Regional Operational Plan No. ROP.SF.2A.2022.28

Operational Plan: Kenai Peninsula Nonnative Fish Control, Monitoring, and Native Fish Restoration, 2022–2024

by

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and

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July 2022

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

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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

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SIGNATURE PAGE

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Approval

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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 support the restoration and monitoring of native fish populations. Nonnative fish detection will be primarily accomplished by gillnet surveys using a standardized protocol that adjusts netting effort to lake littoral area. The prioritization of waterbodies selected for survey will be based on a threat classification. For some waters, gillnet surveys may be undesirable and environmental DNA (eDNA) sampling methods may be used alone or in tandem with reduced gillnet sampling. When an invasive fish species is detected, 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 whenever appropriate to waters where nonnative fish have been removed. This may be accomplished by releasing wild native fish collected from a nearby source, particularly in situations where native fish populations are unlikely to recover quickly on their own. Restored native fish populations will be monitored periodically using gillnet and minnow trap catch per unit effort (CPUE) and length frequency distributions.

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

INTRODUCTION

PURPOSE

This project will provide information to fishery managers about the presence and distribution of invasive northern pike (*Esox lucius*; "pike") 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

Documented nonnative freshwater fish found 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*), and a nonnative strain of rainbow trout (*Onchorhynchus mykiss*; 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 only 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 Alaska (Rutz 1999; Patankar et al. 2006; Sepulveda et al. 2013; Sepulveda et al. 2015; Glick and Willette 2016; Dunker et al. 2018; Massengill 2017b; Massengill 2022). 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). Also, prevalent shallow lake morphology and slow stream velocities throughout much of southcentral Alaska offer prey limited deep-water refugia from northern pike, which typically occupy shallow, vegetated habitat (Inskip 1982; Cook and Bergersen 1988; Dunker et al. 2018).

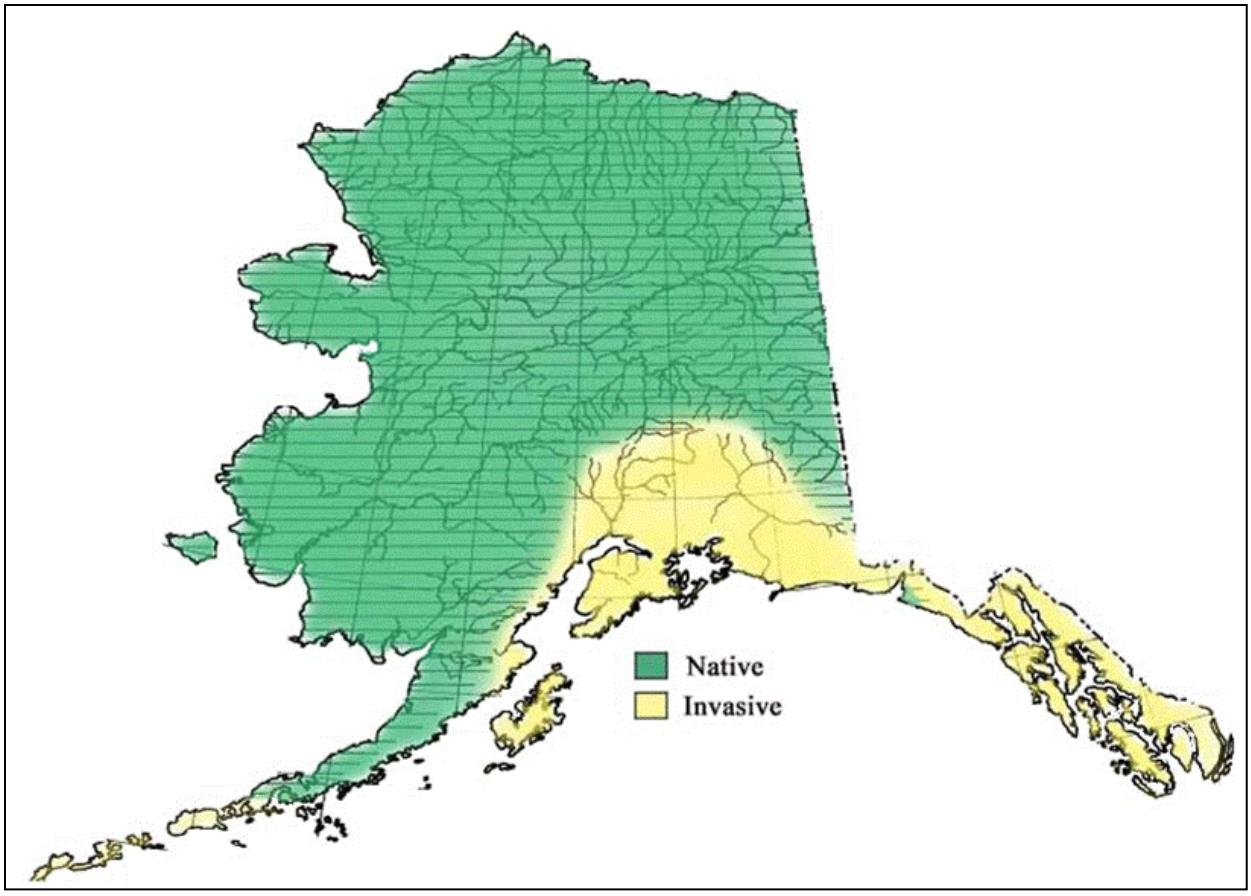

Figure 1.–Map of the native and invasive range of northern pike in Alaska

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 27 Kenai Peninsula waterbodies (Figure 2)[1]. Northern pike or muskellunge were discovered in 16 of these waterbodies since 2000; however, the dates of their 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, 2014b, 2017b, 2022). 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, 2014b) 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 most of the drainage resides 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,

[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.

and North Vogel Lake (Figure 3). A partnership between ADF&G, United States Fish and Wildlife Service (USFWS), and the Kenai Watershed Forum (KWF) was formed to develop a response to the northern pike threat in the Miller Creek drainage. During October of 2021, a rotenone treatment was executed in pike-invaded waters. Just prior to the rotenone treatment, wild native fish, primarily rainbow trout, sculpin (*Cottidae* spp.), and juvenile coho salmon (*O. kisutch*), were collected from the treatment area for temporary safe relocation to a nearby pond (Bird Pond). These native fish will be returned to the treatment area in 2022 to help reestablish native fish populations if the removal of northern pike is deemed successful.

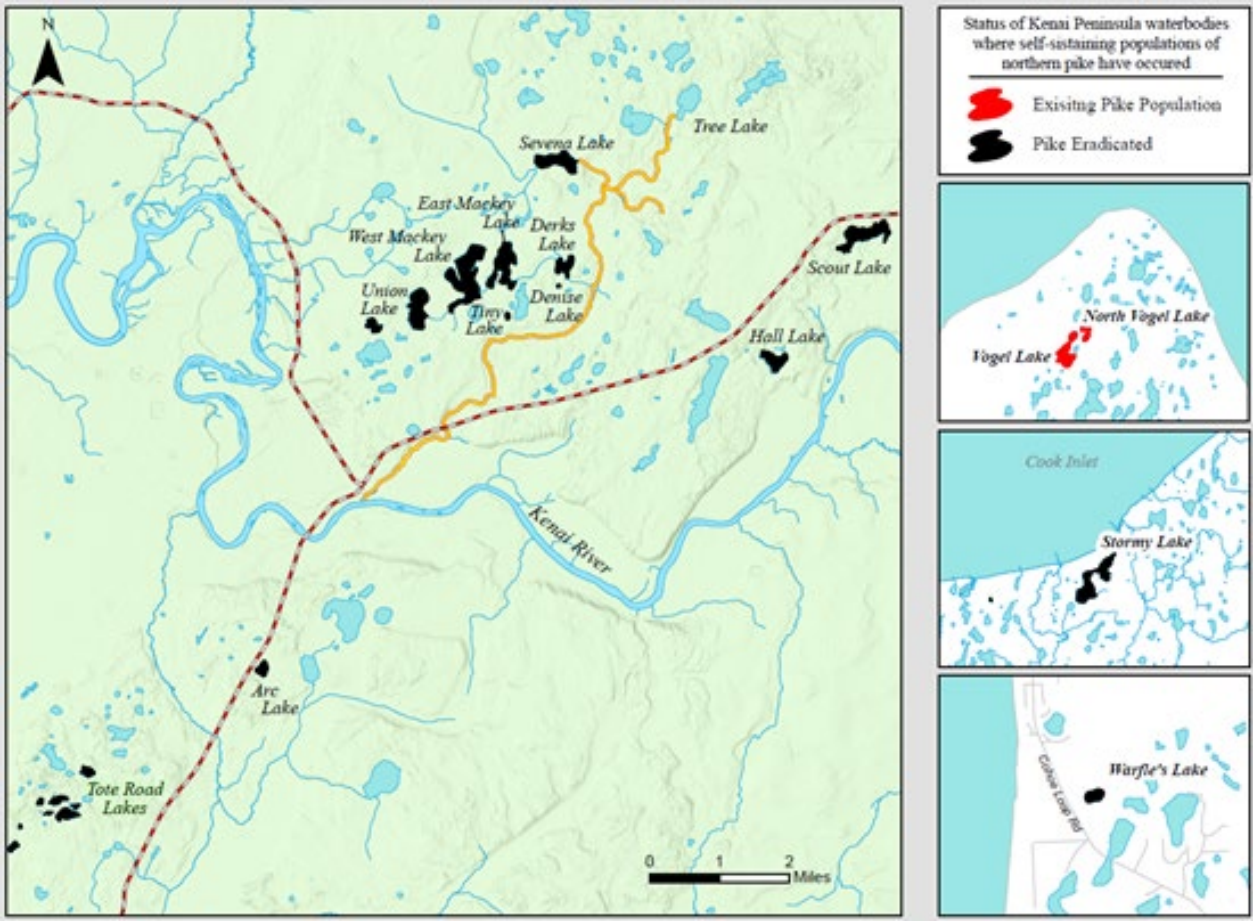

Figure 2.–Map showing the status of Kenai Peninsula waters associated with northern pike. Waters in red color identify areas where the success of northern pike removal is still pending evaluation.

Other nonnative fish previously found on the Kenai Peninsula include blackfish, yellow perch, red swamp crayfish, goldfish, and a nonnative strain of rainbow trout illegally imported from Oregon. The lone yellow perch population existed in a small lake in Nikiski, and that population was eradicated with a rotenone treatment in 2000 by ADF&G. Red swamp crayfish were twice discovered in the Kenai River drainage since 2000; apparently, the live crayfish intended for food were illegally dumped, but ultimately the dumping failed to establish sustaining populations. A single dead goldfish was found in Loon Lake (Soldotna Creek drainage) in 2017 following a rotenone treatment to remove northern pike, and over a dozen live goldfish were found in a flooded gravel pit near Funny River Road (Soldotna) in 2007, which were removed by draining the pond. Blackfish, found in 2 small lower tributaries of the Kenai River, have been present for decades and

remain today (Byker 2019). A small reproducing population of muskellunge was detected in 2017 in a 20-acre lake near Soldotna. The muskellunge were removed with a rotenone treatment in the fall of 2018 in conjunction with a multi-lake northern pike eradication effort in the same vicinity. Genetic analysis of the muskellunge indicated they likely originated from Wisconsin. In 2018, fathead minnows were found in a 1-acre manmade pond in the City of Kenai and subsequently eradicated with a rotenone treatment in 2019 by ADF&G.

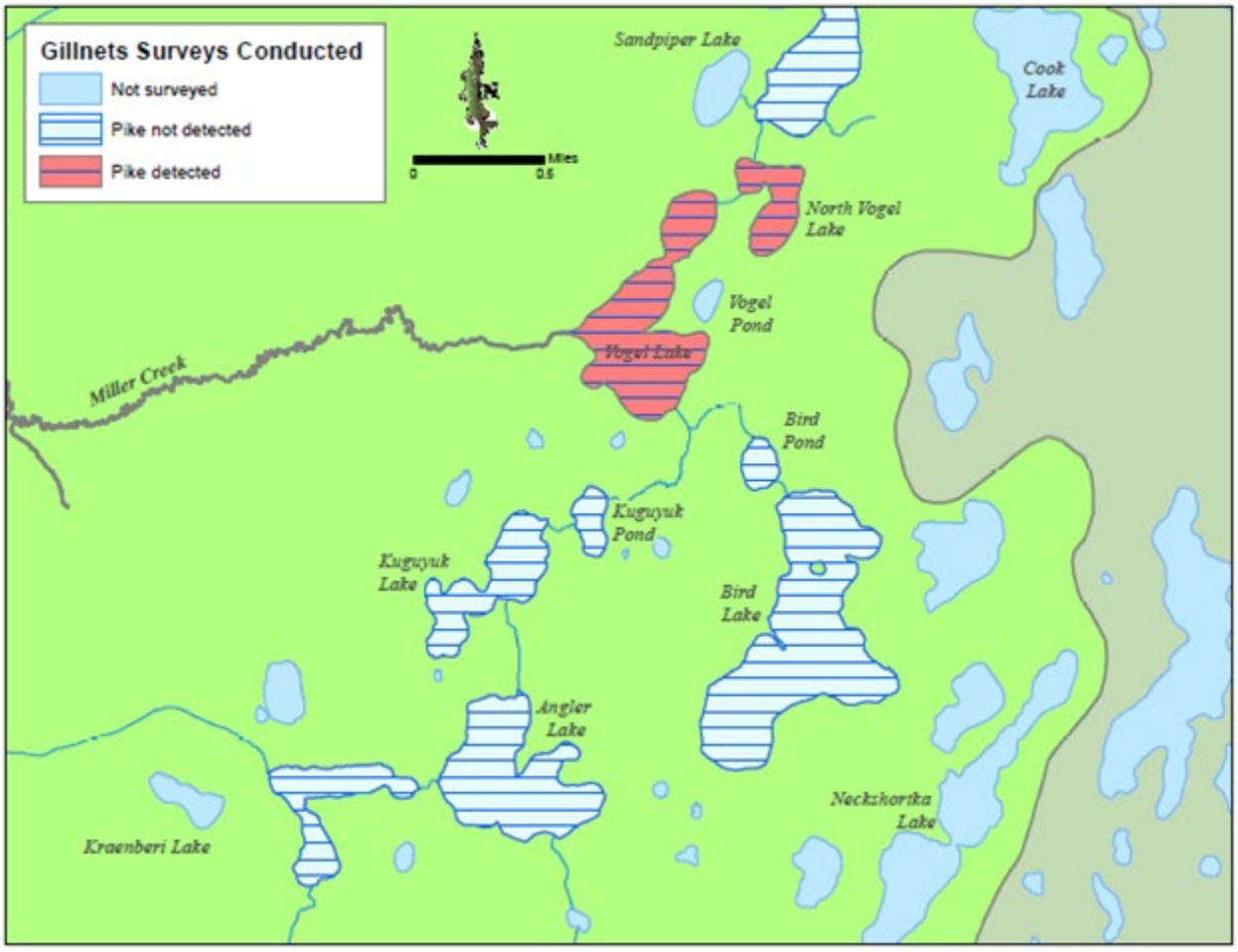

Figure 3.–Map of the Miller Creek drainage and the lakes surveyed for invasive northern pike.

OBJECTIVES

PRIMARY OBJECTIVES

1) Conduct gillnet surveys between 1 July 2022 and 30 June 2024 and within 6 months of any northern pike removal effort to evaluate the success of the removal effort such that the probability of detecting surviving northern pike is 0.80 assuming the population comprises at least 20 fish that are greater than 300 mm fork length.
2) Conduct gillnet surveys between 1 July 2022 and 30 June 2024 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 comprises at least 20 fish that are greater than 300 mm fork length[2].

SECONDARY OBJECTIVES

1) Collect and analyze eDNA samples for northern pike in all waters where gillnet surveys for detection of suspected northern pike populations are undesirable or insufficient to meet precision criteria for Primary 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 at all waters where nonnative fish discoveries are made.
4) Inventory invertebrate taxa at waters where 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.
11) Assist with statewide driessenid mussel monitoring at select Kenai Peninsula waters as outlined in a separate ADF&G operational plan.

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

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), and a flowchart will be used to assign the threat class (Figure 4). When a waterbody qualifies for multiple threat classes, the greater threat class will be used to determine the response time. For example, if a lake satisfies criteria for both a medium and high threat waterbody, it will be considered a high threat water and surveyed as soon as possible.

Netting effort will be based on a lake category assignment that considers the lake's fish assemblage and invasive fish management history. The purpose of assigning lake categories is to limit the bycatch of sport fish (i.e., salmonids) in restored waters or new waters needing investigation while providing an opportunity to detect nonnative fish populations.

Lake category definitions are listed below.

1) Restored (R): A R 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 that initial survey, additional gillnet and minnow trap surveys will occur at least once every 3 years for a 9-year period with enough effort each time to achieve Secondary Objective 8 requirements. These subsequent surveys are primarily designed to monitor restored native fish populations and secondarily to provide an opportunity to detect nonnative fish.
2) Unrestored, salmonids present (USP): A USP waterbody is suspected to have nonnative fish and known to support a salmonid population; netting effort should satisfy Primary Objective 2 precision criteria.
3) Unrestored, salmonids absent (USA): A USA waterbody is suspected to have nonnative fish and not known to support a salmonid population; netting effort should satisfy precision criteria for Primary Objective 1 or Primary Objective 2, depending on the judgement of the project leader after considering bycatch concerns and site-specific issues.

Appendix A1 provides a current list of the NKPMA waterbodies organized by threat class (high, medium, and low) with corresponding lake category assignments (R, USP, and USA).

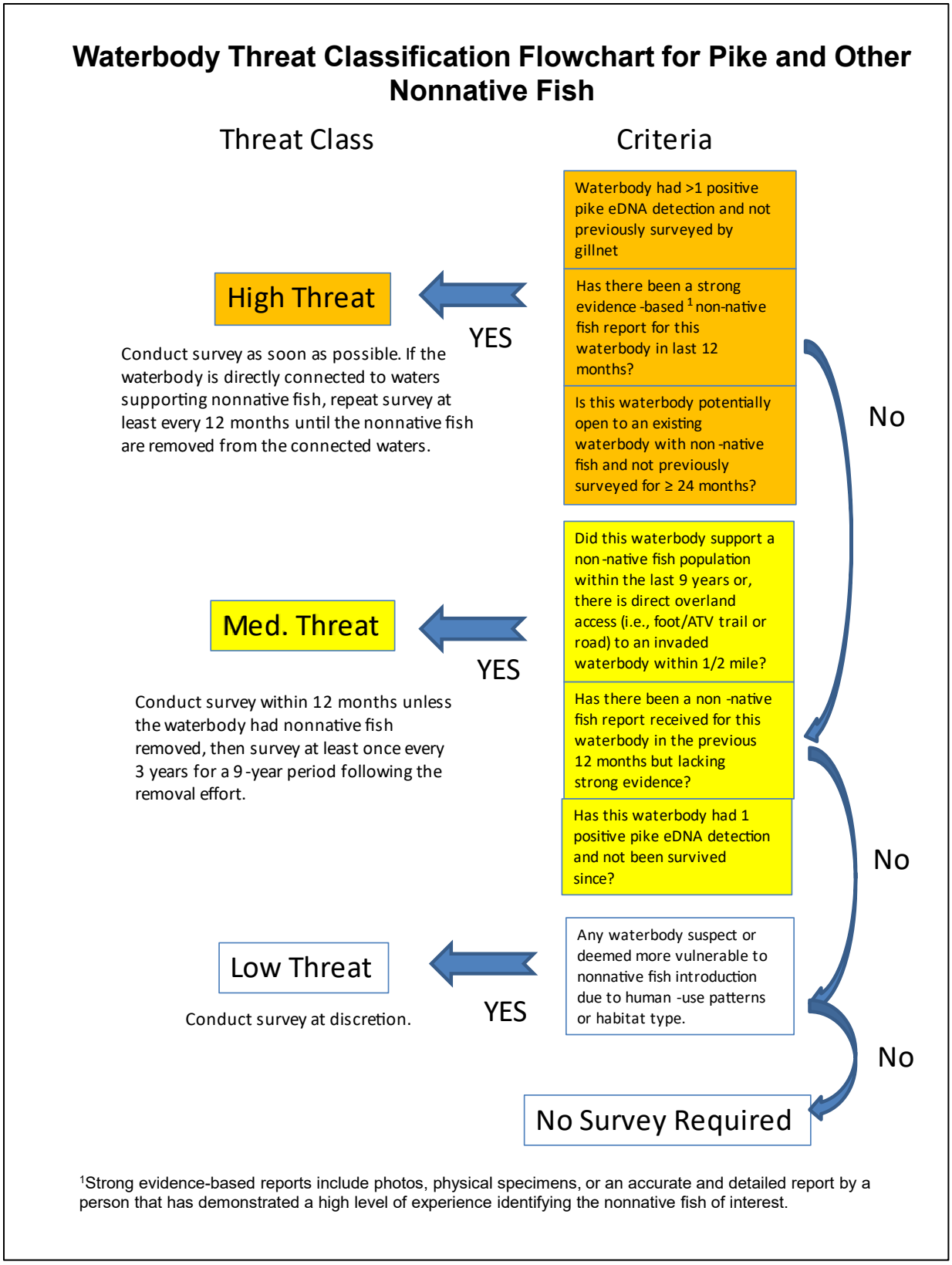

Figure 4.–Flowchart for assessing the invasive fish threat to a waterbody and timeframe for conducting a gillnet detection survey.

Gillnet Sampling Effort

Gillnet surveys designed to detect the presence of northern pike or other nonnative fish in High, Medium, or Low threat waters 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 determine the netting effort needed to detect a northern pike population of at least 20 fish with an estimated detection probability of 80% or 50% for Objectives 1 and 2, respectively, we utilized gillnet capture data from past northern pike abundance estimates.

Between 2018 and 2019, ADF&G conducted gillnet studies to estimate pike abundance at Threemile Lake and Chuitbuna Lake. Data collected from these surveys included catch C_{ij}, effort E_{ij} (in units of net-hours per littoral surface acre), and density D_{ij} (in numbers of fish per littoral surface acre) for sample i (where $i = 1, \ldots, s$) and survey j (where $j = 1, \ldots, 4$). Populations were assumed closed except for captured fish, and fishing was assumed to represent a Poisson process with a constant probability of capture for all individuals larger than 300 millimeters. Each lake was surveyed in 2018 and 2019, and 2 samples were collected in each survey. Data were analyzed using a linear regression method (Pierce et al. 2010) for the response *CPUE* versus the predictor *D*, each calculated at survey *j* and sample *i* to estimate the slope *K*:

$$CPUE_{ij} = KD_{ij} \tag{1}$$

where *K* is the average probability that a northern pike larger than 300 millimeters is captured with 1 unit of effort during survey *j* and sample *i*, D_{ij} is the estimated fish density in survey *j* during sample *i* and $CPUE_{ij}$ is determined as follows:

$$CPUE_{ij} = \frac{C_{ij}}{E_{ij}} \tag{2}$$

The estimate of *K*, the probability of detecting small northern pike populations, was used to inform effort determination for this northern pike project. Under the assumption that fishing represents a Poisson counting process, the probability F_p of failing to detect a population of northern pike of size *N* as a function of net-hours per acre (*E*) was determined as follows:

$$F_p = [\exp(-KE)]^N \tag{3}$$

where

$$E = \frac{H}{A} \tag{4}$$

and *H* is net hours, and *A* is littoral acres fished.

Using Equations 1 and 2, K was estimated at 0.038 from the data collected by the northern pike abundance studies at Threemile and Chuitbuna Lakes in 2018 and 2019. The approximate netting effort found in Table 1 will be used to satisfy the precision criteria found in Objectives 1 and 2. This effort represents the minimum effort needed to detect at least 1 northern pike given a probability of failing to detect any northern pike equal to 0.2 and an initial population size of 20 individuals.

Table 1.–Minimum effort needed to detect at least 1 northern pike provided an initial population of 20 individuals and a probability of failing to detect any northern pike equal to 0.2.

Littoral acres	Net density (nets/acre)						
	0.1	0.4	0.7	1.0	1.3	1.6	1.9
1	21	5	3	2	2	1	1
10	212	53	30	21	16	13	11
50	1,059	265	151	106	81	66	56
100	2,118	529	303	212	163	132	111
200	4,235	1,059	605	424	326	265	223

Gillnetting Methods

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 littoral surface acreage (area of waters <4 m deep, which are more likely to contain pike)
3) applicable objective (Primary Objective 1, 2 or Secondary Objective 8)

Gillnets manufactured by Duluth Nets and made of single-strand monofilament mesh hung from a polypropylene floating line with the net bottom attached to 30 lb lead line will be used to survey lakes. 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 an organism releases 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 approaches for detecting aquatic taxa in low abundance (Ficetola et al. 2008). For aquatic species detection, eDNA is commonly collected within water samples (of about 1000 ml), concentrated by filtration, and then the filtrate is processed further using quantitative polymerase chain reaction (qPCR) amplification.

In circumstances when gillnetting may be an undesirable method for detecting northern pike (due to logistical, safety, or bycatch concerns), eDNA detection methods may be used to supplement or substitute for gillnetting. In those situations where eDNA sampling is warranted, an effort will be made to achieve similar precision criteria listed in Objectives 1 and 2 for netting (see *eDNA Sampling Effort* below).

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[3]).

Processing of the eDNA samples will be done by either of 2 options. The preferred option, when available, is for the processing to be done by USFWS Conservation Genetics Lab in Anchorage using a benchtop laboratory method called quantitative polymerase chain reaction (qPCR). A secondary option, when the USFWS Conservation genetics lab is unable to provide processing, is for the samples to be processed using a portable device called Biomeme Two3, which 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[4]). Many factors can affect the detection and persistence of eDNA. Positive detections may not always represent the presence of a live northern pike. Often called "false positives," misleading positive detections can be caused by sample contamination during handling or processing. False positives can also be caused by eDNA persisting in the environment after the organism is gone, or the transport of eDNA from elsewhere. Sediment-trapped eDNA has yielded positive eDNA results from waters where no live pike were ever detected (Dunker et al. 2016). Windstorms or fall turnover can increase the potential for positive eDNA detection by resuspending eDNA from nonliving sources (Harrison et al. 2019) so surveys should be timed to avoid sampling during those events.

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. Sample locations will be distributed evenly throughout the vegetated littoral (<4 m depth) habitat of the waterbody where it is optimal for pike. Summertime aerial imagery will be used to locate submerged and emergent aquatic standing vegetation beds, and lake bathymetry maps will be used to identify optimal pike habitat as well.

Care will be taken to ensure gear worn by the collectors (e.g., waders, rain gear, life jackets, etc.) and collection equipment (e.g., sample bottles, swing sampler, transport coolers, etc.) are

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

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

decontaminated. Decontamination is done by spraying a 10% bleach solution on the items and allowing it to soak for 10 minutes before rinsing with tap water. All sample containers should be certified sterilized by the manufacturer or should be sterilized by the sampler using a 20% bleach solution soak for at least 10 seconds, followed by a triple rinse of deionized water or distilled water. All decontaminated sample containers will be stored inside a clean plastic bag until use.

To reduce the risk that the boat could cause eDNA contamination, the boat hull, lower unit, and trailer will be decontaminated prior to deployment into a new waterbody. Decontamination involves removal of dirt and debris from outer surfaces of these items using a high-pressure wash followed by a 10% bleach solution spray and 10-minute soak. When samplers are collecting from a boat, they will collect the sample from the bow of the boat before the hull travels atop or past the sample site. Whether sampling from a boat or by foot, samplers will systematically collect samples from the waterbody in a sequential manner (i.e., clockwise or counterclockwise) and avoid traveling over a location prior to it being sampled.

There are typically 2 options used for collecting water or eDNA samples: 1) collection is done by submerging a bottle (sterilized 1-liter Nalgene bottle) near the water surface by hand or by the use of a hand-held sampler (a long pole with a bottle mount) that allows the sampler to sample deep or hard to reach areas, or 2) collection using a battery-powered Smith-Root backpack sampler, which automatically filters the water as it is collected through its intake hose and single-use filter pack. Both options are equally applicable, but the Smith-Root backpack sampler eliminates the need to filter the samples later, which reduces handling time and therefore risk of eDNA contamination. The Smith-Root backpack sampler has a control panel and screen with a variety of water sampling controls for setting target water volume, water flow, and pressure. Operating manuals for Smith-Root eDNA samplers are available online[5].

When collecting directly by hand or with a swing sampler, water samples will be collected by filling a 1000 ml bottle with near-surface water. A single 1000 ml sample per sample location will suffice if traditional benchtop lab analysis is used for the eDNA analysis regardless if the sample is collected in a bottle or by filtering with a Smith-Root backpack sampler. If the Biomeme Two 3 thermocycler is used for eDNA analysis, duplicate 1000 ml grab samples will be collected at each sample location if collecting by hand or by swing sampler, and at least 2,000 ml of water should be filtered if using a Smith-Root backpack sampler. Duplicate sampling or larger filtering volumes will compensate for the lower detection efficiency of the Biomeme Two3 thermocycler compared to traditional benchtop qPCR processing (Sepulveda et al. 2018).

Due to the high filtering efficiency of the Smith-Root backpack sampler, often 2,000 ml can be collected using single filter pack. In dirty water, which may lead to filter clogging, 2 or more filter packs may be needed in succession to achieve the target filtering volume. When sampling a single site with the Smith-Root backpack sampler, it is recommended to switch to a new filter pack whenever the filtering rate slows to less than 0.2 liters/minute. Smith-Root backpack sampler filter packs come in sterilized single-use resealable bags that the filter pack can be stored in after use. After collection, filter packs should be kept in cold storage until processed, unless self-preserving filter packs were used that desiccate the sample for stable, long-term storage at room temperature.

For samples collected in 1,000 ml bottles by hand and destined for benchtop lab analysis, each bottle will be placed in a clean Whirl-Pak bag until filtered. All samples (bottles or filter packs) will be labeled with a location code, unique sample code, collection date, and sampler initials.

[5] Smith-Root eDNA Sampler Manual: https://www.smith-root.com/support/downloads/edna-sampler-manual

Sample bottles will be placed inside a Whirl-Pak bag until filtered. All sample locations will be recorded with a handheld global positioning system (GPS).

Whether or not samples will be analyzed in the field or in a lab setting, on each day of sampling, samplers will collect several control blanks that will help identify if eDNA contamination occurred during handling or transport. Control blanks will be collected using identical containers and sample volumes as the actual water samples, but the control blanks will consist of deionized water. One control, called a field blank, will be filled with deionized water when onsite in the field to assess whether sample contamination has been introduced during field 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 actual water samples. The travel blank will help identify if sample contamination occurred during transport. A lab blank (sometimes called a pump 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 sample filtering and pumping process. If an eDNA backpack sampler is used to collect a sample, a lab blank will not be required.

For laboratory analysis, all water samples must be filtered in less than 2 days of sample collection, unless the samples were filtered onsite with a Smith-Root eDNA sampler. Unfiltered water samples will be filtered in a clean room using a GeoTech series II peristaltic pump and using 1.0–1.2 µm Whatman glass filters. After filtering each sample, all used filters (sometimes filtering requires 2 or more filters due to clogging issues) will be stored together in a sterilized 50 ml centrifuge tube then placed in a Whirl-Pak bag for cold storage (−20°C).

All 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 bath of 50% bleach solution (50% deionized water and 50% household bleach containing 8.25% hypochlorite) 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. These sample filtrates will be sent to the USFWS Conservation Genetics Lab in Anchorage for analysis.

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, the Biomeme Two3 portable real time thermocycler 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 from 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 that 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 positive detection results with a gillnet survey. To conclude that a waterbody supports northern pike, a specimen must be physically obtained.

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) were 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. Duplicate 1-liter samples will be collected if using a Biomeme Two3 device to process samples to account for its lower detection probabilities.

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. 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) and the donut-shaped areas 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, those 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:

$$P(D) = P(D \mid R_1) \times P(R_1) + P(D \mid R_2) \times P(R_2) + P(D \mid R_3) \times P(R_3) \quad (5)$$

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 ($P[D|R_i]$) 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

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

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 a 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 $S = 1, 2, \ldots, n$. Thus, the only change in the probability calculation for S sites is that the proportion of area represented by each subregion is now $S \times P(R_i)$. By another application of the law of total probability and definition of conditional probabilities:

$$\begin{aligned} P(D \text{ at } S \text{ sites}) &= P(D \mid R_1) \times S \times P(R_1) + P(D \mid R_2) \times S \times P(R_2) + P(D \mid R_3) \times S \times P(R_3) \\ &= S \times P(D) \end{aligned} \quad (7)$$

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, $S \times P[D]$) distribution. The probability of at least 1 detection at S sites is $1 - (1 - S \times P[D])^N$. This equation can then be set equal to the desired probability of detection and solved for S. Table 2 displays calculated eDNA sampling requirements for a variety of desired probabilities of detection and acreages assuming a population of 20 northern pike.

Table 2.–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 Nonnative Fish Discoveries

If nonnative fish are discovered in a waterbody, a variety of site-based data will be collected to aid in planning potential control actions and to better assess the ecological threat posed by the nonnative population. Data collection will focus on documenting the following baseline environmental and biological conditions and assessment of containment options.

Lake Mapping

In waterbodies where nonnative fish are newly detected, lake bathymetry data will be collected to produce volume estimates and a bathymetric map useful for planning fish control or eradication efforts. To collect bathymetry data, a boat-mounted Lowrance HDS chart plotter 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 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[6].

Water Quality Monitoring and Stream Discharge

Water quality data will be collected monthly for 1 year in waters where nonnative fish 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 winter.

If the waterbody containing nonnative fish 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 and from 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 complies with United States Geological Survey (USGS) specifications as described in Nolan and Shields (2000).

Invertebrate Surveys and Minnow Trapping

In addition to the biological data obtained for fish by the initial gillnet survey, macroinvertebrate and plankton surveys will be conducted to document the presence of dominant taxa in waters where northern pike are discovered. 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

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

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 nonnative fish 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 nonnative fish, including lands surrounding other waters linked to the infested 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 nonnative fish population will usually require a management response. When feasible, an appropriate initial response to an invasive fish detection is to immediately contain the population when 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.

Most 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 one-quarter-inch to one-eighth-inch mesh. Fabric mesh fyke nets will be shrouded in plastic-coated wire poultry fencing or hardware cloth to reduce animal damage that could compromise the integrity of the barrier. In small lake outlets and inlets with obvious elevation drops (e.g., beaver dams, spillways, perched culverts), often a simple modification to create a more abrupt vertical drop reduces successful upstream passage of northern pike (Massengill 2022). 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 may be effective at reducing upstream pike movement[7]. Ongoing research on northern pike jumping limits suggests barrier heights of 40 cm and greater are highly effective at preventing upstream movement (Taylor Cubbage, University of Fairbanks

[7] 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.

Fisheries Masters Student, American Fisheries Society (AFS) student presentation on results of northern pike jumping trials, 25 March 2022).

Eradication

The decision whether to implement a control or eradication action must weigh the consequences a nonnative 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 for northern pike (unpublished data, Soldotna ADF&G office), but only when the northern pike population is small (<30 individuals) and reproductive success is poor as indicated by the paucity of juvenile age classes during sampling efforts. Successful northern pike eradication using only gillnets has usually involved fishing the gillnets continuously under ice from ice-up until ice-out with gillnet effort representing 0.5 to 2.0 nets/acre (ADF&G unpublished data).

For infestations of nonnative fish where a quick-response action is not feasible, a formal restoration plan should be drafted to facilitate the scoping, permitting, eradication methods, or other control options to be administered.

Native Fish Restoration and Monitoring

Overview

The goal of the native fish restoration component of this project is to reestablish self-sustaining native fish populations that were present historically but were lost or severely reduced by nonnative fish impacts or by the management action (i.e., rotenone application) used to remove the nonnative fish. For open waters that allow natural mechanisms for recolonization of native fish to occur (i.e., migration), planned releases of native fish may be unnecessary for recovery. In waters where natural recolonization is unlikely to occur rapidly or at all, transplanting native fish may be required to successfully reestablish their populations.

Restoration

Recent ADF&G practices that accomplished wild native fish restoration have generally used 2 methods. The first is to collect native fish from the invaded waters, if they are still present in sufficient numbers, and temporarily hold them offsite in a safe area (net pen or small closed pond) until reintroduction can occur after eradication of the nonnative fish (Massengill 2017). The second method is to collect representative native fish from a different waterbody, ideally within the same drainage, and release them into the restored waters following the removal of the nonnative fish population (Massengill 2022). In exceptional circumstances, such as attempting to conserve a population of native fish at very low abundance that might be genetically unique, brood stock may be collected for propagating the population, as was done for Stormy Lake Arctic char (Massengill 2017). Brood stock can be used to collect gametes for producing hatchery-reared offspring that can be released into the wild to reestablish the population.

Rainbow trout (*Oncorhynchus mykiss*), Dolly Varden (*Salvelinus malma*), juvenile coho salmon (*Oncorhynchus kisutch*), and threespine stickleback (*Gasterosteus aculeatus*) have been the native species most impacted by invasive northern pike on the Kenai Peninsula due to their overlapping habitat preferences with northern pike. Past native fish restoration efforts have focused mostly on collecting these species for safe holding and later reintroduction (Massengill 2017, 2022).

Typically, the juvenile salmonid stocking rate goal for restoring waterbodies is based on ADF&G stocking density guidelines for hatchery-stocked rainbow trout fry of about 100 fish/acre for moderately fished lakes (Havens and Sonnichsen 1992). The frequency of salmonid stocking (a single year event vs. multiple years) will depend on fish-specific management goals, observed gillnet CPUE, and population size and age structure of stocked fish. Threespine stickleback reintroductions following northern pike removal have been successful in establishing self-sustaining populations in Kenai Peninsula waters after a single stocking event. Stocking goals for stickleback have varied and range from about 35 to greater than 75 fish per surface acre (Bell et al. 2016; Massengill 2022). Minnow trapping has proven to be the most efficient method for collecting juvenile native salmonids, stickleback, and sculpin but other methods such as backpack electrofishing, hook and line, and fyke traps, are useful.

Sampling restored native fish populations such as salmonids and stickleback using gillnets and minnow trapping on a schedule of every 3 years for a 9-year duration will help assess whether self-supporting native fish populations have been restored and if salmonid populations can support harvest. This project will provide the resources for native fish restoration efforts 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 are native fish restoration efforts underway following the removal of northern pike from the Tote Road Lake complex, Soldotna Creek drainage, and Miller Creek drainage. 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. Between 2019 and 2021, native fish (juvenile coho salmon and rainbow trout) were collected from Kenai River tributaries (e.g., Soldotna Creek, Slikok Creek, or Beaver Creek) and released into the Tote Road Lake complex. Annual stocking via collection and release will continue through at least 2023 (Table 3).

Table 3.–Annual native fish stocking goals for the Tote Road Lakes complex.

		Annual Tote Road Lakes stocking goals for 2022–2023	
Waterbody release site	Surface acres	Rainbow trout	Coho salmon
CC Lake	4	96	345
Crystal Lake	17	363	1,308
Freds Lake	6	133	478
G Lake	17	376	1,355
Hope Lake	27	585	2,108
Leisure Lake	11	242	870
Leisure Pond	2	37	133
Ranchero Lake	8	168	603
Total	92	2,000	7,200

Note: Stocking goals are met by collecting native fish from Kenai River tributaries and releasing at designated waterbodies.

It is hoped the stocked rainbow trout population will eventually become self-sustaining. 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, Canada). Beginning in 2022, native fish surveys will be done in the Tote Road lakes to assess whether the stocked 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 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 and dissection data will be recorded on Rite-in-the-Rain data forms (Appendices C1 and C2) 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 collected sample will be given a unique identifier name in addition to labeling the waterbody name and collection date. All sample data will be recorded in an Excel file on a laptop computer. These data will include the sample

collection and filtering date and time (collection and filter time will be the same when using a Smith-Root backpack sampler), numbers of filters used per sample, waterbody name, unique sample identifier, initials of the collector and the person doing the filtering (if using a Smith-Root backpack sampler, then the collector and filterer are the same), 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 chart plotter 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, 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 D1 and D2, 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 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. Considering this, only eDNA surveys

yielding greater than 1 positive eDNA detection will trigger the need for a follow-up gillnet survey. For eDNA detection survey results having ≤1 positive detection, no further sampling will be necessary unless new information or reliable reports indicate that invasive fish may be present in that waterbody.

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

When applicable, water quality data for 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 fish species, the fraction p_k of fish in length group k will be estimated as follows:

$$\hat{p}_k = \frac{n_k}{n} \tag{8}$$

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

$$\widehat{\text{var}}(\hat{p}_k) = \frac{\hat{p}_k(1-\hat{p}_k)}{n-1} \tag{9}$$

SCHEDULE AND DELIVERABLES

Dates	Activity
2022	
July–October	Tote Road Lakes: continue with native fish releases. East and West Mackey Lakes (Soldotna Creek Drainage): continue with native rainbow trout releases. NKPMA lake monitoring: conduct fish surveys (see Appendix A1). Assist Palmer ADF&G office with northern pike eradication at Fire Lake (Anchorage). Conduct monthly weir maintenance at Miller Creek. Conduct dreissenid mussel monitoring as requested by ADF&G Statewide Invasive Species Coordinator.
November–December	Continue monthly Miller Creek weir maintenance. Complete first draft of Special Report for the Miller Creek Restoration Project. Complete first draft of reporting materials (text and tables) for the Kenai Peninsula Nonnative Fish Control and Native Fish Restoration (lake monitoring, native fish releases, etc.) done during FY19-FY22, this will be inserted as a new section in the upcoming NKPMA AMR.
2023	
December–April	Finalize Miller Creek Restoration Special Report. If needed, seek funding, permits, and prepare treatment plan to remove any new nonnative fish populations discovered in the NKPMA during 2022. Manage existing project permits. Data analysis. Continue monthly Miller Creek weir maintenance.
May–September	Tote Road: continue with native fish releases. East and West Mackey Lakes (Soldotna Creek Drainage): continue with native rainbow trout releases. NKPMA lake monitoring: conduct fish surveys (see Appendix A1). Continue monthly Miller Creek weir maintenance. Conduct dreissenid mussel monitoring as requested by ADF&G Statewide Invasive Species Coordinator.
October–December	Assist with any rotenone application in Region II to remove nonnative fish as needed. Data analysis for FY23-24 field work.
2024	
January–April	Draft reports, treatment plans and operational plans as needed. Acquire permits and funding as needed to support removal of any nonnative fish populations discovered in FY2023.
May–June	Tote Road: continue with native fish releases. East and West Mackey Lakes (Soldotna Creek Drainage): continue with native rainbow trout releases.

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.

Michael Martz, Fishery Biometrician I
Duties: Provides guidance on study design, data analysis, reviews project operational plans and reports.

Kris Dent, 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.

Warren Wyrick, 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.

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APPENDIX A: LAKE INFORMATION SUMMARY

Appendix A1.–History of lake monitoring (2018–2021) with lake category threat classification for northern pike and year when a fish survey is due.

Site name	Lat, long	Invasive fish status	Management action (MA)	Year of MA	Criteria for threat class	Lake cat.[a]	Surface acres	Next fish survey due	Comments
City of Kenai Pond	60.581228, -151.279693	Fathead minnow removed	Eradicate via rotenone	2019	Medium: Fathead minnow present < 9 years	R	1	2023	Final survey due 2027
Warlfe Lake	60.290155, -151.365712	Pike removed	Eradication via gillnet	2017	Medium: Pike were present <9 years	R	3.4	2023	Final survey due 2026
Y Lake	60.391618, -151.170624	Nonnative trout removed	Eradication via gillnet	2021	Medium: : Nonnative rbt were present <9 years	R	3.1	2024	Final survey due 2030
Bird Pond	60.982084, -150.416570	No invasive fish detected	Gillnet survey	NA	Medium: within 0.5 mi of pike water	USP	14	NA	NA
Kuguyuk Pond	60.979667, -150.435943	No invasive fish detected	Gillnet survey	NA	Medium: within 0.5 mi of pike water	USP	14	NA	NA
Miller Creek	60.989477, -150.450037	Pike removed	Eradicate via rotenone	NA	Medium: Pike were present <9 years	R	NA	2024	Final survey due 2030
North Vogel Lake	60.996564, -150.412099	Pike removed	Eradicate via rotenone	2021	Medium: Pike were present <9 years	R	38	2024	Final survey due 2030
Sandpiper Lake	61.003679, -150.407882	No invasive fish detected	Gillnet survey	NA	Medium: within 0.5 mi of pike water	USP	80	NA	NA
Vogel Lake	60.988725, -150.430818	Pike removed	Eradicate via rotenone	2021	Medium: Pike were present <9 years	R	140	2024	Final survey due 2030
Vogel Pond	60.991316, -150.421049	No invasive fish detected	Gillnet survey	NA	Medium: within 0.5 mi of pike water	USP	7.7	NA	NA
Derks Lake	60.529538, -150.968375	Pike removed	Eradicate via rotenone	2014	Medium: Pike were present <9 years	R	37	2022	Final survey due 2023
Derks Pond	60.525671, -150.970904	Pike removed	Eradicate via rotenone	2014	Medium: Pike were present <9 years	R	2	2023	Final survey due 2026
East Mackey Lake	60.530484, -150.994013	Pike removed	Eradicate via rotenone	2014	Medium: Pike were present <9 years	R	100	2023	Final survey due 2023
Loon Lake	60.519361, -151.050500	Pike removed	Eradicate via rotenone	2017	Medium: Pike were present <9 years	R	21	2022	Final survey due 2026
Sevena Lake	60.551596, -150.968029	Pike removed	Eradicate via rotenone	2017	Medium: Pike were present <9 years	R	73	2022	Final survey due 2026
Soldotna Creek	60.520879, -150.957791	Pike removed	Eradicate via rotenone	2016	Medium: Pike were present <9 years	R	NA	NA	NA
Tiny Lake	60.519868, -150.992927	Pike removed	Eradication via gillnet	2011	Medium: Pike were present <9 years	R	5.5	NA	NA
Union Lake	60.521522, -151.031389	Pike removed	Eradicate via rotenone	2014	Medium: Pike were present <9 years	R	79	2022	Final survey due 2023

-continued-

Appendix A1.–Page 2 of 2.

Site name	Lat, long	Invasive fish status	Management action (MA)	Year of MA	Criteria for threat class	Lake cat.[a]	Surface acres	Next fish survey due	Comments
West Mackey Lake	60.527709, -151.009460	Pike removed	Eradicate via rotenone	2014	Medium: Pike were present <9 years	R	184	2022	Final survey due 2023
CC Lake	60.421778, -151.195102	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	4.5	2024	Final survey due 2027
Crystal Lake	60.424205, -151.190872	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	17	2024	Final survey due 2027
Fred's Lake	60.424147, -151.198286	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	6	2024	Final survey due 2027
G Lake	60.429900, -151.177922	Pike removed	Eradicate via rotenone	2018	Medium: Muskellunge were present <9 years	R	17	2024	Final survey due 2027
Hope Lake	60.421483, -151.187683	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	27	2024	Final survey due 2027
Leisure Lake	60.415164, -151.210241	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	11	2024	Final survey due 2027
Leisure Pond	60.419165, -151.207173	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	1.5	2024	Finalsurvey due 2027
Ranchero Lake	60.422857, -151.183571	Pike removed	Eradicate via rotenone	2018	Medium: Pike were present <9 years	R	7.7	2024	Final survey due 2027

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.

APPENDIX B: BIOMEME DNA EXTRACTION AND THERMOCYCLER PROTOCOL

Appendix B1.–Biomeme DNA extraction and thermocycler protocol excerpted from Sepulveda et al. (2018)[8].

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.

.

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APPENDIX C: NETTING SURVEY AND DISSECTION FORMS

Appendix C1.–Netting survey form.

NORTHERN PIKE SURVEY AND MONITOR NET FORM									Page _____ of _____
	WATERBODY: SAMPLERS:		SECTION # :			CAPTURE DEVICE:			START DATE: STOP DATE:
NET #	GPS LOCATION LAT./LONG. (dd.ddddd)	START TIME:	STOP TIME:	# Northern Pike	Bycatch species				Notes
	Air Temp: Precipitation: Wind: % Overcast: Barometric Pressure:		Water Temp: Max Depth/Flow: Substrate Type: % Vegetation:						Habitat Notes:

Appendix C2.–Catch dissection form.

NORTHERN PIKE DISSECTION FORM

Page _____ of _____

WATERBODY: SAMPLERS:				SECTION # :					START DATE: STOP DATE:				
Fish # (NP Only)	NET #	FISH SPECIES	FORK LENGTH (mm)	WEIGHT (g)	SEX (M, F, U)	Maturity (I or M)	Condition (G, R, S)	Stomach contents (Y/N)	STOMACH CONTENTs	Cleithra? (Y/N)	# Otos (0,1,2)	Whatman Card #	Whatman Fish #(1-10)

Common abbreviations for species. KS=king salmon; SS=silver salmon; RS=red salmon; CS=chum salmon; PS=pink salmon; UNS=unknown salmon; UNF=unknown fish; WF=white fish; LNS=long nose sucker; SB=stickleback; RT=rainbow; GR=grayling; NP=northern pike; BB=burbot; DV=dolly varden; SC=sculpin; PL=pacific lamprey; LCH=leech; PDB=predacious diving beetle, FRG=frog. Other catch could be: macro invertebrates, rodents, other mammals, birds.

APPENDIX D: WATER QUALITY AND DISCHARGE FORMS

Appendix D1.–Water quality sampling data form.

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 D2.–Stream discharge data form.

Station:							Date:		
Crew:									
GPS							River		
Coordinates:							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 Point**	**Mean Vertical**	**Cell % Flow**	**Flow (ft^3/s)**
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						