Regional Operational Plan No. ROP.SF.2A.2022.14

Operational Plan: Northern Pike Sex Determination and Trojan YY Investigations in Alaska

by

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and

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January 2022

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

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Weights and measures (metric)

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

Weights and measures (English)

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

Time and temperature

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

Physics and chemistry

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

General

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

Mathematics, statistics

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

REGIONAL OPERATIONAL PLAN NO. ROP.SF.2A.2022.14

OPERATIONAL PLAN: NORTHERN PIKE SEX DETERMINATION AND TROJAN YY INVESTIGATIONS IN ALASKA

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SIGNATURE PAGE

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

This project will complete the development of a sex determination marker for northern pike and investigate the application of a potentially new invasive northern pike control technique (Trojan YY) with model simulations of 3 invasive northern pike populations in Southcentral Alaska. The northern pike sex determination marker is a critical step in the development of Trojan YY males used for invasive species control of northern pike.

Keywords: northern pike, *Esox lucius*, invasive species, sex determination marker, Trojan YY

INTRODUCTION

PURPOSE

The purpose of this project is to finalize the development of sex determination markers for northern pike (*Esox lucius*, hereafter referred to as pike) in Alaska and model potential scenarios where a new invasive fish control technique known as "Trojan YY" could be applied (Schill 2016). The Alaska Department of Fish and Game (ADF&G), Division of Sport Fish, is interested in this technique as an additional tool for controlling invasive pike populations, and this project will help inform decisions about the potential use of Trojan YY pike in Alaska.

BACKGROUND

The introduction and subsequent establishment of invasive fish populations can be a primary conservation challenge for fisheries and aquatic ecosystems where it is difficult to implement selective removal (Britton 2011). This is the case for northern pike in Southcentral Alaska where it is an invasive species that negatively impacts salmonid (*Oncorhynchus* spp.) populations via predation of juvenile salmon in invaded waters (Dunker et al. 2018). The effects of this invasive species are most severe in shallow, slow moving, vegetated lakes and streams where pike and rearing salmonids share complete habitat overlap (Sepulveda et al. 2013).

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 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, pike have been documented from over 150 lakes and rivers in Southcentral Alaska[1].

More recent, smaller-scale "secondary" pike infestations (i.e., likely originating from the Susitna River basin infestation) have been reported widely throughout the Matanuska–Susitna Valley. Some of these infestations are the result of illegal anthropogenic introductions. Over time, pike have continued to spread throughout the watershed. They have also spread into Knik Arm drainages, drainages on the west side of Cook Inlet, and several drainages of the Kenai Peninsula. Most populations on the Kenai Peninsula have been eradicated using traditional fisheries methods (Dunker 2020). However, the last remaining Kenai Peninsula population in the Miller Creek drainage, and potentially others along west Cook Inlet and Knik Arm are thought to be the result of movements of pike through the estuarine waters of Cook Inlet (Mat Wooller, UAF Stable Isotope Lab, unpublished data). Given the complexity and remoteness of areas in which invasive pike are becoming established, control tools beyond the traditional fisheries techniques of piscicides (fish pesticides) and nets might be warranted.

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

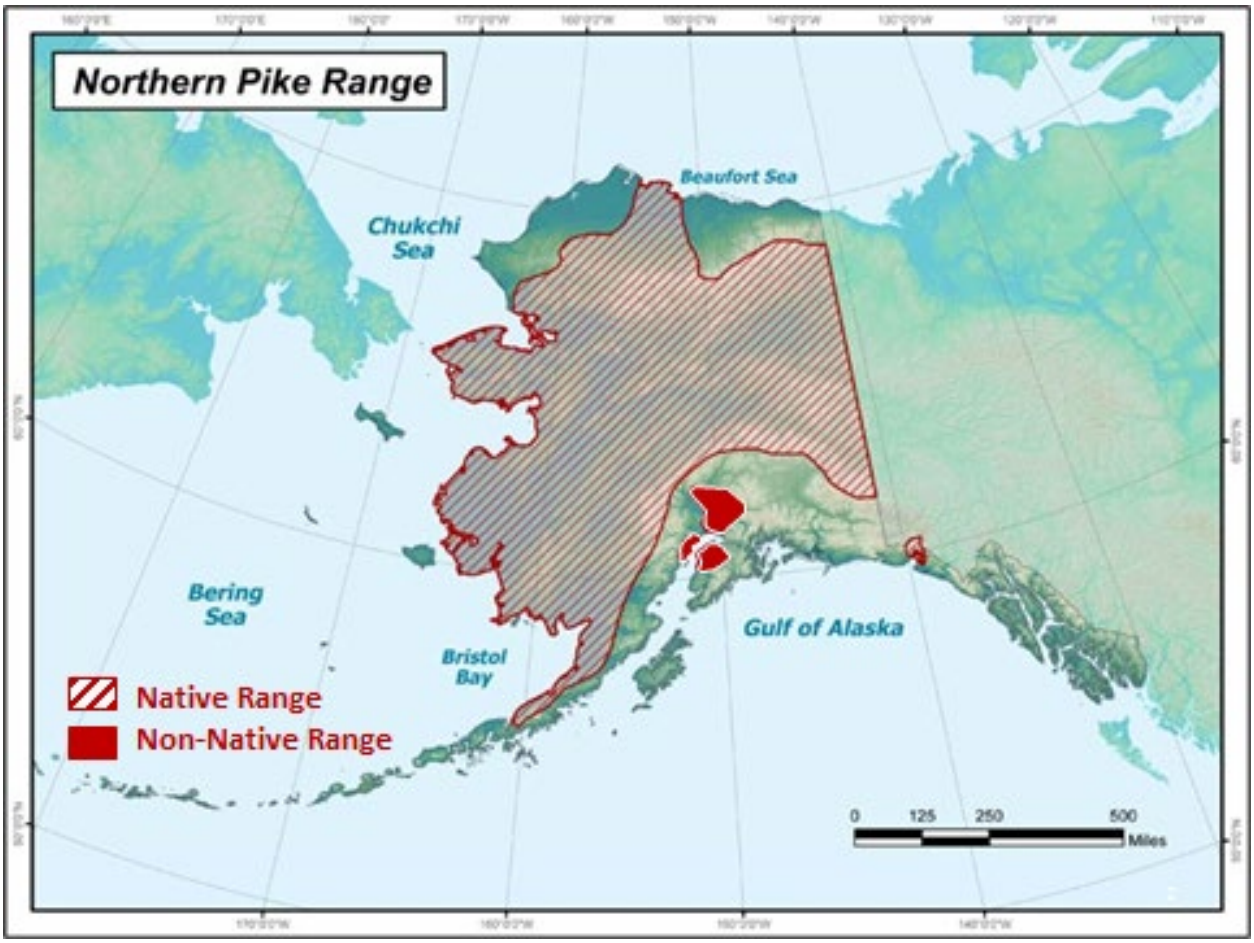

Figure 1.–Northern pike range in Alaska.

The Western Association of Fish and Wildlife Agencies (WAFWA) is coordinating a consortium of state agencies to develop a new technology to add into the toolkit for invasive fish management. The tool is called the Trojan Y-chromosome approach (Gutierrez and Teem 2006) and is used to develop and release males that are genetically YY instead of XY. These "Trojan YY" males, if they survive and reproduce, can skew a population toward males and theoretically drive an invasive fish population to extirpation (Gutierrez and Teem 2006; Schill et al. 2016). To date, the only available management techniques for invasive fish eradication include physical removal, dewatering, and piscicide use. However, these methods are effort-intensive, expensive, and can harm native fish. Continued research into new techniques for controlling and eradicating invasive fish is greatly needed. This project provides a significant step in that direction by contributing to the development of a northern pike sex determination marker to the WAFWA consortium to further develop Trojan YY capabilities for invasive northern pike.

The creation of Trojan YY males for release into wild fish populations involves a step with individuals that have a female phenotype but a male genotype (Schill et al. 2016, 2017). Normal male XY fish are raised in captivity and fed a diet of extradiol that effectively "feminizes" the males. These feminized male fish are then bred to normal males to produce Trojan YY males (Schill et al. 2016). In wild populations with natural XX females and XY males, the stocking of Trojan YY males should cause a disproportionate influx of Y chromosomes into subsequent generations, biasing the sex ratio towards males (Schill et al. 2017). If enough carriers of Trojan

Y chromosomes are introduced, the population should eventually die out as females become scarce. Advantages to this technique include its specificity, reversibility, and flexibility (it can be used for suppression or eradication), and it involves less collateral damage to fish and wildlife than current methods.

The WAFWA Trojan YY Consortium is working to develop this technique for several notorious invasive fish species such as brook trout (*Salvelinus fontinalis*), walleye (*Sander vitreus*), common carp (*Cyprinus carpio*), and northern pike—species desired in their native habitats but which become problematic in nonnative ecosystems. ADF&G has an interest in the potential use of Trojan YY pike to assist in managing invasive pike populations south of the Alaska Range where pike are not native. A requirement of the Trojan YY technology is development of an effective sex determination genetic marker to distinguish sex among fish that are phenotypically similar so that proper broodstock can be selected for the production of Trojan YY males. Development of this marker will benefit both Alaska and other states considering this technology in their invasive northern pike management strategies. In conjunction with developing this marker, ADF&G intends to investigate specific cases where Trojan YY pike might be applied by constructing system-specific models that illustrate stocking requirements, suppression needs, and time to significant reduction or extinction of specific populations.

A study by Pan et al. (2021) found that a male-specific duplication of the anti-Müllerian hormone gene (*amhby*) is the master sex-determination gene in northern pike from Europe and Asia. This gene is located in a small male-specific insertion point on the Y chromosome of northern pike, supporting an XX/XY sex determination system where females entirely lack this gene. However, Pan et al. (2021) also found that sex determination in northern pike populations in Canada and the continental United States outside of Alaska is complicated by the complete loss of the *amhby* gene such that both males and females lack the gene, implying sex determination by other means. In samples analyzed from Alaska, most males possessed the *amhby* gene, but some did not. Thus, it will be critical to find markers that distinguish between male and female northern pike in the invasive Alaskan populations before the Trojan YY approach can be implemented.

OBJECTIVES

1) Develop a genetics-based sex determination marker for northern pike in Alaska.
2) Model Trojan YY stocking scenarios and effectiveness in extirpating 3 invasive northern pike populations in Alaska.

METHODS

NORTHERN PIKE TISSUE SAMPLE COLLECTION

ADF&G Division of Sport Fish biologists and partners will collect 1,045 pike of known sex from several areas of the Alaska including both native and invasive populations (Vogel Lake on the Kenai Peninsula; several lakes in the Matanuska-Susitna Valley including Whiskey, Hewitt, Alexander, Big, Hourglass, Nancy, South Rolly, Milo, and Long Lakes; Threemile and Chuitbuna Lakes on the west side of Cook Inlet; Vogel Lake on the northern Kenai Peninsula; the Minto Flats near Fairbanks; and the Innoko River in western Alaska (Figure 2). Tissue sample collection began in 2020, and 217 samples are already archived with the ADF&G Gene Conservation Lab. The majority of the sample collection will occur during the open water season of 2021. Tissue samples are collected following the Gene Conservation Lab protocol in Appendix A1. Only pike with 100%

confidence in sex identified (ID) in the field will be used for the lab analysis (Figure 2). All data associated with the tissue sample, including location, collectors, specimen ID, length, and sex will be stored in an Excel file and provided to the lab with samples.

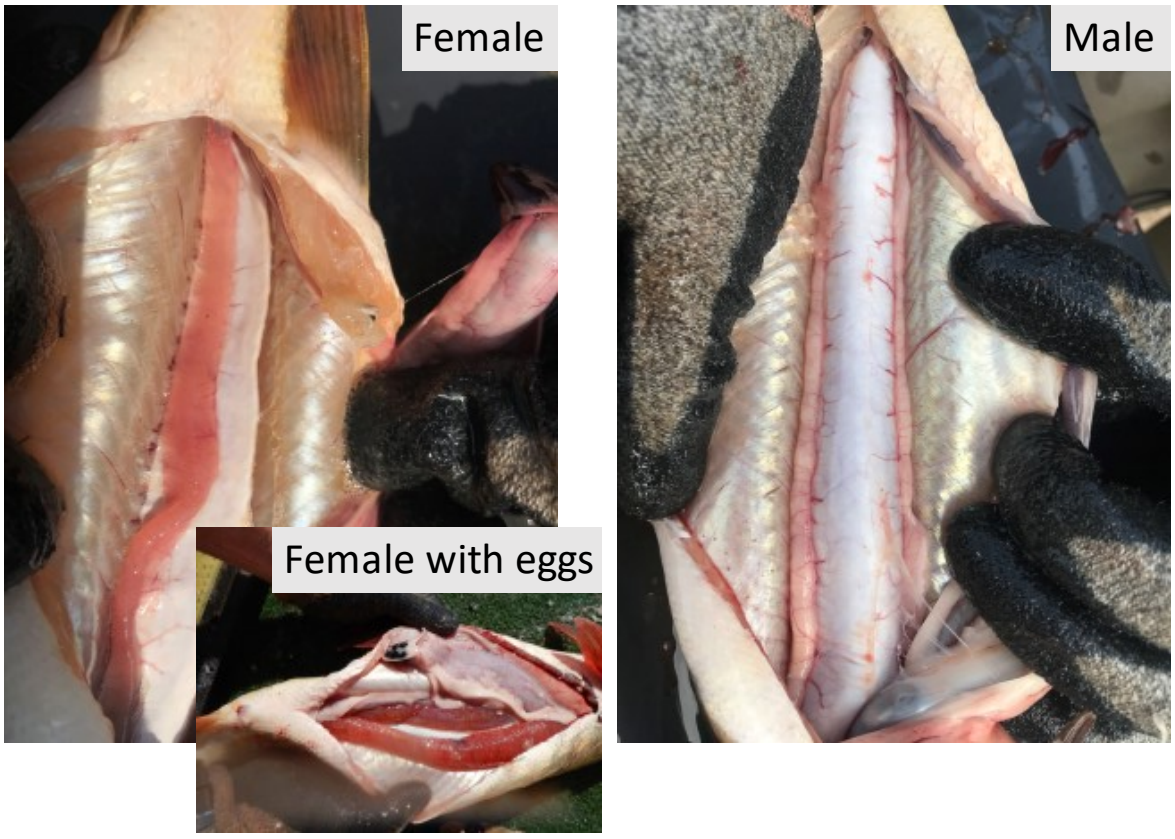

Figure 2.–Identification key for male and female northern pike.

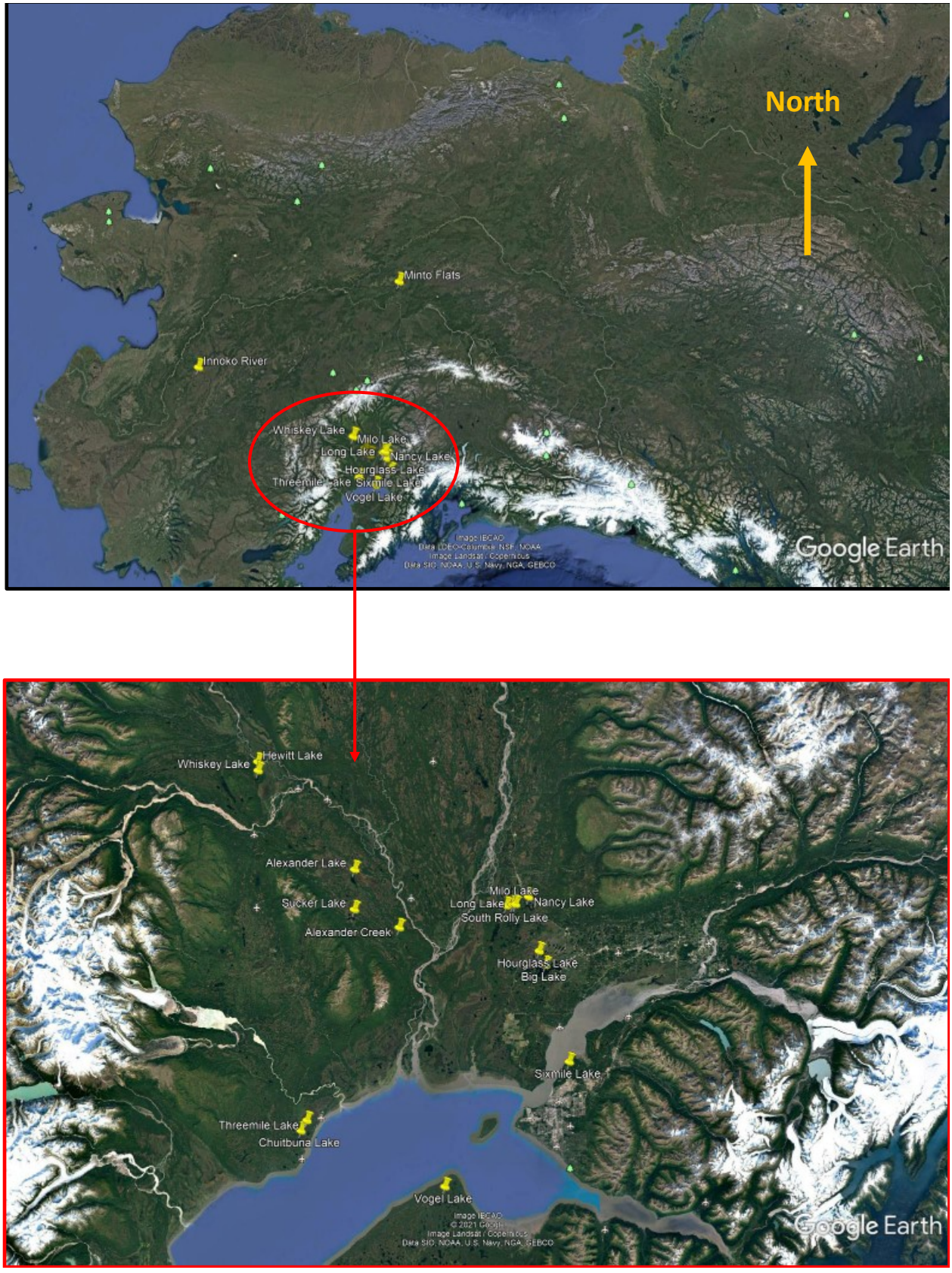

Figure 3.–Locations where northern pike sex marker samples will be collected.

NORTHERN PIKE SEX DETERMINATION MARKER

Prior to this project, the ADF&G Gene Conservation Lab used restriction site–associated DNA sequencing (RADseq) to develop 12 single nucleotide polymorphism (SNP) markers associated with sex from tissue samples of 96 invasive northern pike from 3 locations in Southcentral Alaska (Tote Road Lakes in Soldotna, Threemile Lake near Tyonek, and Alexander Lake in the Matanuska-Susitna Valley). Five of these markers are in a section of the genome that is strongly correlated with sex identified in the field, and the remaining 7 markers are associated with other sections of the genome that are more weakly correlated with sex identified in the field. Together, these 12 potential markers are expected to have a high probability of differentiating sex in northern pike (Chris Habicht, Fish Scientist I, ADF&G, personal communication). The next step is to collect and genetically analyze additional samples from more populations to better assess the effectiveness of these markers to differentiate sex in pike. Once sex can be determined genetically, this simplifies the production of Trojan YY males because genetic screening can be used to generate broodstock of appropriate genetic type (Schill et al. 2016). Although identifying an effective sex determination marker is an important initial step for developing Trojan YY pike for northern pike control (Dan Schill, personal communication), it also has the added benefit of aiding stock assessment research on pike in other areas of Alaska where they are native.

During the fall and winter of 2021–2022 after completion of the tissue sample collection in the field, the ADF&G Division of Commercial Fisheries Gene Conservation Lab will develop TaqMan assays and screen 12 SNP markers with 285 previously extracted northern pike samples to select the best performing 6 SNPs and apply them to all 1,045 new pike samples of known sex. This will identify top markers for Trojan YY sex determination, and these results will be published in a professional journal.

TROJAN YY SIMULATIONS

Three invasive northern pike populations have been chosen for modelling the potential effectiveness of Trojan YY pike to control pike population in Southcentral Alaska. These are Threemile and Chuitbunga Lakes on the west side of Cook Inlet and Shell Lake in the Matanuska–Susitna Valley. The westside Cook Inlet pike populations are of interest because they are believed to be on the pike invasion front in that region. Chuitbuna Lake is a relatively confined population whereas Threemile Lake encompasses a more open system with multiple lakes, wetlands, and stream connections. Both these populations have comprehensive ADF&G data sets available from ongoing Sport Fish Division invasive northern pike suppression projects for model inputs. The third population of interest is in Shell Lake. This pike population has been suppressed with gillnets fished throughout the open water season for many years by the Cook Inlet Aquaculture Association (CIAA), and it also has a substantial data set available. Most of the ecological impacts of pike in Shell Lake are on out-migrating sockeye salmon smolt at the lake outlet each year (Wizik 2018). Gillnet suppression has been highly effective, and the pike population has greatly decreased (Wizik, CIAA, personal communication) although Trojan YY pike are of interest here to keep the population from rebounding, and this area presents a unique scenario within a large open drainage for which Trojan YY could be an ideal tool to prevent further range expansion.

The modeling exercises in this project will aim to answer the following question:

Under various Trojan YY stocking rates and gillnet suppression rates, how long before extirpation can be expected in each of the 3 test populations?

Population Model

To simulate population levels under various population demographic and removal scenarios, an age-structured Leslie matrix model of the following form (after Jensen 1995) will be used:

$$\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \end{bmatrix}_{t+1} = \begin{bmatrix} f_1 & f_2 & f_3 & f_4 \\ s_1 & 0 & 0 & 0 \\ 0 & s_2 & 0 & 0 \\ 0 & 0 & s_3 & s_4 \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \end{bmatrix}_t - \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & h & 0 & 0 \\ 0 & 0 & h & 0 \\ 0 & 0 & 0 & h \end{bmatrix} \begin{bmatrix} f_1 & f_2 & f_3 & f_4 \\ s_1 & 0 & 0 & 0 \\ 0 & s_2 & 0 & 0 \\ 0 & 0 & s_3 & s_4 \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \end{bmatrix}_t$$

where in year t

$[n_i]_t$ is the number of female pike in age category i

f_i is the number of female offspring produced by female pike in age category i

s_i is the survival rate of female pike in age category i

and h is the percent of age 2+ female pike manually harvested per gillnet hour (assumed constant across age categories)

Specific inputs to the model will focus on using data from Threemile and Chuitbana Lakes, which have the most robust pike population data available for lakes in Southcentral Alaska. Age classes will be determined by subsampling cleithra from each year of suppression in each lake and correlating that to length class to assign ages to all pike within the datasets. The ratio of males to females in each age class will also be used to estimate the number of female offspring. Natural survival rates will be input using estimates available in the published literature. Percent harvest in the model will be based on the initial population estimates developed for each of these lakes (ADF&G, unpublished data).

Initial starting values for n_i at time $t = 0$ will be adjustable inputs. To account for Trojan YY stocking rate (a fixed annual input number), it will be assumed that each YY male has a fixed chance of successfully fertilizing 100% of a female's eggs each year (also accounting for the number of pike removed through suppression). Each introduced male's fertilization success or failure will be modelled as a Bernoulli trial with probability of success p. Fecundity rates of female offspring (f_i) will then be reduced by the proportion of the female population that YY males successfully paired with (because all offspring will be male). To account for a wide range of scenarios and parameter values, this model will be implemented in RShiny (Appendix B1), allowing outcomes to be observed as parameters are adjusted. The length of time to extirpation (i.e., population: $n = 0$) will be determined as the number of years it takes for $n_1 + n_2 + n_3 + n_4 < 1$.

SCHEDULE AND DELIVERABLES

Dates	Activity
May–August 2021	Collect northern pike tissue samples.
Fall 2021	Develop Trojan YY model.
Winter 2021	Trojan YY simulations
Spring 2022	Genetics lab conducts northern pike sex determination marker analysis.
Summer 2022	Publish northern pike sex marker(s) in a professional journal and write Trojan YY simulation report in the Fishery Data Series.

RESPONSIBILITIES

Kristine Dunker, Fishery Biologist III, ADF&G
Duties: Primary project biologist; 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.

Robert Massengill, Fishery Biologist II, ADF&G
Duties: Assist with planning and coordinating field logistics

Parker Bradley, Fishery Biologist II, ADF&G
Duties: Assist with planning and coordinating field logistics

Cody Jacobson, Fishery Biologist I, ADF&G
Duties: Assist with planning and coordinating field logistics

Chris Habicht, *Fish Scientist I, ADF&G*
Duties: Coordinate the development of the sex determination marker

Wei Cheng, *Fisheries Geneticist 1, ADF&G*
Duties: Lead the laboratory procedures to develop the sex determination marker

Ben Buzzee, Biometrician IV, ADF&G
Duties: Conduct the Trojan YY scenario modeling

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APPENDIX A: TISSUE SAMPLING

Adult Finfish Tissue Sampling for DNA Analysis

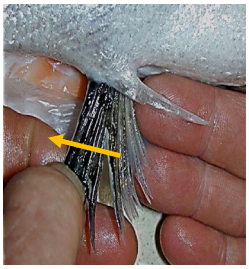
ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use fin tissues as a source of DNA to genotype fish. Genotyped fish are used to determine the genetic characteristics of fish stocks or to determine stock compositions of fishery mixtures. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible.

Preservative used: Silica desiccant bead packet dries and preserves tissues for later DNA extraction. Quality DNA preservation requires **dry storage (**with desiccant packs) in Pelican case or watertight file box.

II. Sampling Method



Pelvic fin located below axillary spine.

To secure fins to card for handling, place one staple across fin clip (shown above).

III. Sampling Instructions

- **Prior to Sampling:**
  - Set up workspace and fill out required collection information (upper left-hand corner and latitude/longitude).
  - Place Whatman genetic card (10WGC) on mini clipboard flat for easy access. One Whatman card per scale card. Same card can be used throughout same day.
- **Sampling:**
  - Wipe excess water and/or slime off the pelvic fin prior to sampling to avoid getting excess water or fish slime.
  - Fin clip will be taken from lower portion of the pelvic fin.
  - Cut off a portion of the fin clip using Fiskar scissors to get roughly a **¾ - 1" inch maximum** piece and/or about the size of a small fingernail (see cutting line to left in orange).
  - Place one clipped fin tissue onto appropriate grid space. Follow sampling order printed on card - **do not deviate**. If large tissue sample, center tissue diagonally on grid space.
  - **Only one fin clip per fish into each numbered grid space**.
  - Fin clips will stick to the 10WGC grid card (see photo).
  - **Staple** fin clip to card; this secures the fin for handling in lab.
  - **DO NOT** staple landscape cloth to paper edge.
  - Sampling complete.
  - Periodically, wipe or rinse the scissors with water so not to cross contaminate samples.
  - Insert the 10WGC card inside Pelican case and layer with blotter cards and desiccant packs.
  - Close and secure the lid of Pelican box so drying begins.
  - Data to record: Record **each fin clip number to paired data** information (i.e. location, lat./long., sample date(s), etc.). Electronic version preferred.
- **Loading Pelican Case:**
  - 1st card: Remove blotter papers and desiccant packs (remove plastic) from Pelican case. Place first card in Pelican case with tissues facing up. Next, place blotter paper directly over card and place one 2 desiccant packs on top. Close and secure lid so drying begins.
  - Up to 4 cards can be added per case. Add them so **tissue samples always face the desiccant pack** through blotter paper: 2nd card facing down between desiccant packs; 3rd card facing up between desiccant packs; and 4th card facing down on top of second desiccant pack. Close and secure Pelican case after inserting each card.
  - All cards must **remain in Pelican 1400 case at all times** to dry flat.
- **Post-sampling storage:**
  - Store dried 10WGC tissue cards in Pelican box at room temperature or below. Two-four desiccant packs fit inside Pelican 1400 case. This helps flatten the cards as they dry out over time.
- **Shipping at end of the season:**
  - Keep all **dried** cards layered inside Pelican box with secured lid, pack inside priority mailing box with returning sampling supplies.

IV. Supplies included in sampling kit:

1. Scissors - for cutting a portion of lower tip of selected fin.
2. Stapler – for stapling fin clip to card; secures for handling
3. Staples – for stapling
4. Whatman genetics card (10WGC) – holds 10 fish/card.
5. Silica packs – desiccant removes moisture from samples.
6. Pelican case – overnight dry storage "day use" (small 1150).
7. Pelican case – long term dry storage prior return shipment (1400).
8. Blotter cards – insert between 10WGC and desiccant pack.
9. Zip ties – to secure closure of the Pelican case for return shipment.
10. Laminated "return address" labels.
11. Sampling instructions.
12. Pencil

V.

Return to ADF&G Anchorage lab:

ADF&G – Genetics
333 Raspberry Road
Anchorage, Alaska 99518

Lab staff: 907-267-2247
Judy Berger: 907-267-2175
Freight code: _____

APPENDIX B: POPULATION MODEL

Appendix B1.–R code for northern pike population model.

```
titlePanel("Pike Supression Modeling"),
  tabsetPanel(
    tabPanel("Plot", plotOutput("distPlot"),
# create one row, each element is a column of inputs
     fluidRow(
      # column 1
      column(3,
      radioButtons(inputId = "growth",
                   label = "Type of Growth",
                   choices = list("Logistic" = 1,
                                  "Exponential" = 2)),
      numericInput(inputId = "K",
                   label = "Carrying Capacity",
                   value = 5000),
                 textInput(inputId = "n",
                  label = "Intial age class abundances (comma sep)",
                  value = "1000,500,200,100"
      )
      # column 2
      column(3,
                     h3("Survival"),
                     sliderInput(inputId = "s1",
                    label = "Yearly Survival % Age 1",
                    min = 0, max = 1, value = .25),
      sliderInput(inputId = "s2",
                    label = "Survival % Age 2-3",
                    min = 0, max = 1, value = .6),
      sliderInput(inputId = "s3",
                    label = "Survival % Age 4-5",
                    min = 0, max = 1, value = .6),
      sliderInput(inputId = "s4",
                    label = "Survival % Age 5+",
                    min = 0, max = 1, value = .4)
      ),
      # column 3
      column(3,
                     h3("Fecundity"),
      numericInput(inputId = "f1",
```

-continued-

```
            label = "Age 1 produced by Age 1",
            value = 0),
      numericInput(inputId = "f2",
            label = "Age 1 produced by Age 2-3",
            value = 5),
      numericInput(inputId = "f3",
             label = "Age 1 produced by Age 4-5",
             value = 10),
      numericInput(inputId = "f4",
             label = "Age 1 produced by Age 5+",
             value = 15)
      ),
      # column 4
      column(3,
                    h3("Intervention"),
                    numericInput(inputId = "num_yy",
                   label = "Number of Age 1 YY males stocked each year",
                   value = 1000),
                    sliderInput(inputId = "materate",
                  label = "Probability of Successful YY Fertilization",
                  min = 0, max = 1, value = .5),
          sliderInput(inputId = "hrate",
                  label = "% of age 2+ harvested each year",
                  min = 0, max = 1, value = .4)
      )

     ) # end widget row
          ), # end panel
  tabPanel("Model Details",
        uiOutput('markdown'))
    ) # end tabsetPanel
  ) # end page
server <- function(input, output) {
   # ================================================================
  # DEFINE POP GROWTH FUNCTION
  # ================================================================
  # grow_pop is the primary modeling function. It is non-reactive (takes no inputs directly from ui)
```

-continued-

```
# outputs a vector of population sizes
 grow_pop <- function(n, A, K, H, nYY, pYY = .5, growth = 1){
    # n is the vector of intial age-class abundances of reproducing females
 # A is the leslie matrix - fecundity is the per capita number of females produced by each age class
 # K is the carrying capacity in terms of female fish
 # H is the harvest matrix: The diagonal elements are the percent of each age class harvested each year
 # nYY is the vector of initial YY male abundances - must be same length as n, and it is assumed the same number are stocked each year
 # pYY is the probability a YY male successfully pairs with a female - same for all YY males
       # sup_mat is to become the A matrix with fecundity supressed
 # N is a vector that keeps track of total population size
 # out is a matrix where each column is the vector of age-class abundances that year (females only)
  sup_mat <- A
I <- diag(length(n))
N <- NULL
out <- matrix(0, nrow = length(n), ncol = 25)
total_YY <- nYY
for (i in 1:25){
       N[i] <- sum(n)
  out[,i] <- n
  # the number of females that emerge next year will be reduced by
  # the percent of females that paired with YY males
  # The original fecundity rates are the ones that need to be suppressed each year
  # by a potentially different number of 'successful' YY males
       # Model mating as a bernoulli proccess
       # sYY is the total number of YY males that are successful
  sYY <- rbinom(n = 1, p = pYY, size = round(sum(total_YY)))
  # this is the proportion of females that pair off with YY males
  supress_rate <- sYY/N[i]
  # this suppression rate applies to all age classses, so we need a vector
  supress_vec <- rep(ifelse(supress_rate  <= 1, supress_rate, 1), times = nrow(A))
  # implicit assumption that YY males mate uniformly across age classes
  # apply suppression rate to fecundity values
  sup_mat[1,] <- A[1,]*(1-supress_vec)
  if(growth == 1){
   # logistic growth
   # source for below: A.L. Jensen 1995 page 46 equation 15
   # The fish population grows - post breeding census
```

-continued-

```
    # This form of logistic growth is only useful when the intial abundances and first generations are below
    # the carrying capacity. Otherwise the numbers shoot off to +- infinity = NAN
    n <- n + ((K-N[i])/K)*(sup_mat - I)%*%n
            }else{
    # exponential growth matrix formulation
    n <- sup_mat%*%n
   }
   # of the ones that survive, remove some
   n <- n - H%*%n
   # next years YY stock consists of a new stock of age 1's plus those that survived from previous years
   # zero fecundity since they produce no females
       B <- A
   B[1,] <- rep(0, times = length(B[1,]))
       total_YY <- nYY + B%*%total_YY
      }
    return(data.frame(pop_size = N))
}
# ================================================================
# Collect Inputs using Reactive Expressions
# ================================================================
 # initial abundances
n <- reactive({
 return(as.numeric(unlist(strsplit(input$n, ","))))
 })
 # leslie matrix
A <- reactive({
    A <- diag(length(n()))
 diag(A) <- 0
  A[2,1] <- input$s1
 A[3,2] <- input$s2
 A[4,3] <- input$s3
 # IMPORTANT NOTE: age 5+ stay in their age class if they survive
 # SO THE LAST AGE CLASS IS YEAR 4'S THAT AGE UP PLUS AGE 5'S THAT SURVIVE
 A[4,4] <- input$s4
  A[1,] <- c(input$f1, input$f2, input$f3, input$f4)
  return(A)
 })
```

-continued-

```
  # supression matrix - diagonal elements are the percents of each age class removed, so n - H%*%n
 # is how we can remove a percent of each age class year to year
 H <- reactive({
  H <- diag(length(n()))
  diag(H) <- c(0, rep(input$hrate, times = length(n())-1))
  return(H)
  })
  # vector of number of yy supermales stocked each year
 nYY <- reactive({
  return(c(input$num_yy,0,0,0))
  })
  # ================================================================
 # CREATE PLOT
 # ================================================================
  output$distPlot <- renderPlot({
     # inputs taken from reactive expressions
     z <- grow_pop(n=n(), A=A(), H=H(), nYY = nYY(), pYY = input$materate, K = input$K, growth = input$growth)
     xint <- ifelse(test = is.finite(min(which(z$pop_size < 1))), yes = min(which(z$pop_size < 1)), no = NaN)
     z %>% ggplot(aes(x = 1:length(pop_size), y = pop_size)) + geom_line(size = 1.05) +
       geom_vline(aes(xintercept = xint, color = paste("Year:", xint)), show.legend=T) + xlab("Year") + ylab("Number of Females") +
       scale_color_manual(name = "Extirpation", values = "red") +
       theme(legend.position = c(.9,.9),
             panel.border = element_rect(colour = "gray", fill=NA, size=1))
  })
  # ================================================================
 # Render details md file
 # ================================================================
 output$markdown <- renderUI({
  HTML(markdown::markdownToHTML(knit('details.Rmd', quiet = TRUE)))
 })
}
# Run the application
shinyApp(ui = ui, server = server)
# ==== TEST CODE ====
#
# leslie_log <- function(n, A, K, H, nYY, pYY = 1){
#
```

-continued-

```
#   # n is the vector of intial age-class abundances of reproducing females
#   # A is the leslie matrix - fecundity is the per capita number of females produced by each age class
#   # K is the carrying capacity in terms of total fish
#   # t is the number of years to run the model
#   # H is the harvest matrix: The diagonal elements are the percent of each age class harvested
#   # nYY is the vector of initial YY male abundances - must be same length as n, and it is assumed the same number are stocked each year
#
#
#   # sup_mat is to become the matrix with fecundity surpressed
#   # N is a vector that keeps track of total population size
#   # out is a matrix where each column is the vector of female abundances that year
#
#   sup_mat <- A
#   I <- diag(length(n))
#   N <- NULL
#   out <- matrix(0, nrow = length(n), ncol = 25)
#
#   for (i in 1:25){
#
#     out[,i] <- n
#     N[i] <- sum(n)
#
#
#     # print(c("n= ", n))
#     # print((K-N[i])/K)
#     # print(sup_mat-I)
#     # print((sup_mat-I)%*%n)
#     # print(((K-N[i])/K)*(sup_mat - I)%*%n )
#
#
#     # source for below: A.L. Jensen 1995 page 46 equation 15
#     # The fish population grows, post breeding census
#
#     n <- n + ((K-N[i])/K)*(sup_mat - I)%*%n
#
#     # of the ones that survive, remove some
#     n <- n - H%*%n
```

-continued-

```
#
#    # after removing some, add YY stock
#    # Fish released are Myy supermales, producing only xy males
#    # only a fraction of the YY stock (pYY) will successfully mate
#    # Dampen the number by the percent expected to successfully mate with a female
#    nYY <- nYY*pYY
#
#
#    # if there are more successful males than females, 90% of the age class will mate with a YY male
#    age_p <- ifelse(nYY/n < .99, nYY/n, 1)
#
#    # the number of females that emerge next year will be reduced by
#    # the percent of females that paired with YY males
#    # The original fecundity rates are the ones that need to be suppressed each year by a potentially different number of YY males
#    sup_mat[1,] <- A[1,]*(1-age_p)
#
#    # a year passes and apply survival rates to YY males (same as females) and add new stock
#    # zero fecundity since they produce no females
#    B <- A
#    B[1,] <- rep(0, times = length(B[1,]))
#    nYY <- nYY + B%*%nYY
#
#  }
#
#  #list(age_vec = out, pop_size = N)
#  return(list(out = out, pop_size = N))
# }
#
#
#
#
# A <- diag(4)
# A[2,1] <- .25
# A[3,2] <- .6
# A[4,3] <- .6
# A[4,4] <- .4
# A[1,] <- c(7,5,10,15)
```

-continued-

```
#
# H <- diag(4)
# diag(H) <- c(0)
#
# nYY <- c(100,0,0,0)
#
# n <- c("1000,50,40,10")
# n <- as.numeric(unlist(strsplit(n, ",")))
#
# z <- leslie_log(n=n, A=A, K=5000, H = H, nYY = nYY)
#
# sum(z$out[,12])
# plot(z$pop_size)
# diag(A) <- 0
# A[1,] <- rep(0, times= 4)
# diag(A) <- 0
# A[4,4] <- .6
#
#
# nYY <- c(100,0,0,0)
#
# bYY <- c(0,0,0,0)
#
# for (i in 1:10){
#   bYY <- nYY + A%*%bYY
#   print(bYY)
# }
# N <- NULL
# K <- 5000
# n <- c(200,100,100,900)
# sup_mat <- diag(c(.25,.6,.6,.6))
# I <- diag(length(n))
#

# for(i in 1:30){
#   N[i] <- sum(n)
#   n <- n + ((K-N[i])/K)*(sup_mat - I)%*%n
# }
```