Regional Operational Plan SF.2A.2020.02

Operational Plan: Kenai Peninsula Invasive Northern Pike Monitoring and Native Fish Restoration

by

Robert Massengill

Robert N. Begich

and

Kristine Dunker

March 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.2020.02

OPERATIONAL PLAN: KENAI PENINSULA INVASIVE NORTHERN PIKE 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

March 2020

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Robert Massengill,
Alaska Department of Fish and Game, Division of Sport Fish,
43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669-8276, USA

Robert N. Begich,
Alaska Department of Fish and Game, Division of Sport Fish,
43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669-8276, USA

and
Kristine Dunker
Alaska Department of Fish and Game, Division of Sport Fish,
333 Raspberry Rd, Anchorage AK 99518-1599, USA

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SIGNATURE/TITLE PAGE

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Field Dates:	1 July 2018–31 October 2018; 1 April 2019–30 June 2019 1 July 2019–31 October 2019; 1 April 2020–30 June 2020
Plan Type:	Category II

Approval

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

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ABSTRACT

This project will conduct surveys to detect invasive northern pike and evaluate the success of efforts to eradicate them. Where northern pike have been successfully eradicated, this project will aid in restoring and monitoring native fisheries. Northern pike detection will be accomplished primarily by gillnet surveys using a standardized protocol that adjusts netting effort to lake surface area. Prioritizing which waters to survey for northern pike will be founded on a risk assessment. In waters where gillnetting is undesirable, environmental DNA (eDNA) detection methods may be used alone or in tandem with gillnetting efforts. When northern pike are detected in a waterbody, this project will collect the baseline environmental and biological data necessary to inform decisionmakers who will plan a control action. Native fish restoration will often be accomplished by collecting wild fish from a source area and releasing them to affected waters whenever natural recolonization is difficult or unlikely. Waters that had been previously stocked with hatchery fish prior to invasion by northern pike will resume hatchery stocking once the northern pike population is removed. Assessments of restored native fish populations will utilize gillnet and minnow trap surveys to produce catch per unit effort (CPUE) estimates and length frequency distributions for each species present.

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

INTRODUCTION

PURPOSE

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

BACKGROUND

In Alaska, south and east of the Alaska Range, northern pike are considered an invasive species (Figure 1) and are implicated in the decline of native fisheries throughout the region (Rutz 1999; Patankar et al. 2006; Sepulveda et al. 2015; Sepulveda et al. 2013; Glick and Willette 2016). There is evidence that northern pike prefer soft-finned juvenile salmonids over other available prey species in southcentral Alaska (Pankatar et al. 2006; Sepulveda et al. 2013). Consumption of native juvenile salmonids by introduced northern pike has also 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 evolved adaptations for predator-avoidance (Oswood et al. 2000). Prevalent shallow lake morphology throughout much of southcentral Alaska also offers limited deep-water refugia for northern pike prey because northern pike typically occupy habitats that are shallow and vegetated (Inskip 1982; Cook and Bergersen 1988).

Introduced northern pike were first documented on the Kenai Peninsula in the Soldotna Creek drainage in the 1970s (ADF&G unpublished). Over decades, subsequent dispersal and more illegal introductions resulted in northern pike occurring in at least 24 Kenai Peninsula waterbodies (Figure 2). Northern pike were first detected in 11 of these waterbodies since 2000, however the date of these introductions remains unknown. Kenai Peninsula northern pike have reduced or eliminated wild and hatchery-produced fish populations from some lakes (Begich and McKinley 2005; Begich 2010; McKinley 2013; Massengill 2014a; Massengill 2014b). Beginning in 2008, the Alaska Department of Fish and Game (ADF&G) initiated a program to

eradicate northern pike from the Kenai Peninsula. Initial efforts focused on eradicating northern pike from landlocked lakes (Massengill 2014a; Massengill 2014b) followed by eradication efforts in progressively complex and open waterbodies within the Swanson River and Soldotna Creek drainages. Currently, the Tote Road Pike Lakes (TRPL) harbors the last known northern pike population on the Kenai Peninsula.

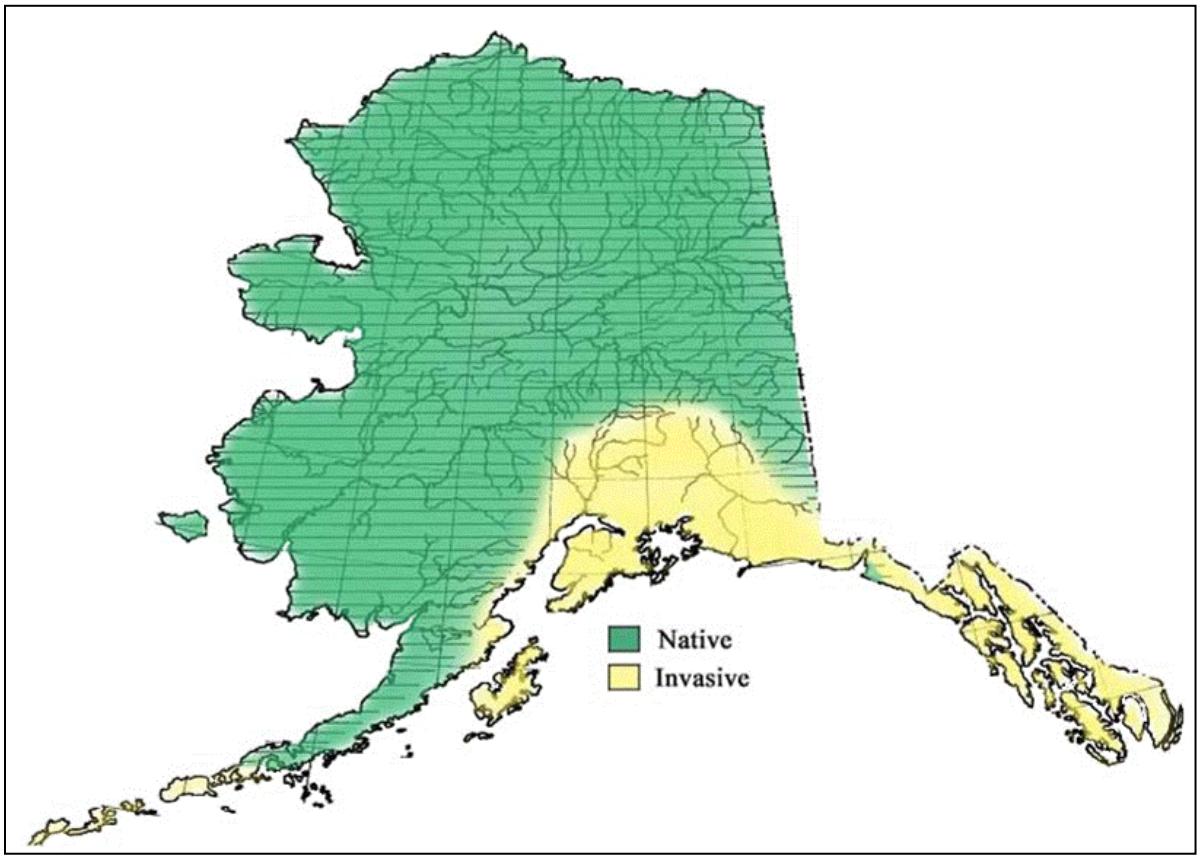

Figure 1.–Map of the native and invasive ranges of northern pike in Alaska

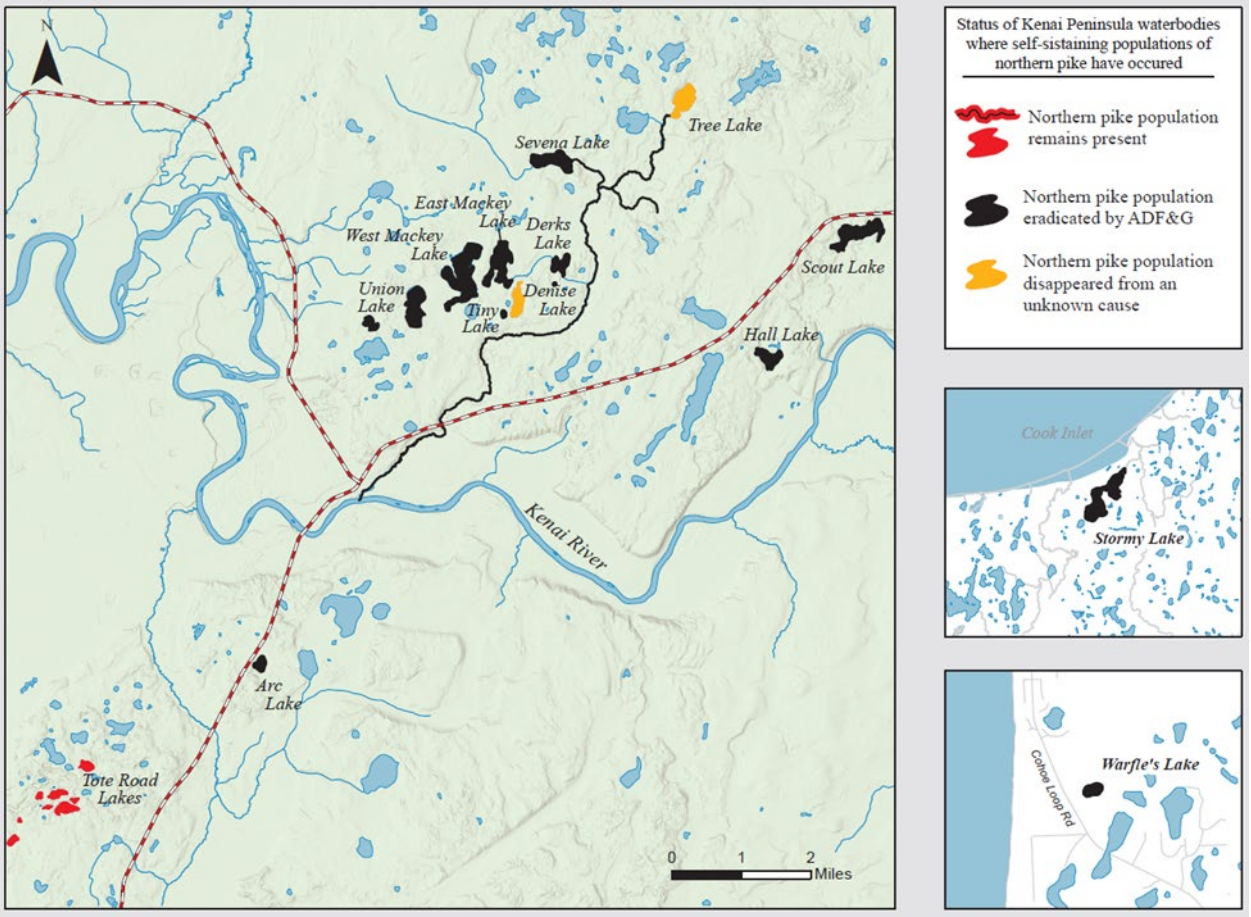

Figure 2.–Map showing the status of Kenai Peninsula northern pike waters.

OBJECTIVES

PRIMARY OBJECTIVES

1) During the open water season each year between July 1, 2018 and June 30, 2020, survey a minimum of 8 high threat waters that are void of native salmonids to detect the presence of northern pike such that the probability of detection is 0.80 given the population is at least 20 northern pike >300 mm fork length.
2) During the open water season each year between July 1, 2018 and June 30, 2020, survey a minimum of 4 high threat waters that have native salmonids present to detect the presence of northern pike such that the probability of detection is 0.50 given the population is at least 20 northern pike >300 mm fork length.

SECONDARY OBJECTIVES

1) Collect and analyze northern pike eDNA samples for all waters where gillnet surveys are undesirable or insufficient to meet precision criteria for Objectives 1 or 2.
2) Map all waters where new northern pike discoveries are made to verify surface acreage and volume.

3) Measure water quality (temperature, dissolved oxygen, pH, specific conductance) monthly for 1 calendar year from any waters where new northern pike discoveries are made.
4) In every waterbody where northern pike have been removed, at least once every 3 years, for a 6-year period following their removal, calculate the mean gillnet and minnow trap catch per unit effort (CPUE) of all collected salmonids.
5) In every waterbody where northern pike have been removed, at least once every 3 years for a 6-year period following their removal, measure and record the fork length (FL) of all salmonids collected in gillnets and minnow traps to determine length composition.
6) Inventory dominant invertebrate taxa from any waters where new norther pike discoveries are made.
7) Prepare a northern pike control and eradication plan for all waters where new northern pike discoveries are made.
8) When feasible, implement a quick-response northern pike control and eradication plan as soon as practical.
9) Collect wild native fish and release them into waters where restoration of the native fish assemblage is appropriate following the removal of invasive northern pike.
10) Estimate the fork length composition of all salmonid species present in surveyed lakes in 50 mm increments during FY19 and FY20.

METHODS

STUDY AREA

The study area encompasses the entire Northern Kenai Peninsula Management Area (NKPMA) with an emphasis on waters categorized as high threat for the presence of northern pike. In general, high threat waters include the Soldotna Creek drainage, Moose River drainage, Swanson River drainage and any waters where northern pike have ever been confirmed.

STUDY DESIGN

Primary Objectives 1 and 2

The primary objectives are to determine northern pike presence in Kenai Peninsula waters considered most at risk for invasion primarily using gillnet surveys. Appendix A1 provides a list of unrestored lakes where no invasive fish have been confirmed or eradicated that could be surveyed for northern pike, and Appendix A2 lists the criteria for ranking the threat of northern pike presence in selected waters on the Kenai Peninsula.

Gillnets are frequently used for the detection and suppression of invasive northern pike in Alaska (Rutz et. al. *In prep* a, b; Glick and Willette 2016; Sepulveda et al. 2013; Massengill 2010). Gillnets are most effective when fished in the optimal habitat for northern pike which typically includes slow flow or lentic waters, side sloughs, embankments, and densely vegetated littoral zones (Inskip 1982). This study will conduct detection surveys (primarily gillnetting but potentially assisted with eDNA surveys) to assess the presence or absence of northern pike. Different survey protocols will be followed according to which of 3 categories the waterbody is assigned. Definitions for the waterbody categories are as follows:

Restored (R): A Restored waterbody is one where northern pike eradication has been conducted. All restored waters must have a detection survey, satisfying precision criteria for Primary

Objective 1, completed within 6 months of the eradication effort to assess the success of the eradication effort (objective). Additional gillnet and minnow trap surveys will occur at least once every 3 years for a 6-year period following eradication with enough effort to satisfy Secondary Objective 4 requirements. These subsequent surveys are designed to monitor restored native fish populations. A survey schedule for R waters is found in Appendix B1.

Unrestored–Salmonids Present (USP): A USP waterbody is one where northern pike presence is unconfirmed and a survey to detect them is warranted. The waterbody is also known to contain salmonids so the netting effort will be reduced to satisfy precision criteria for Primary Objective 2.

Unrestored–Salmonids Absent (USA): A USA waterbody is one where northern pike presence is unconfirmed and a survey to detect them is warranted. The waterbody is not known to contain salmonids so the netting effort will be sufficient to satisfy precision criteria for Primary Objective 1.

Unrestored waterbodies will also be given a threat ranking that prioritizes them for how quickly they are surveyed. There are 3 threat rankings (high, medium and low). A threat rank is assigned if just 1 criterion for that rank is met (Appendix A2). In instances where a waterbody meets criteria for 2 different rankings, the waterbody will be assigned the highest ranking of those it qualifies for. For instance, if a lake satisfies criteria for both a medium and high threat waterbody, it will be assigned a high threat waterbody. When a waterbody receives a threat ranking, that waterbody must be surveyed within the time period described in Appendix A2. All northern pike and salmonids caught in any survey will be measured for fork length (FL).

Gillnet Sampling Effort for Primary Objectives 1 and 2

Gillnet surveys designed to detect northern pike presence will be conducted with enough effort to satisfy precision criteria for Objective 1 or 2 according to category (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%, respectively, for each objective, we utilized data from past northern pike removal experiments.

Between 2005 and 2010, ADF&G conducted 12 removal experiments with northern pike populations on the Kenai Peninsula using similar gillnetting methods. Data collected from these experiments included catch C_{ij} and effort E_{ij} (in units of net-hours per surface-acre) for sample i $(i = 1, \dots, s)$ and experiment j $(j = 1, \dots, 12)$. Populations were assumed to be closed except for fish caught, 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):

$$CPUE_{ij} = K_j N_j - K_j C_{ij}^* \tag{1}$$

where

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

and

$$C_{ij}^* = \sum_{k=1}^{i-1} C_{kj} \; for \; (i \; in \; 2, \dots, s+1) \; with \; C_{1j}^* = 0 \tag{3}$$

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

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 (R Development Core Team 2011). 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 the purpose of estimating the probability of detecting small pike populations in future removal experiments.

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 3.

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

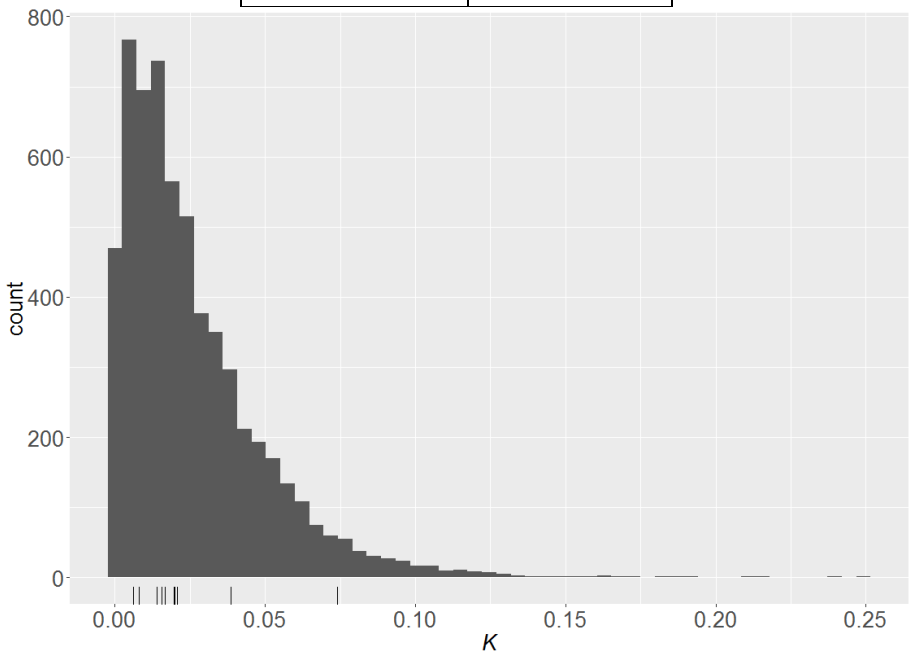

Figure 3.–Prediction distribution for *K*, the average probability a fish is captured in a new removal experiment with 1 unit of effort.

Note: Tick marks along the *x*-axis show the median values for K_i which is 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 pike of size N as a function of net-hours per acre (E) is as follows:

$$D_p = \exp(-KE)^N \quad (4)$$

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 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 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
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

Based on lake surface acreage and status of salmonid presence the minimum gillnet survey effort (hours and number of nets) will be determined using the detection probabilities found in Table 3 such that the probability of failing to detect a northern pike population of at least 20 individuals will be 0.20 for lakes without salmonid presence (Objective 1) and 0.50 for lakes with native salmonid presence (Objective 2).

Gillnets used for northern pike surveys will be identical to those used in the 12 removal experiments mentioned previously. The gillnets are 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. Each net is 120 ft long, 6 ft deep, with six 20 ft wide panels of mesh in the following sizes tied with number 9 twine: 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. Gillnets will be deployed in vegetated littoral areas and fished continuously as practical. When continuous gillnetting 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 tend the nets

continuously, and at a minimum, nets will be tended daily. If a northern pike is captured in a waterbody where the sole purpose of the survey was to determine northern pike presence, the netting will be halted if bycatch becomes a concern.

Substitute eDNA Sampling

Background

Environmental DNA (eDNA) is the DNA from an organism shed into the environment. Organisms may shed their DNA nearly 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., in about a 1,000 ml bottle), which are then concentrated by filtration and the filtrate 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 large bycatch concerns), eDNA detection methods may be used to supplement reduced netting effort or to supplant gillnetting effort altogether. In those situations, an effort will be made to achieve similar precision criteria listed for Objectives 1 and 2.

ADF&G and the United States Fish and Wildlife Service (USFWS) developed and tested several genetic markers for use in detecting northern pike eDNA, which resulted in the selection of a preferred marker (*EluCOI*) located in the cytochrome oxidase 1 gene of mitochondrial DNA (Olson 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 (ADF&G unpublished[1]). Processing of the eDNA samples was done by USFWS Conservation Genetics Lab in Anchorage using a benchtop qPCR laboratory method.

A portable device called Biomeme Two[2] 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 takes 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 (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 reduce the risk of contaminating or degrading eDNA samples (Wolt et. al. 2015; Carim et. al 2014; Laramie et. al. 2015). 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 organism is 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

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

[2] Product names in the publication are included for completeness but do not constitute product endorsement.

amplification), a lack of target DNA in the sample, or degradation of the eDNA in the sample prior to processing (Evans 2017).

A number of eDNA samples (determined following methods in the eDNA Sampling Effort section) will be collected either by foot travel along the shoreline or from a boat. Great care will be taken to ensure outer gear worn by collectors (waders, life jackets) and collection equipment (swing sampler, transport coolers) have been decontaminated with a 10% bleach solution allowing for a 10-minute soak before rinsing with tap water. All sample containers will be purchased presterilized 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 used for sampling.

To reduce the risk of eDNA transfer by boat, 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[3]. When samplers collect 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 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 1,000 ml surface water grab samples collected in either a sterilized 1-liter Nalgene bottle or Whirl-Pak bag. Duplicate samples will be collected if sample processing is done using a Biomeme Two3 thermocycler to compensate for its lower detection efficiency 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 GPS.

Each day of sampling, we 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 sample with deionized water. One control, called a field blank, will help assess whether sample contamination is 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 is 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 is introduced during the filtering processes.

Within 2 days of sample collection, all samples will be filtered using a GeoTech series II peristaltic pump and 0.45 µm nitrocellulose membrane filters. After filtering, all filters from each unique sample will be stored together in a vial sterile Whirl-Pak bag and placed into cold storage. Each vial or Whirl-Pak bag will be given a unique sample ID. All field water sampling,

[3] United States. Fish and Wildlife Service. 2018. Quality Assurance Project Plan. https://www.fws.gov/midwest/fisheries/eDNA/documents/QAPP.pdf

equipment decontamination, sample filtering, and storage follow established eDNA protocols (Laramie et. al. 2015). These decontamination procedures will include: 1) wearing new nitrile or latex gloves each time a new sample is handled, 2) using only sterilized tweezers to handle filters, and 3) sterilizing all filtering assemblies prior to use in a 50% bleach solution (50% deionized water: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, the pump and associated work area will be sprayed with a 10% bleach solution or DNA AWAY and then the space will be wiped dry with a sterilized tissue. Filtered samples will be placed on ice until processed by the Biomeme Two3. Samples that are collected and filtered will be processed at the Soldotna ADF&G office. Sample filtrate will be extracted and analyzed using methods described by Sepulveda (2018) and summarized below.

Filtrate extraction will be done with a Biomeme Field Test Kit which is designed for use only with 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 C1). The purified DNA is then stored in the elution buffer until qPCR.

A Biomeme Two3 portable real-time thermocycler will be used to analyze DNA extract for presence of northern pike DNA. The Biomeme Two3 has 2 channels (FAM and Cy5) and 3 wells so duplicate reactions can be run for 3 samples simultaneously.

We will pipette 20 µl of the purified DNA into each well, which is prefilled with a lyophilized assay that includes the EluCOI marker specific to norther pike DNA (Olsen et al. 2015). Biomeme's recommended thermocycler protocol for this assay is found in Appendix C1.

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 (Cy5 channel). Samples that are positive for northern pike DNA will be those which amplified. Samples determined to be inhibited will be those for which the IPC failed to amplify.

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 to 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 we conclude that northern pike are present in a waterbody.

Prior to collecting eDNA samples, approximate sample locations will be numbered and identified 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 sufficiently robust to detect northern pike populations with low abundance, we relied on the estimated mean detection probabilities of northern pike eDNA. The detection probabilities were estimated from the results of replicate 1-liter water samples collected at 1, 10, and 40 meters from a single, caged, live northern pike and were estimated to have a 0.89, 0.57, and 0.27 probability of detection, 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 lake acreage is known and no gillnet sampling occurs. Calculations will be based on three assumptions: 1) fish are randomly distributed throughout the sampling area, 2) there are no false detections, and 3) the probability of detection beyond 40 meters is zero, since no estimates are available for this region.

To account for differences in the probability of detection due to the distance between a northern pike and the sample site, we will divide the 40-meter circle around each sample site into 3 distinct subregions. These subregions will be the circular areas less than 1 meter, between 1 and 10 meters, and between 10 and 40 meters from the sample site, which we will label subregions 1, 2, and 3, respectively. Because previous work estimated the probability of detection at 1, 10, and 40 meters, we will use their estimates as conservative proxies for the probability of detection within the respective subregions.

If P represents the probability of detecting a northern pike, D is the event a northern pike is detected, and R_i is the event that a single northern pike is present in subregion i for $i = 1,2,3$, we note by the law of total probability and the definition of conditional probabilities, the following relationship can be used to calculate the probability of detection:

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

Thus, the probability a northern pike is detected is equivalent to the probability a northern pike can be detected, given it is within a subregion, times the probability it is in the subregion summed over all subregions. The probability a northern pike can be detected in subregion i, given it is present in the subregion, $\mathrm{P}(\mathrm{D} \mid R_i)$, is 0.89, 0.57, or 0.27 for subregions 1–3, respectively. Under the assumption that northern pike are randomly distributed, the probability a northern pike is present in a subregion is the proportion of total area represented by that region:

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

where the fixed areas of the subregions are …..for subregions 1–3, respectively, and the total surface area of the lake is taken from Appendices A1 or B2.

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 northern pike is at 1 of S sample sites for $S = 1, 2, \ldots, n$. Thus, the only change in our probability calculation for S sites is that 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, the probability of detection at S sites is as follows:

$$P(\text{detection at } S \text{ sites}) = P(D \mid R_1) \times S \times P(R_1) + P(D \mid R_2) \times S \times P(R_2) + P(D \mid R_3) \times \mathrm{S} \times P(R_3) = S \times P(D) \quad (7)$$

Because the N pike are assumed to be randomly distributed, the number of northern pike that are successfully detected follows a Bin[N, S × P(D)] distribution. The probability of at least 1 detection at S sites is $1 - \left[1 - \mathrm{S} * \mathrm{P(D)}\right)]^N$. We then set this expression to the desired probability of detection and solve for S. Table 4 displays calculated eDNA sampling requirements for a variety of desired probabilities of detection and acreages assuming a population of $N = 20$ northern pike.

Table 4.–Number of samples required to achieve the desired probability of detection.

	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
0.90	4	8	16	23	31	61
0.95	4	10	20	30	39	78

Protocol for New Northern Pike Discoveries

Site Evaluations

If northern pike are discovered in a waterbody, data will be collected to aid in planning a control and 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 new waterbodies where invasive northern pike are detected, lake bathymetry data will be collected to produce volume estimates and a bathymetric map useful for planning northern pike control and eradication efforts. To collect bathymetry data, we will use a boat-mounted Lowrance HDS chartplotter and transducer to record x, y, z mapping data. Mapping will begin with the lake perimeter as nearshore as feasible followed by a repeat of the perimeter circuit about 20 m farther offshore. After 2 complete lake perimeter circuits, 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 can be run by dividing the lake into sections. Typically, transects lines should be 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 in Appendix D1.

Water Quality Monitoring

Water quality data will be collected monthly for 1 year following the discovery of northern pike. 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

start near the deepest area of each lake and thereafter will be collected in 1-meter increments upwards 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 and by drilling through the ice during the winter.

Stream Discharge

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 following the discovery of northern pike. In addition, stream discharge measurements will be collected monthly from streams linking the infested waterbody to other waterbodies, from the headwaters to the drainage's terminus at the mainstem of a 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 according to the ADF&G Statewide Aquatic Resources Coordination Unit training course titled "How to Measure Stream Discharge" that complys with United States Geological Survey (USGS) specifications as described in Nolan and Shields (2000).

Invertebrates Surveys

In addition to gillnet surveys, macroinvertebrate and plankton surveys will be collected in waters found to have northern pike to document the dominant taxa and their relative abundance. In each lake, zooplankton evaluations will be made by replicate vertical tows using a 0.5-meter diameter Wisconsin net with 153 µm mesh at 2 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 the lowest reasonable degree of taxonomic resolution and relative abundance will be determined by counts. 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. Attempts will be made to visually locate and collect freshwater mussels and snails opportunistically. 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.

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

Minnow Trapping

At each waterbody where northern pike are discovered, 5 minnow traps baited with salmon eggs will be fished continuously for at least 1 hour in an attempt 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.

Land Status

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 Actions

Containment

Based on gillnet survey results and an assessment of connectivity to other waters, the physical detection of a northern pike population will require an appropriate control action. When feasible, an initial response to a northern pike detection is to immediately contain the population. This response aligns with universal early detection rapid response (EDRR) protocol for control of invasive species as found online at: https://www.invasive.org/edrr/index.cfm. In most instances, containment of northern pike 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 one-quarter-inch to one-eighth-inch mesh. Fyke nets should 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 northern pike passage. Little information is available quantifying the jumping ability of northern pike but anecdotal information suggests vertical drops greater than 0.3 feet are effective to contain upstream movement (Diebel 2013).

Eradication

The decision to implement a control or eradication action must weigh the potential or realized consequences the northern pike population poses to ecological and economic concerns. When 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 emergency exempted. In small closed lakes (<40 acres) intensive under-ice gillnetting has also proven to be an effective eradication alternative (unpublished data, Soldotna ADF&G) but only when the northern pike population is small (<30 individuals) and reproduction success is low as noted by the lack of multiple age-classes or juvenile northern pike during sampling efforts. Successful eradication using gillnets alone has involved fishing gillnets continuously from fall ice-up until spring ice-out with gillnet densities of 0.5–2.0 nets/acre (ADF&G unpublished data).

For infestations where a quick-response eradication plan is not possible, a 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 native fish restoration is to reestablish self-sustaining native fish populations historically present but lost or severely reduced by the presence of invasive northern pike. For waters that are sufficiently open to allow natural recolonization of native fish via migration and dispersal, planned releases of native fish may not be necessary for fish populations to recover. Conversely, transplanting or stocking fish may be required to successfully restore fish to some waters where natural recolonization is impeded or impossible.

Recent ADF&G practices to accomplish wild native fish restoration has generally been accomplished by 2 methods. The first is by collecting native fish from the pike-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 posteradication (Massengill 2017). The second method is by collecting representative native fish from a different waterbody, ideally from within the same drainage, and releasing them into waters following the removal of northern pike (ADF&G unpublished manuscript[4]). In rare circumstances, native fish broodstock may be collected from the pike-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 and collecting enough individuals for reintroduction is impractical, particularly if the population is suspected of being genetically unique based on phenotypic or morphological traits (Massengill 2017).

Rainbow trout (*Oncorhyncvhus mykiss*), Dolly Varden (*Salvelinus malma*), juvenile coho salmon (*O. kisutch*), and threespine stickleback (*Gasterosteus aculeatus*) are the most common native species impacted by invasive northern pike on the Kenai Peninsula. Past native fish restoration efforts have focused mostly on collecting these species for reintroduction (ADF&G unpublished data). Maximum annual stocking densities for juvenile salmonids for this project will be based on the recommended 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 characteristics of the lake, management goals, and biological information gathered from poststocking fish surveys. Previous threespine stickleback reintroductions in Alaska following northern pike removal have been successful (Bell et al. 2016). These threespine stickleback introductions typically have a stocking goal of releasing several thousand reproductively mature sticklebacks into a waterbody during 1 stocking event (Bell et al. 2016).

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

This project will provide the resources and support for native fish restoration efforts for Restored waters (defined above) as needed. Native fish restoration efforts will typically be planned and described in a "treatment plan" that is developed specifically for each eradication project. Currently, there is an active treatment plan for eradicating northern pike and restoring native fish species in the Tote Road area south of Soldotna titled "Tote Road Pike Lakes Restoration: Northern Pike Eradication" archived at the Soldotna ADF&G office.

Survey Effort in Restored Waters

In every Kenai Peninsula waterbody where northern pike are removed and native fish populations restored, at least once every 3 years, for a 6-year period following the removal of northern pike, we will conduct gillnet and minnow trap surveys to monitor native fish populations. To avoid excessive impacts to restored native fish populations, gillnetting effort will be at the discretion of the project leader. In most instances, not more 24 hours of cumulative gillnetting will be applied to each lake being surveyed. Minnow trap surveys will be conducted such that 5 minnow traps (18-inch long galvanized mesh screen traps with funnel entrances at

[4] Soldotna Creek Drainage Restoration: Northern Pike Eradication (2013). Unpublished and located at the ADF&G Soldotna Office

both ends) baited with salmon eggs are fished continuously for at least 1 hour each in nearshore locations near protective cover such as weed beds, snags, or tributary mouths.

Estimating Length Composition

In lakes surveyed with gillnets, all captured fish will be sampled for length. Length composition by size class (50 mm increments) will be estimated for all salmonid species present using the method described by Thompson (1987). Accordingly, confidence intervals will only be created when enough sample sizes are obtained.

Estimating CPUE

For Secondary Objective 4, mean CPUE by gear type (gillnet and minnow trap) will be calculated using standard statistical methods.

DATA COLLECTION AND REDUCTION

Gillnet and Minnow Trapping

All fish captured in gillnets will be identified by species, counted, and measured for fork length (FL; tip of nose of fork of tail). Data will be recorded on Rite-in-the-Rain notebooks and later transcribed into an Excel file. We will release all native fish species, if alive, but will dispatch all captured northern pike on site and will record their sex, maturity, stomach contents, and collect cleithra bones for determining age and otoliths for possible determination of otolith microchemistry. We will record each net's set and pull date and time and the collector's initials. Set locations will be recorded on a handheld GPS and labeled with a unique identifier.

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 and labeled with the waterbody name and collection date. During sample filtration, an array of 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, waterbody name, unique sample identifier, initials of the collector and 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 concluding the mapping survey, the mapping data, which is stored by the Lowrance chartplotter as an .sl2 file on an external memory SD card, can be downloaded to a computer and uploaded to a cloud-based subscription service (BioBase). BioBase will run algorithms on the data and generate a report that includes the lake volume, surface area estimates, and a printable bathymetric map.

Water Quality Monitoring

All data will be recorded on data sheets in the field (Appendix E1) and later entered into an Excel file to graph seasonal patterns.

Stream Discharge

All data will be recorded on data sheets in the field (Appendix E2) and later entered into an Excel file.

Invertebrate Surveys

During invertebrate surveys, invertebrates will be collected in the field and later identified down to the lowest taxonomic level as possible and entered into an Excel file in the lab. 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 of northern pike. If no northern pike are caught, we will conclude either no northern pike are present or that the population is less than 20 individuals. For lakes surveyed with gillnets 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 gillnets under Objective 2 precision criteria, the probability of failing to detect a population of 20 individuals will be less than 0.50.

eDNA Sampling

Analyzing 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 where only a single sample tested positive (N = 7) have never been associated with a live northern pike population following subsequent gillnet surveys. Therefore, only eDNA surveys yielding greater than 1 positive eDNA detection will trigger the need for a follow-up gillnet survey. For instances when there is a complete lack of positive eDNA detections in a survey, we will conclude the probability of failing to detect a northern pike population of 20 individuals is less than 0.20.

CPUE

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

Length Composition

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

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

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

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

Lake Mapping

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

Water Quality and Stream Discharge Monitoring

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

Invertebrate Surveys

After identification of taxa identified in all 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.

SCHEDULE AND DELIVERABLES

Dates		Activity
2018		
	Jul–Oct	Conduct pretreatment gillnet surveys at 8 lakes where salmonids are not present (Tote Road Lakes), conduct minnow trap CPUE surveys in Soldotna Creek and Sevena Lake. Conduct invertebrate survey at Hope Lake in July. Conduct gillnet survey at Derks Lake and McLain Lake where salmonids are present.
	Oct–Nov	Conduct native fish (Arctic char) gillnet and (or) entanglement net survey at Stormy Lake.
	Jul–Dec	Collect monthly water quality and stream flow measurements at Tote Road lakes.
2019		
	Jan–Jun	Collect monthly water quality and stream flow measurements at Tote Road lakes.
	May–Jun	Conduct gillnet surveys at 4 lakes with salmonids present.
	May–Oct	Conduct native fish restoration at Tote Road lakes. Conduct gillnet surveys in up to 8 lakes with no salmonids present and up to 4 lakes with salmonids present. Conduct invertebrate surveys at Hope Lake in July.
2020		
	May–Jun	Conduct native fish restoration at Tote Road lakes. Conduct gillnet surveys at 4 high threat lakes with salmonids present.

RESPONSIBILITIES

Robert Massengill, Fishery Biologist II

Duties: Project biologist; coordinates all field logistics, purchasing, and project implementation; enters and manages data; prepares project report and presentations to the public

Robert Begich, Fishery Biologist III

Duties: Provide oversight and make recommendations on study designs and project plans; assist with data analysis and project reporting; coordinate and assist with the completion of project deliverables; assist with field work as needed.

Kristine Dunker, Fishery Biologist III

Duties: Provide guidance on study design; review project operational plans and reports; assist with field work as needed.

Ben Buzzee, Biometrician I

Duties: Provide guidance on study design; review project operational plans and reports.

Jerry Strait, FWTIII

Duties: Assist with all aspects of field work and sampling; record and edit raw data; perform basic maintenance and inventory of equipment and supplies.

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APPENDIX A: THREAT RANKING OF UNRESTORED WATERBODIES

Appendix A1.–Threat ranking of unrestored waterbodies that could be surveyed for northern pike.

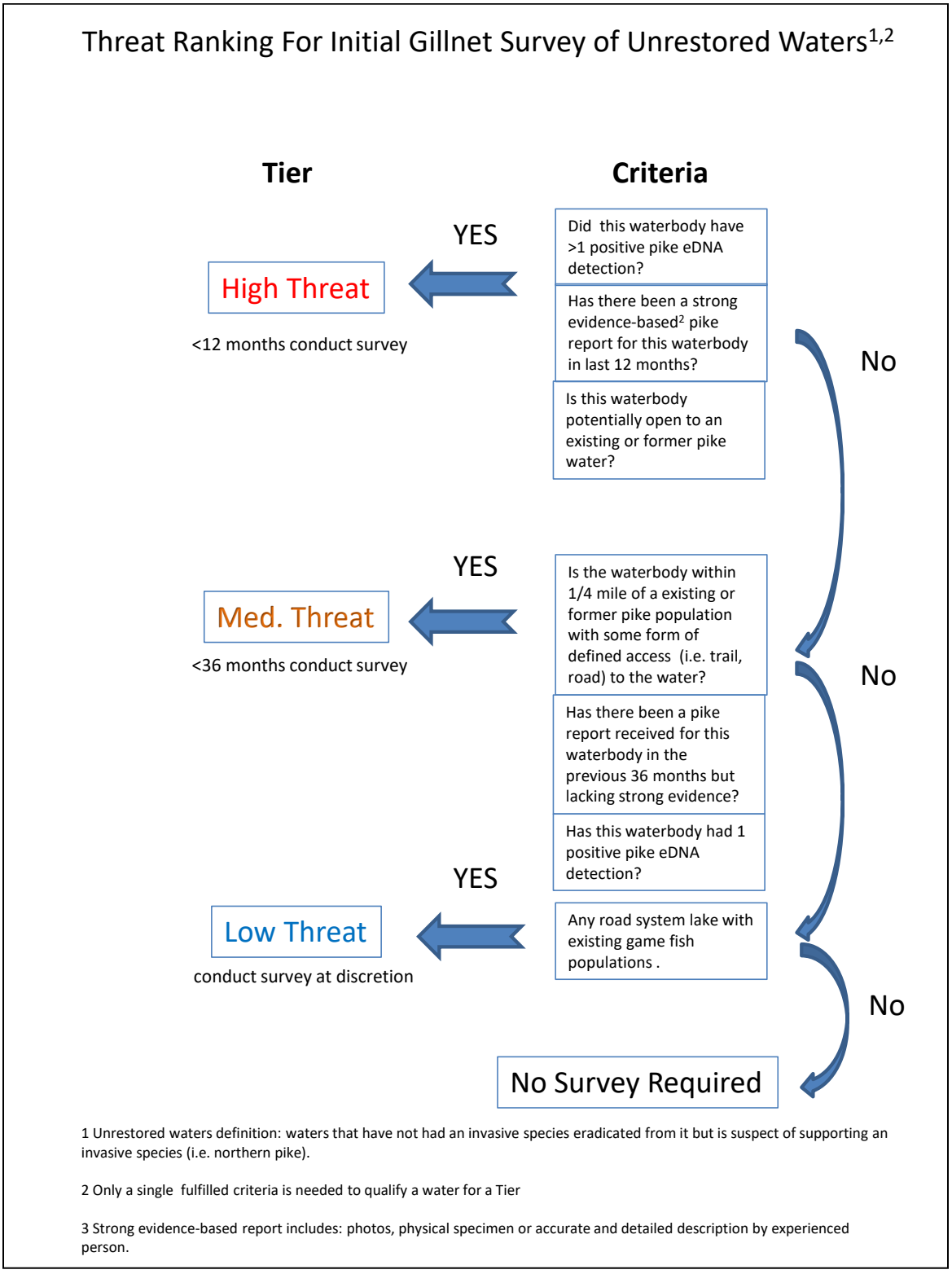
Drainage	Waterbody name	Salmonids present?	Description	Latest survey results	Threat ranking	Surface acres	Surface hectares
Miscellaneous closed lakes							
	Independence Road Ponds	No	No information	No information	Low	2	0.8
Kenai River							
	Hall Lake	No	Intensively netted in 2011 when last pike was confirmed	No pike caught in net survey 2017	Medium	42.7	17.3
Moose River							
	Afonsai Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2001	Medium	112	45.3
	Clam Lake	Yes	Periodic pike rumors in area	A single positive eDNA detection in 2013	Medium	357	144.5
	Engumen Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2001	Medium	32	12.9
	Imeri Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2001	Medium	16.5	6.7
	Kelly Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2014	Medium	146	59.1
	Peterson Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2001; a single positive eDNA detection in 2013	Medium	92	372
	Watson Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2001; a single positive eDNA detection in 2014	Medium	58	23.5
Swanson River							
	McLain	Yes	No previous reports of pike	Four positive eDNA detections in 2013	High	281	113.8
	Crane Lake	Yes	Periodic pike rumors in area	No pike caught in net survey 2017	Medium	51	20.6
	Middle Crane Lake East	Yes	Periodic pike rumors in area	No pike caught in net or eDNA survey 2017	Medium	36	14.6

-continued-

Appendix A1.–Page 2 of 2.

Drainage	Waterbody name	Salmonids present?	Description	Latest survey results	Threat ranking	Surface acres	Surface hectares
Swanson River							
	Middle Crane Lake West	Yes	Periodic pike rumors in area	No pike caught in net or eDNA survey 2018	Medium	38	15.4
	Salmo Lake	Yes	Periodic pike rumors in area	No pike caught in net or eDNA survey 2017	Medium	125	50.6
	Salmo Pond	Yes	Periodic pike rumors in area	Never surveyed	Medium	30.6	12.4
	Snipe Lake	Yes	Periodic pike rumors in area	No pike caught in net or eDNA survey 2017	Medium	128	51.8
Tote Road drainage	A Lake	No	150 yards from known pike lake with intermittent surface connection	No pike caught in net survey 2017	Medium	5.5	2.2
	Lingren Pond	No	Surface linkage to Leisure Lake that has a pike population	No surveys conducted	High	1	0.4
	Orphea Lake	No		No pike detected in net survey; single eDNA detection 2017	Medium	53	21.4
	Oxford Ave. Pond	No	Unverified report of pike capture in 2012	No pike captured in gillnet survey 2018	Medium	3.5	1.4
Miller Creek	Vogel Lake	Yes	Angler reported catching a pike in lake in fall of 2018	N/A	Medium	120	48.6

Appendix A2.–Unrestored waterbody threat ranking flowchart.



1 Unrestored waters definition: waters that have not had an invasive species eradicated from it but is suspect of supporting an invasive species (i.e. northern pike).

2 Only a single fulfilled criteria is needed to qualify a water for a Tier

3 Strong evidence-based report includes: photos, physical specimen or accurate and detailed description by experienced person.

APPENDIX B: SURVEYS FOR RESTORED WATERBODIES

Appendix B1.–Survey schedule for restored waterbodies.

Survey Schedule for Restored Waters[1]

Treatment success evaluation: complete treatment success evaluation (gillnet survey) ≤ 6 months post-TX.

Post-stocking monitoring[1]: complete fish surveys (gillnetting and minnow trapping) at least every 36 months post-stocking for at least 72 months (2 cycles).

1 Post-stocking waters are waters where an invasive fish populations was successfully removed and a replacement fishery using wild native fish or hatchery-reared fish was restored.

Appendix B2.–List of restored waterbodies requiring gillnet surveys to monitor native fish restoration.

Drainage	Waterbody name	Salmonids present?	Description	Latest survey results	Surface acres	Surface hectares
Closed Lake						
	Arc lake	Yes (hatchery stocked)	Treated with rotenone in 2008 when last pike were confirmed	No pike caught in spring 2009 net survey	18	7.3
	Scout Lake	Yes (hatchery stocked)	Treated with rotenone in 2009 when last pike were confirmed	No pike caught in spring 2010 net survey	85	34.4
	Warfle Lake	No	Intensively netted in spring 2011 when last pike was confirmed	No pike caught in fall 2017 net survey	7.5	3.1
Kenai River						
	Hall Lake	No	Intensively netted in spring 2011when last pike was confirmed	No pike caught in spring 2017 net survey	42.7	17.3
Soldotna Creek						
	Derks Lake	Yes	Treated with rotenone in 2014 when last pike were confirmed	No pike detected in spring 2018 net survey, 50% eDNA detections in fall 2017 survey	37.4	15.1
	Derks Pond	No	Treated with rotenone in 2014 when last pike were confirmed	No pike detected in spring 2017 survey	2	0.8
	East Mackey Lake	Yes	Treated with rotenone in 2014 when last pike were confirmed	No pike detected in net and eDNA surveys in 2017	100.3	40.6
	Loon Lake	Yes (hatchery stocked)	Treated with rotenone August 2017, pike last confirmed in June 2017	No pike detected in October 2017 survey		
	Sevena Lake	Yes	Treated with rotenone in June 2016 and June 2017	No pike detected in 2017 net survey following rotenone treatment	76	30.8
	Union Lake	Yes	Treated with rotenone in 2014 when last pike were confirmed	No pike detected in net survey, single eDNA	84	34.0
	West Mackey Lake	Yes	Treated with rotenone in 2014 when last pike were confirmed	No pike detected in net and eDNA surveys in 2017	183.7	74.3
	Tiny Lake	No	Intensively netted in 2011 when last pike was confirmed	No pike detected in spring 2013 net survey or summer 2013 eDNA survey	5.5	2.2
	Tree Lake	Yes	Winterkill prone; pike last confirmed in 2000	No pike caught in net or eDNA surveys during 2015	68.4	27.7

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Appendix B2.–Page 2 of 2.

Drainage	Waterbody name	Salmonids present?	Description	Latest survey results	Surface acres	Surface hectares
Swanson River						
	Stormy Lake	Yes	Treated with rotenone in 2012 when last pike were confirmed	No pike detected in net surveys 2013–2018	402	162.7
	Stormy Lake	Yes	Treated with rotenone in 2012 when last pike were confirmed	No pike detected in net survey 2017	403	163.1
Tote Road drainage						
	CC Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2013	4.5	1.8
	Crystal Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2013	16.7	6.8
	Freds Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2013	6.1	2.5
	G Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2017	17.3	7.0
	Hope Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2017	26.9	10.9
	Leisure Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2006	11.1	4.5
	Leisure Pond	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2017	1.5	0.6
	Ranchero Lake	No	Scheduled for 2018 rotenone treatment	Pike detected in net survey 2013	7.7	3.1

APPENDIX C: BIOMEME PROTOCOL

For DNA extraction protocol: Biomeme 6-step protocol, which takes about 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 1 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 1 time.
3) Draw up Biomeme wash buffer through the syringe and push back out 1 time.
4) Draw up Biomeme drying wash through the syringe and push back out 1 time.
5) Draw air through the syringe and sample prep column by quickly and vigorously pumping back out for greater than 20 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 5 pumps. The purified DNA is then stored in the elution buffer until PCR.

Biomeme Two3 thermocycler protocol

Biomeme's recommended Two3 thermocycler protocol for this assay was used as follows: 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.

APPENDIX D: LAKE MAPPING

Appendix D1.–Lake mapping quick reference for standard operating procedure provided by BioBase.

ECOSOUND

Quick Reference – Standard Operating Procedure (Updated 03/29/2018)

Installation

- We recommend following the Lowrance™ HDS and Elite Ti unit installation instructions that accompany the unit. Failing to install the transducer correctly may interfere with the sonar signal, and prevent the gathering of accurate data.
- Please ensure your unit has the latest firmware – see https://downloads.lowrance.com
- ***Always ensure a clear signal (both Sonar and GPS) prior and during logging.*** Periodic disruptions due to various causes are ok; sustained disruptions of signal will result in inaccurate or no data. Always monitor the 200 kHz page

Front controls

1 **Touch screen**

2 **Pages**

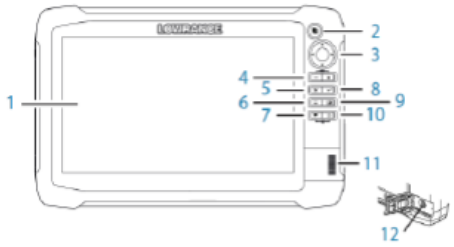
3 **Zoom out / Zoom in (combined press = MOB)**

4 **New waypoint (long press = Find dialogue)**

5 **Power button**
Press and hold to turn the unit ON/OFF.
Press once to display the System Controls dialog.

6 **Card reader** (behind logo)

Elite Ti Controls



No.	Key	Function
1	Touchscreen	
2	Pages key	Activates the home page
3	Cursor keys	Pans the cursor, moves through menu items and adjusts values
4	Zoom out/ Zoom in keys	Zooms the screen; press keys simultaneously to save a Man Overboard (MOB) waypoint
5	Exit (X) key	Exits dialogs, returns user to the previous menu level and removes the cursor from the screen
6	Menu key	Activates the panel menu; press twice to access the Settings menu; press and hold to hide the panel menu
7	Waypoint key	Opens the new waypoint dialog; press twice to save a waypoint; press and hold to access the Find menu
8	Enter key	Confirms selections and saves settings
9	Panel key	Switches the active panel on a multiple-panel display; press and hold to expand the active panel to a full-page panel
10	Power key	Opens the System Controls dialog, adjusts the backlight level and powers the unit on/off
11	Card reader door	
12	microSD card readers	

HDS Controls

Lowrance™ HDS and Elite Ti Unit Settings (Recommended)*

- *Transducer "Installation"*
  - Press Pages button, select SETTINGS, select SONAR; Select INSTALLATION; Select Transducer (check the silver tag on your transducer cable for the model)
- *Fishing Mode = Shallow Water* (for vegetation detection or depths < 60ft, if deeper, use Fresh Water setting)
  - Press Pages button, select SETTINGS, select SONAR
- *Sonar Range = Auto (Default);* critical for optimal bottom and vegetation detection in lakes deeper than 15 ft (4.6 m). Fixing the range to twice the max bottom depth might aid bottom tracking in very shallow ponds.
  - Press Pages button, select SONAR
- *Frequency of Broadband Sonar = 200KHz* ***Mandatory for all three layers*** – other freqs. will create depth maps only
  - Press Pages button, select SONAR
- *WAAS Differential Correction Enabled on GPS*
  - Press the Pages button, select SETTINGS, select SYSTEM, select SATELLITES, select CONFIGURE, enable WAAS
- Recommended Speed = ≤ 5.5 mph. Faster is ok for slowly changing bottom, go slower during rapid depth changes
- ***Monitor your SONAR page; if signal becomes interrupted at faster speeds, slow down.***
- *Maximum Speed = 20 mph (Bathymetry), 12 mph (Vegetation), 10 mph (Bottom Composition).*
- *Bytes per sounding = Default 3200*

BIOBASE

-continued-

ECOSOUND

Quick Reference – Standard Operating Procedure (Updated 03/29/2018)

Recording Sonar

- We recommend carrying two 8-32 (not 64) gb microSD cards. We recommend logging no longer than one hour per file. ***Ensure SD card compatibility/function prior to recording sonar by ensuring the card is recognized in the Log Sonar menu dialog***
- Logging sonar:
  - Press the Pages button, select SONAR, select ADVANCED, select LOG SONAR.
- File Format = .slg (Sonar only, smallest file, min needed for EcoSound maps), .sl2 (Sonar + Structure med sized file - RECOMMENDED), .or .sl3 (Sonar + 3D Structure largest file). Review/select other logging options. Select RECORD.
- Stop recording:
  - Select LOG SONAR, select STOP LOGGING
  - **DO NOT select STOP/START SONAR during recording –** this will only stop/start pinging, not recording and may corrupt your file resulting in lost data
- Do not split both 83 & 200 kHz frequencies or adjust SONAR file/frequency/logging settings. If adjustments need to be made, stop the file, make the adjustment, and then resume logging a new file. Changing displays and zoom levels is ok

Transects

- Transects can be any spacing and depends on the user coverage needs. Users can adjust the buffer in BioBase. 40-m spacing is sufficient for most needs
- Use a design (perpendicular to shore, parallel, concentric) that results in the most efficient coverage of water and maps features to your acceptable level of detail
- Monitor your Lowrance Chart and record a trail to monitor coverage. For pre-planning purposes, transects can be created in GIS, saved to a .gpx file, and then imported into your HDS or Elite Ti unit.

Data Upload

- Once recorded, files can be saved to the user's local computer or uploaded directly from the SD card using the BioBase upload client or web upload tool available at http://www.cibiobase.com/ under "upload tools" on the right-hand side of the page.
- Time required to upload and process the files will depend on a number of factors including the size of the file, size of the area surveyed, and internet connection speed. An email will be sent to you once processing is complete.
- Prior to merging trips, we recommend users review and verify individual trips with the sonar log. Sonar logs are not generated with merged trips

**Refer to the Lowrance™ HDS and Elite Ti manuals or full EcoSound Operator's Guide for additional details on depth finder options. © C-MAP USA Inc.*

APPENDIX E: FIELD DATA SHEETS

Appendix E1.–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					

Visiblity (m): _____

Ice Thickness (In): _____

Comments:

Appendix E2.–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						