Regional Operational Plan SF.2A.2020.19

Operational Plan: Matanuska-Susitna Valley Invasive Northern Pike Surveying and Monitoring

by

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and

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October 2020

Alaska Department of Fish and Game **Division of Sport Fish**

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

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SIGNATURE/TITLE PAGE

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Approval

Title	Name	Signature	Date
Regional Invasive Species Coordinator	Kristine Dunker		
Project coleader	Parker Bradley		
Project coleader	Cody Jacobson		
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Research Coordinator	Tim McKinley		

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ABSTRACT

This project will conduct surveys to detect invasive northern pike *Esox lucius* (“pike”) and determine baseline catch per unit effort (CPUE) throughout the Matanuska-Susitna (Mat-Su) Valley. Mat-Su water bodies will be prioritized for sampling based on whether they fall into 1 of 4 categories: Waters with known pike presence, waters with unconfirmed or suspected pike populations, waters with new credible reports of pike, and vulnerable waters with no known pike populations. For waters in the first 3 categories, northern pike will be detected by means of gillnet surveys using a standardized protocol that adjusts netting effort to lake littoral area. In vulnerable waters where no known pike population occurs, environmental DNA (eDNA) detection methods will be the primary monitoring method. Results from this survey and monitoring project will be used to refine the known distribution of pike in the Mat-Su Valley, increase the likelihood of early detections of new populations, and help prioritize future pike suppression and eradication projects in the Mat-Su Valley.

Key words: invasive species, northern pike, *Esox lucius*, Matanuska-Susitna Valley, eDNA

INTRODUCTION

PURPOSE

The mission of the Alaska Department of Fish and Game (ADF&G), Division of Sport Fish (SF) is “to protect and improve the state’s recreational fisheries resources,” and an objective of the SF strategic plan is to “minimize impacts of invasive species on fish stocks, recreational fisheries, and fish habitat.” Removing northern pike (*Esox lucius*; referred to here as “pike”) from vital salmon rearing habitat directly addresses this objective. ADF&G has an aquatic nuisance species management plan (Fay 2002) and an invasive northern pike management plan (ADF&G 2007). Goals and objectives in these plans address the need to remove invasive northern pike where possible and improve salmon populations that have been impacted by northern pike. The activities proposed in this project are aligned with several plans and initiatives, and ADF&G believes this project will provide the first step in a long-term initiative to effectively survey, monitor, and prioritize pike suppression and eradication projects in the Matanuska-Susitna (Mat-Su) Valley.

BACKGROUND

The northern pike is an invasive species in Southcentral Alaska that negatively impacts salmonid (*Oncorhynchus* spp.) populations via predation of juvenile salmon in invaded waters (ADF&G 2007). The effects of this are most severe in shallow, slow moving, vegetated lakes and streams where northern pike and rearing salmonids share complete habitat overlap (Sepulveda et al. 2013; Sepulveda et al. 2015). Northern pike are native throughout much of Alaska but do not naturally occur south and east of the Alaska Range (Figure 1). It is thought that northern pike were first introduced by an air charter operator to the Yentna River drainage (Bulchitna Lake, Lake Creek drainage) in the late 1950s and subsequently spread throughout the Susitna River basin via natural migration and further illegal stockings. Currently, northern pike have been documented from over 120 lakes and rivers in Southcentral Alaska[1].

More recent, smaller-scale “secondary” northern pike infestations (i.e., originating from the Susitna River basin infestation) have been reported widely throughout the Mat-Su Valley. Some of these infestations are the result of illegal actions by people intentionally transporting pike. Over time, pike have continued to spread throughout the watershed. Many reports of northern pike in

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[1] ADF&G “pike mapper” https://adfg.maps.arcgis.com/apps/webappviewer/index.html?id=ad27ebc052814b66a60d0e52701e64f7&_ga=2.30854847.1642248700.1601938699-959016251.1583185835

the Mat-Su Valley have come from sport anglers; however, most reports lack live or dead specimens, preventing conclusive identification and knowledge of the extent of infestation.

To effectively prioritize and design northern pike suppression and eradication projects, it is necessary to begin with baseline information about the northern pike population in a given area, and what other species may occur (e.g., Baxter and Neufeld 2015). This project will lay the foundation for a long-term northern pike surveying and monitoring program for the Mat-Su Valley.

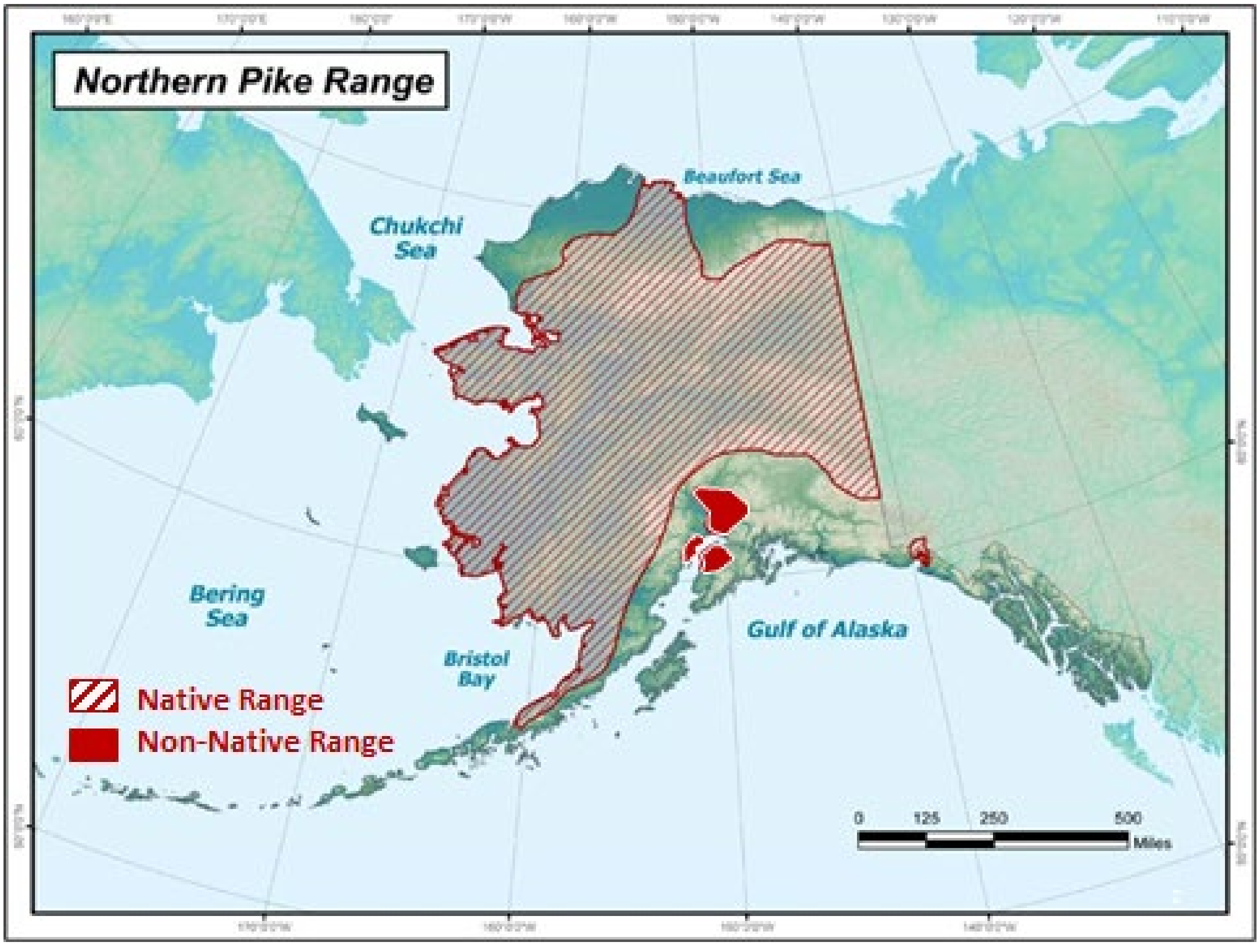

Figure 1.–Northern pike range in Alaska.

OBJECTIVES

PRIMARY OBJECTIVES

1) Document catch-per-unit-effort (CPUE) of northern pike in waters where they are confirmed to be present and document the presence of other fish species.
2) Document presence or absence of northern pike in unconfirmed or suspected infested waters.
3) Document presence or absence of northern pike in waters following a creditable report or greater than 1 positive northern pike eDNA detection.
4) Monitor highly vulnerable water bodies for northern pike presence with annual eDNA surveys.

Secondary Objectives

1) Document length, stomach contents, and sex for all northern pike captured and collect otoliths and cleithra from each.
2) Generate bathymetric maps of water bodies containing northern pike populations.

METHODS

Study Area

This project will take place in the Mat-Su Valley. The Mat-Su Valley is made up of 2 watersheds totaling approximately 25,000 square miles and containing thousands of lakes and miles of river. The Mat-Su Valley also contains the fastest-growing human population in the state and is home to approximately 100,000 residents. There is a wide variety of aquatic habitats ranging from high gradient clear streams to large glacial rivers to low gradient swamp and marsh habitat. This low gradient habitat, which is optimal for northern pike, primarily occurs on the west side of the Parks Highway and supports many of the confirmed and suspected northern pike populations (Figure 2). The watershed also supports all 5 species of Pacific salmon in addition to many resident fish species such as rainbow trout (*Oncorhynchus mykiss*), burbot (*Lota lota*), Arctic grayling (*Thymallus arcticus*), and longnose suckers (*Catostomus catostomus*). Many of these waters are also stocked with rainbow trout and other hatchery fish to provide additional angler opportunity.

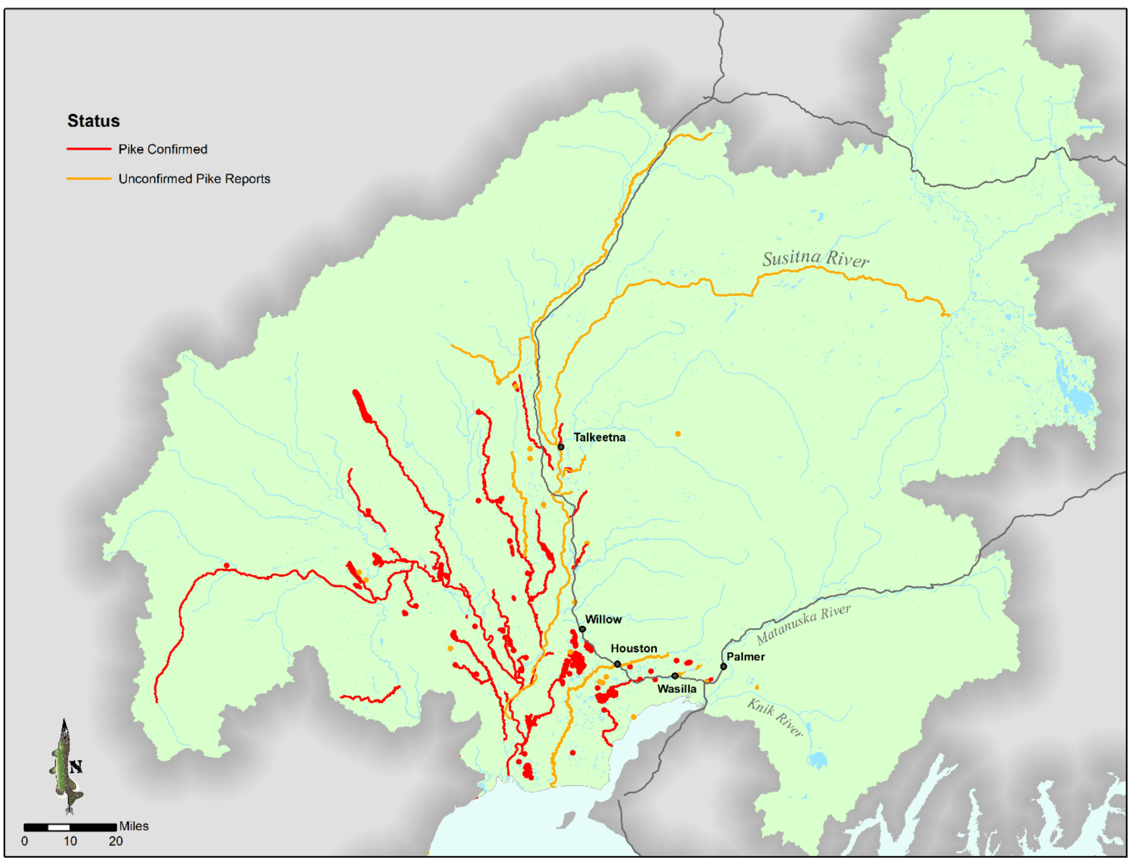

Figure 2.–Map of the Mat-Su drainage and confirmed and unconfirmed northern pike waters.

STUDY DESIGN

Primary Objective 1: Documenting CPUE of Northern Pike in Confirmed Waters

Waters falling in in the “confirmed pike waters” category are shown in red in Figure 2. The primary purpose of documenting CPUE is to establish a baseline for later comparison and to document the degree of infestation. By utilizing repeatable and consistent sampling techniques and effort, comparisons can be made between water bodies and future efforts can be prioritized. However, circumstances such as severe bycatch or high public use of lakes may prevent use of certain gear types and methods. In these cases, alternative gear or methods (see examples below) will be used to keep sampling effort in that given water body consistent between years.

Sampling

For open water sampling, the water body will be divided into sections, with each section containing approximately 1 mile of shoreline (Figure 3). One gillnet per each 0.1 mile of shoreline will be fished in each section (10 gillnets per mile of shoreline). Gillnets will be set overnight along the littoral zone and checked the following day approximately 24 hours later. Nets will be distributed uniformly in the littoral zone of each section, and locations will be recorded with GPS for repeatability, noting the start and stop times for each set. The nets will be pulled as they are checked and reset in the next section of the lake. This will repeat until all sections have been sampled.

Lakes with significant bycatch concern will have the same density of nets per section, but the nets will be set for a shorter duration (approximately 4–5 hours) and they will be closely monitored. Throughout the sampling period, the crew will continuously boat along the nets and remove any northern pike or bycatch that are captured. A new section will be sampled each day until all sections have been sampled.

Some lakes (e.g., Nancy Lake or Big Lake) will be too big to sample with this amount of effort. In these cases, netting effort may be limited to specific areas of the waterbody that provide good pike habitat. In addition, other gear types may be used depending on the circumstances. This includes, but is not limited to, fyke nets, hook and line, and jug sets. Use of these gear types will be specific to the water body, but efforts will be made to remain consistent between sampling years at that given water body.

Sampling frequency at each water body in this category will occur at least once every 5 years.

Lake Mapping

Lake bathymetry data will also 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 take place in roughly 100-surface-acre sections. For each section, the perimeter will be mapped as near shore as safely feasible, followed by a repeat of the perimeter circuit about 20 m farther offshore. After 2 complete section perimeter circuits, the rest of the lake section will be mapped by sequential line transects, typically orientated along the greatest length of each section. Typically, transects lines should be no greater than 40 m apart and all mapping will be done traveling at a slow speed (<5 mph); this can be gauged by watching the GPS track on the Lowrance unit’s monitor.

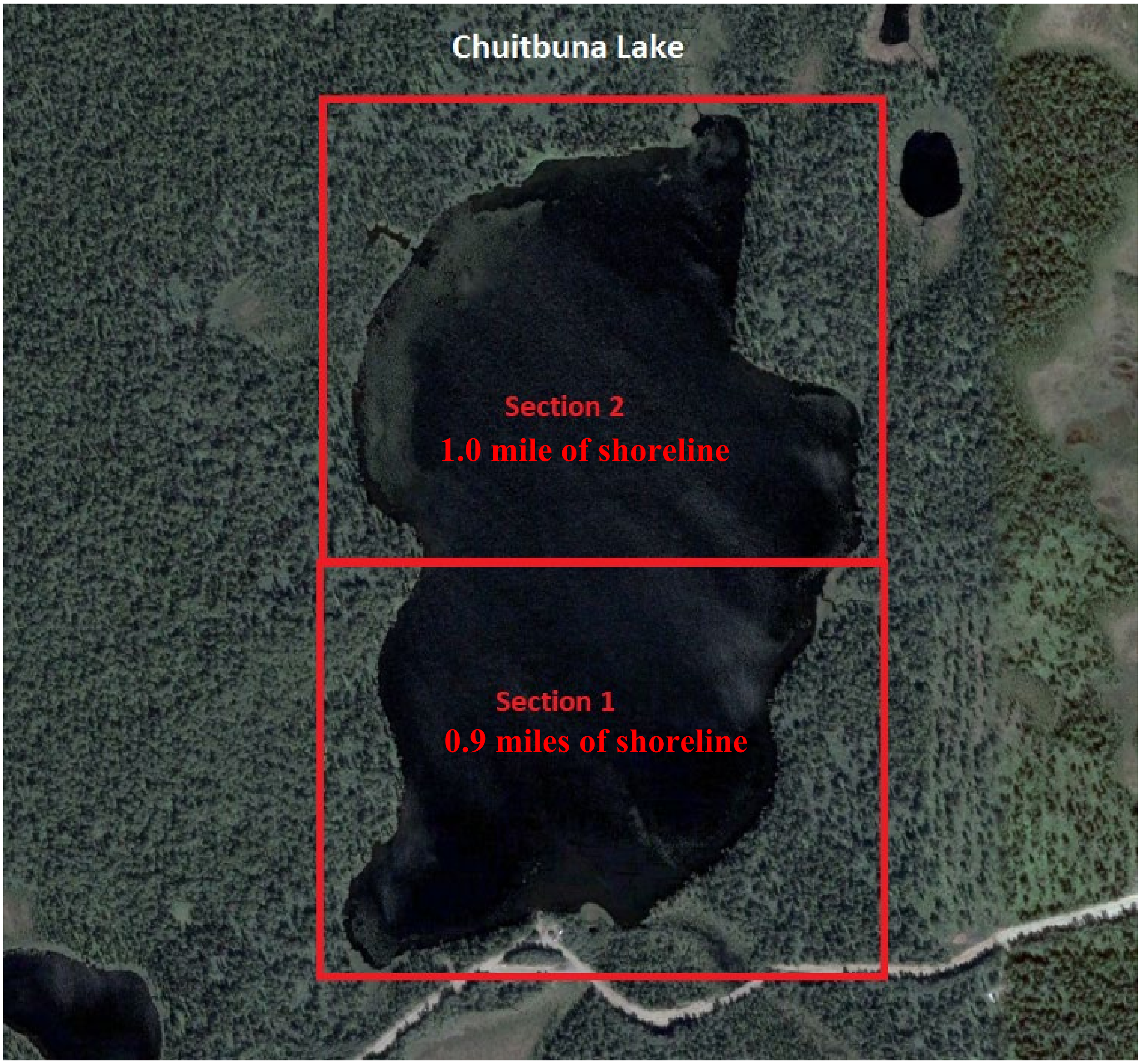

Figure 3.–Example of a lake broken into sections, each with approximately 1 mile of shoreline.

Primary Objective 2: Documenting presence or absence in suspected pike waters

Water bodies with unconfirmed pike reports (orange in Figure 2) and any water bodies with a surface connection to confirmed pike waters will be sampled according to Primary Objective 2. The primary purpose of Objective 2 is to confirm pike presence or absence in suspected pike waters and to further refine the known distribution of northern pike in southcentral Alaska. Methods will be geared toward determining with confidence whether or not pike are present. However, circumstances such as severe bycatch concern or lakes highly used by the public may prevent use of certain gear types and methods.

Sampling

For open water sampling, the water body will be divided into sections, with each section containing approximately 1 mile of shoreline (Figure 3). As for Objective 1, gillnetting effort for each section will be 1 gillnet per 0.1 mile of shoreline. Gillnets will be set overnight along the littoral zone and checked the following day approximately 24 hours later. Nets will be distributed relatively uniformly, with high effort placed in ideal pike habitat. Locations will be recorded with GPS for repeatability, noting the start and stop times. The nets will be pulled as they are checked and reset in the next section of the lake. This will repeat until all sections have been sampled.

Lakes with significant bycatch concern will have a similar netting scheme, except the nets will be set for a shorter duration (approximately 4–5 hours) and they will be closely monitored. Throughout the sampling period, the crew will continuously boat along the nets and remove any northern pike or bycatch that are captured. A new section will be sampled each day until all sections have been sampled.

If netting takes place and no northern pike are captured, eDNA samples may be taken to confirm their absence. The number of eDNA samples will be based on the eDNA detection probabilities calculated in Appendix A1.

Some water bodies are more easily accessed in the winter. Additionally, some water bodies may have high waterfowl bycatch concerns and are best sampled in the winter. For winter sampling, under-ice gillnets and (or) tip ups will be used. Up to 20 tip ups baited with artificial lures or hotdogs will be used at a time. Under-ice gillnets will be 100 ft long, 4 ft deep, and composed of 1.25 in mesh. Nets will either be set individually, or 2 may be tied together as a pair so that there is 200 feet of gillnet per set. Different numbers of nets and types of configurations will be set depending on the lake bathymetry (Figures 4 and 5). Depending on ambient temperatures, nets will be checked every 24 to 48 hours to avoid nets freezing in and becoming unrecoverable. In the spring with warmer temperatures, net sets can be checked once per week.

Once pike are confirmed, the water body will be added to the list for lake mapping, following methods outlined under the previous objective.

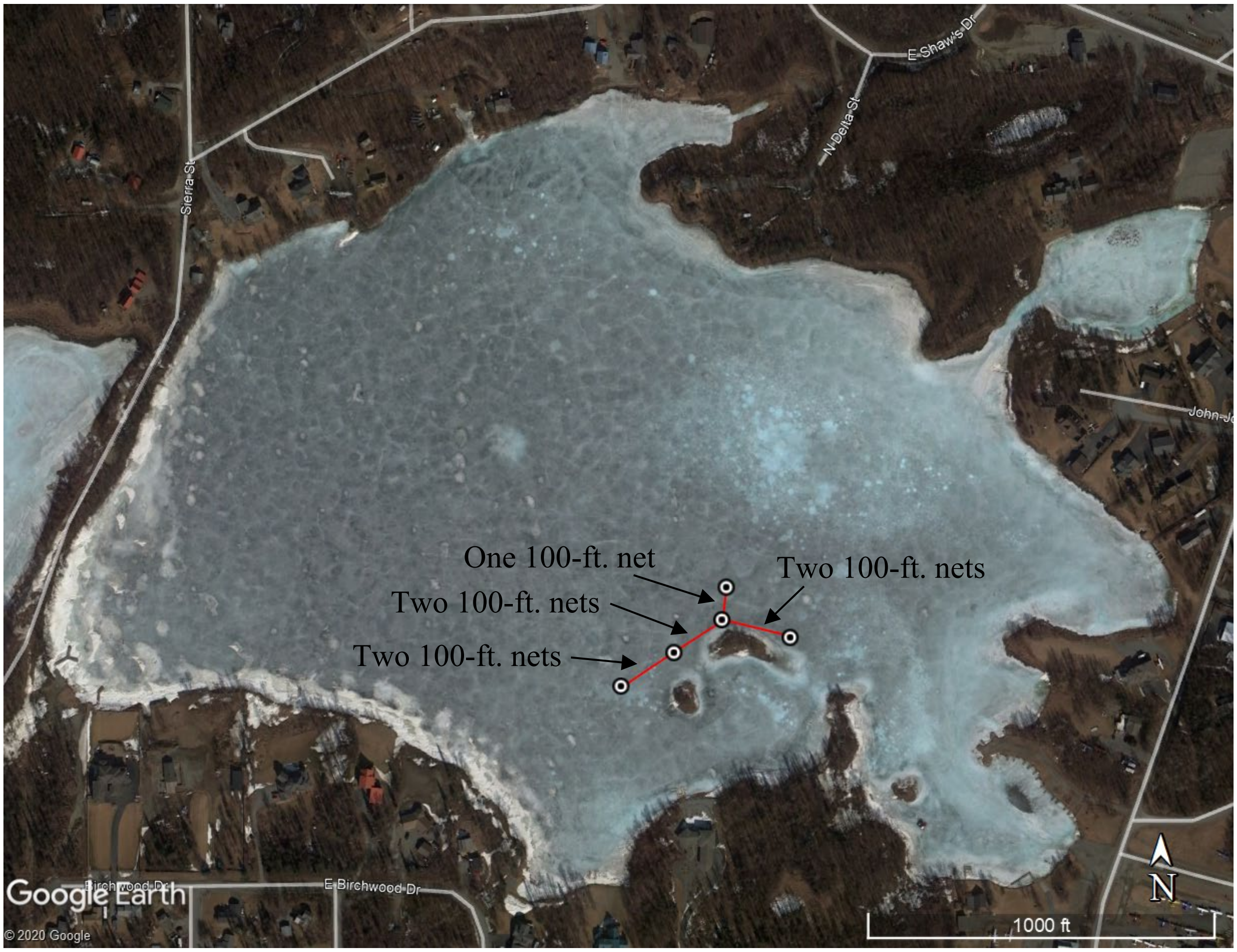

Figure 4.–Anderson Lake showing under-ice netting, with gillnets indicated by red lines and holes drilled in the ice indicated by dots.

Figure 5.–Sand Lake in Anchorage showing under-ice gillnet sets, with gillnets indicated by red lines and holes drilled in the ice indicated by dots.

Primary Objective 3: Documenting pike presence in waters following a report

Water bodies sampled under this objective are those not known to contain pike but have either received a creditable report about their presence or have greater than 1 eDNA detection for pike. Depending on the water body, this situation may be considered a higher priority than other surveying and monitoring because this could be the result of a relatively recent introduction for which removals might prevent the establishment of a self-sustaining population. The goal of Primary Objective 3 is to confirm presence and remove as many pike as possible. Bycatch may be a significant concern in this situation, so sampling gear will need to be selected based on the unique situation. The season in which a pike is reported will further dictate gear type as well.

Sampling

For open water sampling, gillnets will be the primary gear type. Because of the variety of habitat and conditions present in the Mat-Su Valley, ranging from small stocked lakes to large remote interconnected wetland habitats with wild fish, the level of gillnetting effort a water body receives will be highly variable. For small stocked lakes, or lakes with low bycatch concern, each 1-mile section of shoreline will be set with 20 gillnets overnight (24-hour sets). Locations will be recorded with GPS for repeatability, noting the start and stop times. The nets will be pulled as they are checked and reset in the next section of the lake. This will repeat until all sections have been sampled.

For large waterbodies with wild fish and abundant wildlife, netting effort will probably need to be reduced and nets will be closely monitored. However, at a minimum, efforts will be made to set 10 gillnets for each 1-mile section of shoreline for 4 to 5 hours. Nets will be continuously monitored for bycatch.

Should 1 or more pike be captured in this netting, and it is believed few individuals are present and there little evidence of a reproducing population (e.g., only a couple larger adults are captured, all pike captured are the same sex), netting effort will continue until pike are no longer captured or until each section has been sampled twice.

If a pike report is received in winter, under-ice gillnets and (or) tip ups will be used. Up to 20 tip ups baited with artificial lures or hotdogs will be used at a time. Under-ice gillnets will be 100 ft long, 4 ft deep, and composed of 1.25 in mesh. Nets can either be set individually or in pairs so that there is 200 feet of gillnet per set. Different numbers of nets and types of configurations can be made depending on the lake bathymetry (Figures 4 and 5). Duration of gillnet sets will vary depending on circumstances. However, if 1 or more northern pike are captured during under-ice netting, the nets may be left to soak until they can be retrieved in the spring. This will increase the chance of removing reproductively viable individuals before they can spawn in the spring.

Should no pike be captured during the netting effort, a follow-up eDNA survey will take place with sample numbers based on the eDNA detection probabilities in Appendix A1.

Primary Objective 4: Monitoring highly vulnerable waters

Water bodies that fall into this category typically had prior pike populations that have been eradicated or are water bodies with a fishery resource that would be vulnerable if pike became established, and where pike would be difficult and (or) expensive to eradicate. Early detection of pike in these water bodies could result in successful eradication with nets if the response is quick and sufficient. In small closed lakes (<40 acres), intensive under-ice gillnetting has 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).

Sampling

Primary sampling will be collection of eDNA samples annually in the fall. The number of samples will be based on the eDNA detection probabilities outlined in Appendix A1.

DATA COLLECTION AND REDUCTION

Fish Sampling

Gillnet set and check times will be recorded for each gillnet set. Start and stop times will also be recorded for other gear types used. All capture data will be recorded on water-resistant paper following the format in Appendix B1.

All northern pike will be dispatched and processed for fork lengths (nearest millimeter), age (by counting annuli on cleithra bones), sex, and stomach contents, and otoliths will be removed and archived. All data will be recorded on the northern pike sampling forms (Appendix B2). Data will be transferred from the data sheets to Microsoft Excel worksheets for analysis.

eDNA Sampling

Each eDNA sampling location will be recorded with a handheld GPS and given a unique identifying name. Control blank samples will be similarly labeled. Each duplicate water sample collected will be given a unique identifying 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 dates, filtering time, numbers of filters used, waterbody name, unique sample identifier, initials of the collector and person doing the filtering, collection site location (lat and 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 will be stored by the Lowrance chartplotter as an .sl2 file on an external memory SD card, will 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.

DATA ANALYSIS

Northern Pike Surveys

Gillnet Sampling

The capture of a northern pike during a gillnet survey will confirm the presence of northern pike. If no northern pike are caught, we will conclude either no northern pike are present or that the population is very small.

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 (R. Massengill, Fishery Biologist, ADF&G, Soldotna, personal communication). 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.

Catch-Per-Unit-Effort Comparison

Northern pike CPUE can be calculated for each net or each waterbody using Equation 1. Efforts will be made to keep a consistent net soak time per water body between years.

$$CPUE = \frac{Number\ of\ fish\ captured}{Total\ Net\ Hours\ (Duration\ of\ net\ sets * Number\ of\ net\ sets)} \tag{1}$$

Simple linear regression analysis will be used to evaluate if CPUE of northern pike changes for a given water body between years. In addition, maps will be generated showing relative CPUE of netting efforts for each waterbody (e.g., Figure 6).

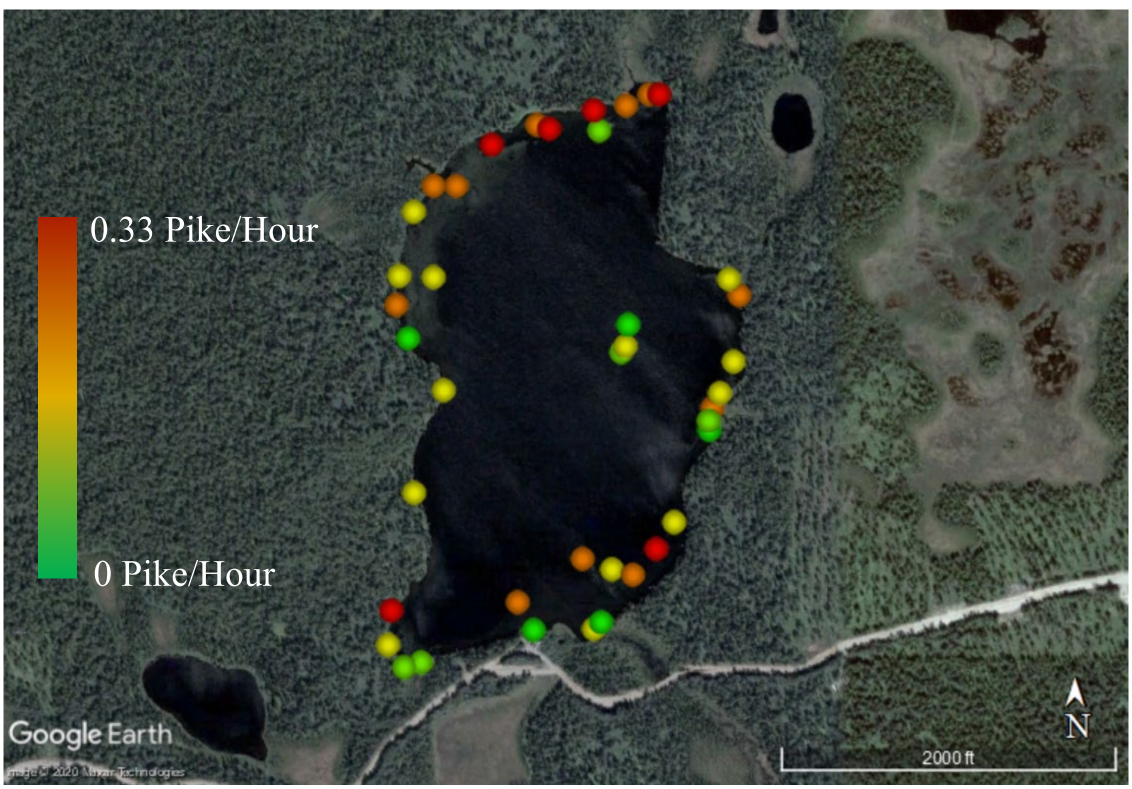

Figure 6.–Netting locations on Chuitbuna Lake in 2019 showing relative CPUE for each net.

SCHEDULE AND DELIVERABLES

Dates	Activity
June–Sept 2020	Perform open water netting and sampling
October 2020	Collect eDNA samples
Winter 2020–2021	Data Entry Otolith/cleithra prep/aging Perform under-ice netting Analyze data and write performance report for our State Wildlife Grant

RESPONSIBILITIES

Kristine Dunker, Fishery Biologist III, ADF&G
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.

Parker Bradley, Fishery Biologist II, ADF&G
Duties: Serve as the primary project biologist; assist with planning and coordinating field logistics; author State Wildlife Grand project report and presentations to the public.

Cody Jacobson, Fishery Biologist I, ADF&G
Duties: Assist with planning and coordinating field logistics and equipment procurement; supervise field activities and technicians.

Ben Buzzee, Biometrician IV, ADF&G
Duties: Provide guidance on study design; assist with postseason data analysis; review project operational plans and reports.

Fish and Wildlife Technicians, ADF&G
Duties: Assist with field activities.

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APPENDIX A: ENVIRONMENTAL DNA SAMPLING

To develop an eDNA sampling effort sufficiently robust to detect northern pike populations with low abundance, the estimated mean detection probabilities of northern pike eDNA were used. 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 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 meters is zero because 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 area less than 1 meter from the sample site and the donut-shaped areas 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 (Dunker et al. 2016) 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 a sampling 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:

$$P(D) = P(D \mid R_1) \times P(R_1) + P(D \mid R_2) \times P(R_2) + P(D \mid R_3) \times P(R_3) \qquad \text{(B1)}$$

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, $P(D \mid R_i)$, is 0.89, 0.57, or 0.27 for sampling 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:

$$\mathrm{P}(R_i) = \frac{area\ of\ region\ i}{total\ area\ of lake} \qquad \text{(B2)}$$

where the fixed areas of the subregions are divided by the known total surface area, and the total surface area of the lake is taken from Biobase results.

-continued-

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, …, *n*. Thus, the only change in our probability calculation for *S* sites is that the proportion of area represented by each subregion is now $S \times P(R_i)$. By another application of the law of total probability and definition of conditional probabilities, the probability of detection at *S* sites is as follows:

$$P(\text{detection at } S \text{ sites}) = P(D \mid R_1) \times S \times P(R_1) + P(D \mid R_2) \times S \times P(R_2) + P(D \mid R_3) \times S \times P(R_3) = S \times P(D) \quad \text{(B3)}$$

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 $1 - [1 - S * P(D))]^N$. This expression is then set to the desired probability of detection and solved for *S*. Table B1 displays calculated eDNA sampling requirements for a variety of desired probabilities of detection and acreages assuming a population of *N* = 20 northern pike.

Table B1.–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

APPENDIX B: SAMPLING FORMS

Appendix B2.–Northern pike capture form.

Northern Pike Capture Form 2019							
Lake: Samplers:		Section #		Start Date: Stop Date:			
Net #	GPS	Start Time	Stop Time	Fish Spp.	Fork Length	Tag #	Comments

Note: This is a standard form. Tag # column not used for this project.

Appendix B3.–Northern pike stomach sampling form.

Northern Pike Stomach Sampling Form, 2019										
Date:				**Lake:**					**Samplers:**	**Page:**
Number	Tag #	Recap **(R or Blank)**	Section #	Net #	Fork Length (mm)	Weight (g)	Sex **(M, F, U)**	Stomach contents **(Yes/No)**	Stomach Content	Comments about fish or stomach content; deformatie

Note: This is a standard form. Tag # and Recap columns not used for this project.