

$$\widehat{var}(\hat{p}_k) = \frac{\hat{p}_k(1-\hat{p}_k)}{n-1} \tag{10}$$

SCHEDULE AND DELIVERABLES

Dates	Activity
2020	
July–October	Miller Creek–Vogel Lake: assess composition of native fish populations, survey for pike in neighboring waters, revise treatment plan, maintain fish barriers, survey invertebrates. Tote Road Pike Lakes: continue with native fish releases. NKPMA lake monitoring: conduct fish surveys (see Appendix A1). Stormy Lake: assess Arctic char (*Salvelinus alpinus*) stock in fall. Possibly conduct nonnative rainbow trout eradication at Y Lake near Kasilof.
November–December	Vogel Lake–Miller Creek: initiate permitting for rotenone treatment, begin helicopter service solicitation and contract, assist with environmental assessment. Tote Road: begin drafting Special Report on restoration project.
2021	
December–April	Vogel Lake–Miller Creek: continue with permitting, continue helicopter service solicitation and contract, assist with environmental assessment, conduct public scoping meeting, supply and equipment procurement.
May–June	Tote Road: continue with native fish restoration, conduct various lake monitoring surveys. Miller Creek: fish barrier maintenance, site and equipment preparations.
July–September	Tote Road: continue with native fish restoration. Miller Creek: conduct fish rescue work, invertebrate surveys as needed. NKPMA lake monitoring: conduct fish surveys (see Appendix A1). Stormy Lake char monitoring.
October	Conduct Miller Creek drainage rotenone treatment and posttreatment monitoring. Stormy Lake char monitoring.
November–December	Miller Creek: posttreatment monitoring.
2022	
January–April	Miller Creek: posttreatment monitoring.
May–June	Miller Creek: release rescued native fish in treatment area. NKPMA lake monitoring: conduct fish surveys (see Appendix A1).

RESPONSIBILITIES

Robert Massengill, Fishery Biologist II, Project Leader
Duties: Develops study design, oversees field logistics, purchasing, and project implementation. Enters and manages data, prepares project reports, manages project budget, and gives presentations to the public and provides management recommendations.

Robert Begich, Fishery Biologist III, Project Supervisor
Duties: Provides oversight and make recommendations on study designs and project plans, assists with data analysis and project reporting, coordinates and assists with the completion of project deliverables. Assists with field work as needed.

Kristine Dunker, Fishery Biologist III
Duties: Provides guidance on study design, reviews project operational plans and reports, assists with field work as needed.

Ben Buzzee, Fishery Biometrician I
Duties: Provides guidance on study design, reviews project operational plans and reports.

Vacant, Fish and Wildlife Technician III, Crew Leader
Duties: Assists with all aspects of field work and sampling, records and edits raw data, performs basic maintenance and inventory of equipment and supplies.

Vacant, Fish and Wildlife Technician II
Duties: Assists with all aspects of field work and sampling, records and edits raw data, performs basic maintenance and inventory of equipment and supplies.

REFERENCES CITED

Bachmann, R. W. 1973. Laboratory manual for limnology. Department of Zoology and Entomology, Iowa State University, Ames, Iowa

Begich, R. N. 2010. Control and removals of invasive northern pike on the Kenai Peninsula, 2004. Alaska Department of Fish and Game, Fishery Data Series No. 10-59, Anchorage. http://www.adfg.alaska.gov/FedAidpdfs/FDS10-59.pdf

Begich, R. N., and T. R. McKinley. 2005. Restoration of salmonid habitat by control and removals of invasive northern pike, Kenai Peninsula, 2003. Alaska Department of Fish and Game, Special Publication No. 05-07, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/sp05-07.pdf

Bell, M. A., D. C. Heins, M. A. Wund, F. A. v. Hipple, R. Massengill, K. Dunker, G. A. Bristow, and W. E. Aguirre. 2016. Reintroduction of threespine stickleback into Cheney and Scout Lakes, Alaska. Evolutionary Ecology Research 17:157-178.

Bradley, P., C. Jacobson, and K. Dunker. 2020. Operational Plan: Threemile Lake invasive northern pike suppression and Chuitbuna Lake population assessment. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.2A.20.01, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/ROP.SF.2A.2020.01.pdf

Carim, K. J., T. Padgett-Stewart, T. M. Wilcox, M. K. Young, K. S. McKelvey, and M. K. Schwartz. 2015. Protocol for collecting eDNA samples from streams. U.S.D.A. Forest Service, National Genomics Center for Wildlife and Fish Conservation. V2.3. https://www.fs.fed.us/research/genomics-center/docs/edna/edna-protocol.pdf

Cook, M. F., and E. P. Bergersen. 1988. Movements, habitat selection, and activity periods of northern pike in Eleven Mile Reservoir, Colorado. Transactions of the American Fisheries Society 117(5):495-502.

Dunker, K., and D. Rutz. 2014. Alexander Creek northern pike suppression. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.2A.2014.13, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/ROP.SF.2A.2014.13.pdf

Dunker, K., A. Sepulveda, R. Massengill, and D. Rutz. 2018. The northern pike, a prized native but disastrous invasive. Pages 356-398 [*In*] C. Skov, and P. A. Nilsson, editors. Biology and ecology of pike. CRC Press, Boca Raton, FL.

Dunker, K. J., A. J. Sepulveda, R. L. Massengill, J. B. Olsen, O. L. Russ, J. K. Wenburg, and A. Antonovich. 2016. Potential of environmental DNA to evaluate northern pike (*Esox lucius*) eradication efforts: an experimental test and case study. PLOS One 11(9):e0162277. doi:10.1371/journal.pone.0162277

Evans, N. T., P. D. Shirey, J. G. Wieringa, A. R. Mahon, and G. A. Lamberti. 2017. Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. Fisheries 42(2):90-99.

Fay, V. 2002. Alaska aquatic nuisance species management plan. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Informational Report 5J02-10, Juneau. http://www.adfg.alaska.gov/FedAidpdfs/RIR.5J.2002.10.pdf

Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. Species detection using environmental DNA from water samples. Biology Letters 4:423-425.

Glick, W. J., and T. M. Willette. 2016. Relative abundance, food habits, age, and growth of northern pike in 5 Susitna River drainage lakes, 2009-2012. Alaska Department of Fish and Game, Fishery Data Series No. 16-34, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/FDS16-34.pdf

Havens, A. C., and S. Sonnichsen. 1992. Evaluation of enhancement efforts for rainbow trout in Southcentral Alaska, 1991. Alaska Department of Fish and Game, Fishery Data Series No. 92-37., Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/fds92-37.pdf

Inskip, P. D. 1982. Habitat suitability index models: northern pike. U.S. Department of Interior, Fish and Wildlife Service FWS/OBS-82/10.17

REFERENCES CITED (Continued)

Koenings, J. P., G. B. Kyle, J. A. Edmundson, and J. E. Edmundson. 1987. Limnology field and laboratory manual: methods for assessing aquatic production. Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement, and Development, Report No. 71, Juneau.

Laramie, M. B., D. S. Pilliod, C. S. Goldberg, and K. M. Strickler. 2015. Environmental DNA sampling protocol—Filtering water to capture DNA from aquatic organisms. U.S. Geological Survey Techniques and Methods, book 2, chap. A13. https://pubs.er.usgs.gov/publication/tm2A13

Massengill, R. 2014a. Control efforts for invasive northern pike on the Kenai Peninsula, 2009. Alaska Department of Fish and Game, Special Publication No. 14-11, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/SP14-11.pdf

Massengill, R. 2017a. Stormy Lake northern pike distribution and movement study to inform future eradication efforts. Alaska Department of Fish and Game, Special Publication No. 17-17, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/SP17-17.pdf

Massengill, R. 2017b. Stormy Lake restoration: invasive northern pike eradication, 2012. Alaska Department of Fish and Game, Special Publication No. 17-18, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/SP17-18.pdf

Massengill, R. L. 2010. Control efforts for invasive northern pike on the Kenai Peninsula, 2005-2006. Alaska Department of Fish and Game, Fishery Data Series No. 10-05, Anchorage. http://www.adfg.alaska.gov/FedAidpdfs/Fds10-05.pdf

Massengill, R. L. 2014b. Control efforts for invasive northern pike on the Kenai Peninsula, 2008. Alaska Department of Fish and Game, Special Publication No. 14-12, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/SP14-12.pdf

McKinley, T. R. 2013. Survey of northern pike in lakes of the Soldotna Creek drainage, 2002. Alaska Department of Fish and Game, Special Publication No. 13-02, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/SP13-02.pdf

McMahon, T. E., and D. H. Bennett. 1996. Walleye and northern pike: boost or bane to northwest fisheries? Fisheries 21(8):6-13.

Muhlfeld, C. C., D. H. Bennett, R. K. S. B. Marotz, and M. Boyer. 2008. Using bioenergetics modeling to estimate consumption of native juvenile salmonids by nonnative northern pike in the upper Flathead River System, Montana. North American Journal of Fisheries Management 28(3):636-648.

Nolan, K. M., and R. R. Shields. 2000. Measurement of stream discharge by wading. Water Resources Investigations Report 00-4036. United States Geological Survey. https://pubs.er.usgs.gov/publication/wri20004036

Olsen, J. B., C. J. Lewis, R. L. Massengill, K. J. Dunker, and J. K. Wenburg. 2015. An evaluation of target specificity and sensitivity of three qPCR assays for detecting environmental DNA from Northern Pike (*Esox lucius*). Conservation Genetics Resources 7(3):615-617.

Oswood, M. W., J. B. Reynolds, J. C. I. III, and A. M. Milner. 2000. Distributions of freshwater fishes in ecoregions and hydroregions of Alaska. Journal of the North American Benthological Society 19(3):405-418.

Patankar, R., F. A. V. Hippel, and M. A. Bell. 2006. Extinction of a weakly armored threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. Ecology of Freshwater Fish 15(4):482-487.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States: Protozoa to Mollusca. 3rd edition. Wiley and Sons, Inc., United States of America.

Plummer, M. 2013. R package version 3-11. rjags: Bayesian graphical models using MCMC. http://CRAN.R-project.org/package=rjags

Rich, B. A. 1992. Population dynamics, food habits, movement, and habitat use of northern pike in the Coeur d'Alene system, Idaho. Master's thesis. University of Idaho, Moscow.

REFERENCES CITED (Continued)

Rutz, D. S. 1999. Movements, food availability and stomach contents of northern pike in selected Susitna River drainages, 1996-1997. Alaska Department of Fish and Game, Fishery Data Series No. 99-5, Anchorage. http://www.adfg.alaska.gov/FedAidPDFs/fds99-05.pdf

Schmetterling, D. A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River Drainage, Montana. North American Journal of Fisheries Management 21(3):507–520.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. 2nd edition. Griffin and Company, Ltd. London.

Sepulveda, A. J., P. R. Hutchins, R. L. Massengill, and K. J. Dunker. 2018. Tradeoffs of a portable, field-based environmental DNA platform for detecting invasive northern pike (Esox lucius) in Alaska. Management of Biological Invasions 9(3):253-258.

Sepulveda, A. J., D. S. Rutz, A. W. Dupuis, P. A. Shields, and K. J. Dunker. 2015. Introduced northern pike consumption of salmonids in Southcentral Alaska. Ecology of Freshwater Fish 24(4):519-531.

Sepulveda, A. J., D. S. Rutz, S. S. Ivey, K. J. Dunker, and J. A. Gross. 2013. Introduced northern pike predation on salmonids in southcentral Alaska. Ecology of Freshwater Fish 22(2):268-279.

APPENDIX A: LAKE INFORMATION SUMMARY

Appendix A1.–Lake information summary including threat classification for northern pike and year when a fish survey is due.

Threat class	Site name	Drainage	Criteria for threat class	Lake category [a]	Surface acres	Invasive fish removal year	Year last surveyed	Survey type	Invasive fish captured in last survey	Next fish survey due
High	Vogel Lake [b]	Miller Creek	Verified pike water	USP	140	2019	NA	Gillnet	Y	Pending
	North Vogel Lake [b]	Miller Creek	Verified pike water	USP	38	2019	NA	Gillnet	Y	Pending
	Bird Pond [c]	Miller Creek	1/4 mile from pike waters	USP	14	NA	2019	Gillnet	N	2021
	Vogel Pond [c]	Miller Creek	1/4 mile from pike waters	USP	7.7	NA	2019	Gillnet	N	2022
	Kuguyuk Pond [c]	Miller Creek	1/4 mile from pike waters	USP	14	NA	2019	Gillnet	N	2021
	Sandpiper Lake [c]	Miller Creek	1 eDNA detection, 1/4 mile from pike waters	USP	80	NA	2019	Gillnet	N	2021
Medium	Warlfe Lake	Closed Lake	Former pike water	R	3.4	2017	2017	Gillnet	Y	**2020**
	Hall Lake	Closed Lake	Former pike water	R	43	2011	2017	Gillnet	N	**2020**
	Arc Lake	Closed Lake	Former pike water	R	18	2008	2009	Gillnet	N	**2020**
	Scout Lake	Closed Lake	Former pike water	R	85	2009	2010	Gillnet	N	**2020**
	Sevena Lake	Soldotna Ck	Former pike water	R	73	2016	2019	Gillnet	N	2022
	Tree Lake	Soldotna Ck	Former pike water	R	64	Unknown	2015	Gillnet	N	**2020**
	Union Lake	Soldotna Ck	Former pike water	R	79	2014	2019	Gillnet	N	2022
	Denise Lake	Soldotna Ck	Former pike water	R	38	Unknown	2010	Gillnet	N	**2020**
	West Mackey Lake	Soldotna Ck	Former pike water	R	184	2014	2019	Gillnet	N	2022
	East Mackey Lake	Soldotna Ck	Former pike water	R	100	2014	2019	Gillnet	N	2022
	Derks Lake	Soldotna Ck	Former pike water	R	37	2014	2019	Gillnet	N	2022
	Loon Lake	Soldotna Ck	Former pike water	R	21	2014	2017	Gillnet	N	**2020**
	Tiny Lake	Soldotna Ck	Former pike water	R	5.5	2014	2012	Gillnet	N	**2020**
	Soldotna Creek	Soldotna Ck	Former pike water	R	NA	2016	NA	Gillnet	N	NA
	Derks Pond	Soldotna Ck	Former pike water	R	2	2017	NA	Gillnet	N	**2020**
	Stormy Lake	Swanson River	Former pike water	R	403	2012	2019	Gillnet	N	2022
	Leisure Lake	Tote Road	Former pike water	R	11	2018	2019	Gillnet	N	2022
	Leisure Pond	Tote Road	Former pike water	R	1.5	2018	2019	Gillnet	N	2022
	Hope Lake	Tote Road	Former pike water	R	27	2018	2019	Gillnet	N	2022
	Ranchero Lake	Tote Road	Former pike water	R	7.7	2018	2019	Gillnet	N	2022

-continued-

Appendix A1.–Page 2 of 2.

Threat class	Site name	Drainage	Criteria for threat class	Lake category [a]	Surface acres	Invasive fish removal year	Year last surveyed	Survey type	Invasive fish captured in last survey	Next fish survey due
Medium	Crystal Lake	Tote Road	Former pike water	R	17	2018	2019	Gillnet	N	2022
	CC lake	Tote Road	Former pike water	R	4.5	2018	2019	Gillnet	N	2022
	Fred's Lake	Tote Road	Former pike water	R	6	2018	2019	Gillnet	N	2022
	G Lake	Tote Road	Former pike water	R	17	2018	2019	Gillnet	N	2022
	Bird Pond	Miller Creek	1/4 mile from pike waters	USP	14	NA	2019	Gillnet	N	2022
	Kuguyuk Pond	Miller Creek	1/4 mile from pike waters	USP	14	NA	2019	Gillnet	N	2022
	Sandpiper Lake	Miller Creek	1 eDNA detection, 1/4 mile from pike waters	USP	80	NA	2019	Gillnet	N	2022
	McLain Lake [d]	Moose River	2 eDNA detections	USP	275	NA	2017	Gillnet	N	NA
	Lingren Pond [d]	Tote Road	Connectivity to pike water	USA	0.2	NA	2017	eDNA	N	NA
	City of Kenai Pond	Closed Lake	Former fathead minnow waters	R	1	NA	2019	Minnow trap, dip net	N	
Low	Longmere Lake	Closed Lake	High use fishery with road access	USP	70	NA	Not surveyed	NA	NA	**2020**
	Sport Lake	Closed Lake	Repeated rumors of pike	USP	29	NA	Not surveyed	NA	NA	**2020**
	Kelly Lake [d]	Moose River	1 unverified pike observation	USP	170	NA	2014	Gillnet	N	NA
	Peterson Lake	Moose River	1 eDNA detection	USP	95	NA	2013	eDNA	N	**2020**
	Watson Lake	Moose River	1 eDNA detection	USP	58	NA	2013	eDNA	N	**2020**
	Clam Lake	Moose River	1 eDNA detection	USP	360	NA	Not surveyed	eDNA	N	**2020**

Note: NA means data not available or not applicable.

[a] R means restored waterbody (nonnative fish removed) and typically resurveyed before native fish have been reintroduced. USP means unrestored waterbody (no nonnative fish removal has occurred) with salmonids present. USA means unrestored waterbody where salmonids are absent.

[b] Pike removal is planned.

[c] Resurvey no later than July 2021.

[d] Northern pike not believed to be present.

APPENDIX B: BIOMEME DNA EXTRACTION AND THERMOCYCLER PROTOCOL

Appendix B1.–Biomeme DNA extraction and thermocycler protocol excerpted from Sepulveda et al. (2018)[10].

For DNA extraction protocol: Biomeme's six-step protocol, which takes ~ 5 minutes, ensures that all fluid in the syringe is expelled before moving onto to the next step:

(1) Shake filter sample tube containing the filter sample vigorously for one minute to loosen DNA off the filter, then draw up the fluid in the filter sample tube with a syringe through the sample prep column and push the fluid back out for a total of 20 pumps.

(2) Draw up Biomeme protein wash through the syringe and push back out one time.

(3) Draw up Biomeme wash buffer through the syringe and push back out one time.

(4) Draw up Biomeme drying wash through the syringe and push back out one time.

(5) Draw air through the syringe and sample prep column by quickly and vigorously pumping back out for greater twenty times, until the pump is warm to the touch and the sample prep column does not spray fluid droplets.

(6) Draw up Biomeme elution buffer all the way up through the syringe and pump back out for a total of five pumps. The purified DNA was then stored in the elution buffer until PCR.

Biomeme thermocycler protocol

We followed Biomeme' s recommended thermocycler protocol for this assay: initial denaturation at 95°C for 1 minute followed by 45 cycles of 95°C denaturation for 1 second, and 20 seconds at annealing temperatures starting at 60°C.

[10] Authors retain copywrite for Management of Biological Invasions publications. Content is excerpted with permission from R. Massengill and K. Dunker.

APPENDIX C: DATA SHEETS

Appendix C1.–Water quality field data sheet.

Lake: **Sampler:**

Date: **Time:**

	Temperature ºC	Specific Conductance S/cm	Dissolved Oxygen mg/L	Dissolved Oxygen %	pH
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					

Visibility (m):

Ice Thickness (In):

Comments:

Appendix C2.–Stream discharge field data sheet.

Station: _____ Date: _____

Crew: _____

GPS Coordinates: River Mile: _____

Description: _____

_____

_____ Meter: _____

Weather: Rating:

Distance from Head Pin (ft.) L or REW	Angle Coef.	Total Depth (ft.)	Vel Obs. Depth %	No. Revo-lutions	Time (sec)	Velocity fps		Cell % Flow	Flow (ft^3/s)
						Point	Mean Vertical		
0.0			0.6						
0.5			0.6						
1.0			0.6						
1.5			0.6						
2.0			0.6						
2.5			0.6						
3.0			0.6						
3.5			0.6						
4.0			0.6						
4.5			0.6						
5.0			0.6						
5.5			0.6						
6.0			0.6						
6.5			0.6						
7.0			0.6						
7.5			0.6						
8.0			0.6						
8.5			0.6						
9.0			0.6						
9.5			0.6						
10.0			0.6						
10.5			0.6						
11.0			0.6						
11.5			0.6						
12.0			0.6						
12.5			0.6						
13.0			0.6						
13.5			0.6						
14.0			0.6						
14.5			0.6						
15.0			0.6						
15.5			0.6						
16.0			0.6						
16.5			0.6						
17.0			0.6						