Regional Operational Plan No. ROP.SF.2A.2023.08

Operational Plan: Early Detection Monitoring for Dreissenid Mussels in Southcentral Alaska

by

Tammy Davis

Kristine Dunker

Robert Massengill

Eric Wood

and

Parker Bradley

June 2023

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.2023.08

OPERATIONAL PLAN: EARLY DETECTION MONITORING FOR DREISSENID MUSSELS IN SOUTHCENTRAL ALASKA

Tammy Davis
Alaska Department of Fish and Game, Division of Sport Fish, Juneau
Kristine Dunker
Alaska Department of Fish and Game, Division of Sport Fish, Anchorage
Robert Massengill and Eric Wood
Alaska Department of Fish and Game, Division of Sport Fish, Soldotna
and
Parker Bradley
Alaska Department of Fish and Game, Division of Sport Fish, Palmer

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

June 2023

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Tammy Davis,
Alaska Department of Fish and Game, Division of Sport Fish,
1125 W. 8th, Juneau, AK 99801

Kristine Dunker,
Alaska Department of Fish and Game, Division of Sport Fish,
333 Raspberry Rd., Anchorage, AK 99518-1565

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

and

Parker Bradley,
Alaska Department of Fish and Game, Division of Sport Fish,
1801 S. Margaret Dr, Suite 2, Palmer, AK 99645-6736, USA

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SIGNATURE/TITLE PAGE

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Project leader(s):	Tammy Davis, Invasive Species Program Coordinator (Project lead) Kristine Dunker, Regional Invasive Species Coordinator (Area lead); Eric Wood, Fisheries Biologist II (Area lead); Parker Bradley, Fisheries Biologist II (Area lead)
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Approval

Title	Name	Signature	Date
Project leader	Tammy Davis		
Co -Project leader	Kristine Dunker		
Co -Project leader	Eric Wood		
Co -Project leader	Parker Bradley		
Research Coordinator	Timothy McKinley		

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF APPENDICES

ABSTRACT

This project will conduct early detection monitoring for dreissenid mussels in Southcentral Alaska with regional invasive species program staff. Surveys will be completed in 20 lakes in 2023. During this first year of monitoring, each lake will be surveyed for infrastructure, water quality, and settling of invasive dreissenid mussels. Dreissenid mussels are commonly known as zebra mussels *Dreissena polymorpha* and quagga mussels *Dreissena bugensis* (collectively referred to as ZQM). Survey protocols will be implemented to determine suitability for ZQM establishment based on water quality thresholds, and to detect presence of juvenile and adult life stages of ZQM. Water bodies have been prioritized for surveys using the Alaska Lakes Prioritization database. All water bodies will be sampled using standard protocols. If mussels are detected during implementation of this operational plan, rapid response efforts will be executed according to regional management priority.

Key words: aquatic invasive species, dreissenid mussels, *Dreissena polymorpha, Dreissena bugensis*, ZQM, Southcentral Alaska.

INTRODUCTION

PURPOSE

The Alaska Department of Fish and Game (ADF&G), Division of Sport Fish (SF) strategic plan identifies the need to minimize impacts of aquatic invasive species (AIS) on sport fish stocks and habitat. Further, it specifically calls on staff to develop and implement AIS field detection protocols. Similarly, the 2015 Alaska Wildlife Action Plan recognizes invasive species as a priority threat to native species, particularly in marine, freshwater, and island ecosystems (ADF&G 2015). In 2022, ADF&G piloted preliminary surveys for dreissenid mussels (zebra mussel *Dreissena polymorpha* and quagga mussel *Dreissena bugensis*) by field testing recognized protocols implemented in the Western US (Culver et al. 2009; Western Regional Panel on Aquatic Nuisance Species 2020). With lessons learned from feasibility work, this project will implement these field detection protocols for dreissenid mussels beginning in 2023. Early detection of AIS is widely accepted as one of the most cost-effective management actions to manage AIS before they become widespread and improves the possibility of eradication when feasible.

This plan describes how ADF&G will implement early detection protocols for long-term monitoring of invasive zebra and quagga mussels (ZQM; Figure 1) in lakes in Southcentral Alaska. Data collected during this project will contribute to existing water quality datasets (e.g., U. S. Environmental Protection Agency's Storage and Retrieval Data Warehouse, U. S. Geological Survey's National Integrated Water Information System, and Alaska Aquatic Nonindigenous Species Clearinghouse), establish baselines for dissolved calcium concentration in Southcentral water bodies, and verify the presence or absence of ZQM. Most importantly, these data will inform ADF&G's assessment and prioritization of waters suitable for sustaining ZQM survival, reproduction, and establishment and improve future project planning to maximize monitoring effort in locations with greatest risk of infestation, and to the extent possible, allow for rapid response. This plan outlines field sampling methods for target life stages of ZQM. The applicable protocols for preserving field samples and decontaminating equipment are also outlined and align with the minimum recommended standard protocols implemented in western states and provinces (Culver et al. 2009; Western Regional Panel on Aquatic Nuisance Species 2020).

Figure 1.–Image of dreissenid mussels: zebra (left) and quagga mussels (right), collectively referred to as ZQM throughout this plan.

Reprinted with permission by Colorado Department of Parks and Wildlife.

BACKGROUND

History of ZQM Introductions. Dreissenid mussels were introduced into the Great Lakes in the 1980s, arriving in ballast water of ships traveling from the Black Sea region (Pennak 1989; Mackie and Schlosser 1996; Mills et al. 1996). Suitable habitat and a lack of natural predators in these new locations facilitated rapid spread throughout the region, where ZQM formed massive colonies with densities of up to 75,000 organisms/ft^2 (McMahon 1996). Because of their ability to disrupt food chains, degrade water quality, and outcompete native species in Midwestern freshwater systems, ZQM quickly became notorious aquatic invasive species. In 2007, ZQM were detected for the first time west of the 100th Meridian at Lake Mead (AZ and NV) more than 1,200 miles from the nearest known infestation at the time. Since 2007, ZQM have spread to other water bodies in the west, primarily via trailered watercraft (Lucy et al. 1999, Johnson et al. 2001).

Western states have led initiatives to slow the spread of invasive ZQM, beginning with the Quagga-Zebra Mussel Action Plan (QZAP) for Western U.S. waters, which was written to identify priority actions needed to prevent and control the spread of ZQM. QZAP divides strategic actions into prevention, early detection, and rapid response. After QZAP was adopted by the Congressionally established Aquatic Nuisance Species Task Force (in 2010), many western states began active prevention, early detection, and rapid response programs that included interception of watercraft at water bodies, state lines, and main artery highways in the west. Watercraft Inspection and Decontamination Stations (WIDS) have been established in most western states. Depending on jurisdiction, WIDS provide an opportunity for mandatory or voluntary inspections for AIS, particularly ZQM on and in boats. When ZQM are found, watercraft are decontaminated or quarantined, depending on jurisdictional requirements. Millions of boats have been inspected and decontaminated in the west in the past decade, showing WIDS are a proven means to slow the spread of dreissenid mussels (Drury and Rothlisberger 2008; Fischer et al. 2021).

Vectors of ZQM Introductions to Alaska. Recreational boating continues to be the primary vector for overland transport of ZQM and many other AIS (Lucy et al. 1999; Johnson et al. 2001). The ongoing discovery of trailered recreational watercraft harboring dreissenid mussels at inspection stations in British Columbia and Alberta, Canada, within the Columbia River Basin, and throughout the western United States, corroborates the importance of this pathway (Karatayev et al. 2007). Thus, trailered recreational watercraft are considered a primary means of transporting ZQM from infested water bodies in the Lower 48 states and Canadian provinces to Alaska.

From 2014 to 2020, the U. S. Fish and Wildlife Service (USFWS) positioned law enforcement staff at the Alcan Port of Entry (POE) to inspect boats entering Alaska for a single 10- to 14-day operation annually (pers. Com Aaron Martin, USFWS). Inspectors collected data on boats and

their movements, including locations from which they departed, prior visitation to any known ZQM infested waters, and the last water body from which it launched

Starting in 2021, USFWS inspectors have annually operated a WIDS between May and September. During hours of operation, all watercraft arriving at the Alcan POE from Canada are intercepted. Boats are inspected for signs of ZQM, and when observed, watercraft are decontaminated with pressurized hot water. Watercraft entering Alaska through other POEs and during periods without ZQM surveillance at the Alcan POE are not inspected and pose a risk to Alaska waters. As of today, an approved method for large-scale eradication of ZQM does not exist; therefore, early detection at sensitive waterbodies followed by containment are essential for preventing widespread distribution of these harmful aquatic invasive species. Should ZQM be detected in Alaska, state and federal entities are currently not prepared to contain this aquatic invasive species to source locations. Data collected during the implementation of this project will inform risk assessments and water body prioritization schemes. The spread of AIS, including ZQM, is a major social, environmental, and economic concern (Escobar et al. 2017). Based on data from western states, surveillance efforts in tandem with boat inspection and decontamination have been proven to protect state waters, aquatic resources, and facilities from the deleterious effects of ZQM establishment.

ZQM Morphology. Dreissenid mussels are small, triangular-shaped freshwater bivalves that display a diversity of shell morphologies. Adults are typically 1 to 3 cm in shell length, and juveniles range between 350 µm to about 5 mm in size.

The zebra mussel is named for the striped pattern of its shell. However, color patterns can vary from striped to plain dark or light-colored shells with no stripes. Zebra mussels are typically found attached to objects, surfaces, or other mussels by byssal threads extending from underneath the shells (Fig. 1). Although similar in appearance to quagga mussels, the two species can be distinguished by their shell morphology. The ventral plane of the shell is a reliable diagnostic feature for zebra and quagga mussels (Figure 2). When placed on a surface, zebra mussels are stable on their flattened ventral underside whereas quagga mussels, lacking a flat ventral plane, will fall over. When both zebra and quagga mussels occur in the same area, differentiation can be more difficult due to the phenotypic plasticity seen in quagga mussels, and thus genetic identification is necessary at times (Karambrun et al. 2018).

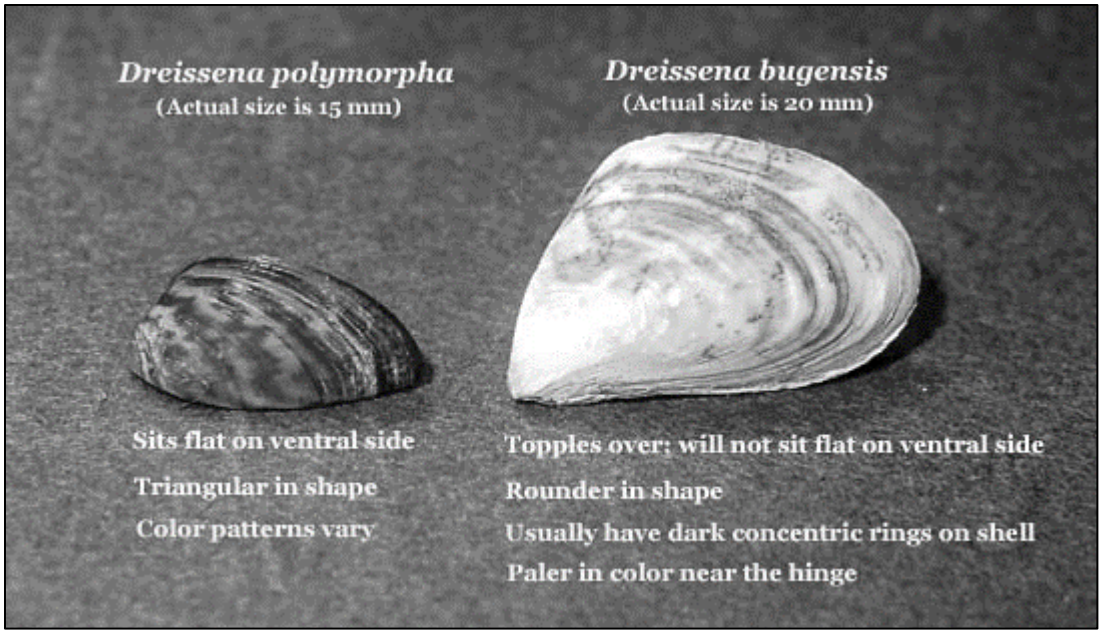

Figure 2.–Shell morphology of zebra (left) and quagga (right) mussels.

Note: Ventral side is flat on zebra mussel shells and quagga mussels lack a ventral side.
Photo credit: U. S. Geological Survey, Myriah Richerson.

Endemic Freshwater Mussels. Several species of freshwater mussels are native to Southcentral Alaska. Prior to 2004, the distribution, abundance, and ecological roles of the dominant, native freshwater mussel species in Alaska had been poorly documented. That year, the University of Alaska surveyed a collection of Southeast and Southcentral Alaska lakes and streams. *Anodonta beringiana* was detected in 54 systems surveyed and of those, 29 were in Southcentral Alaska. Because it is a relatively common species, *A. beringiana* is anticipated to be encountered during monitoring efforts for this project.

ZQM Life Cycle. In suitable conditions, ZQM can live up to 9 years (average life span is 4–5 years) and become reproductive in their second year. A single female mussel can release millions of gametes. The dreissenid mussel life cycle consists of 3 primary life stages: veligers (free-swimming microscopic larvae, usually lasting 2 to 3 weeks), juveniles (various stages of development occurring after a veliger has settled and begins to develop a shell), and adult (reproductive stage). If ZQM were introduced into a water body in Southcentral Alaska, the water quality and other habitat parameters must be suitable for survival, settlement, and reproduction for establishment to occur.

Risk Factors for ZQM Establishment. Total recreational use of a water body, presence of boat ramps and marinas, water body size and types of access, and the presence of motorized boating and fishing activities, are all important determinants in how likely a water body will become infested with ZQM. Additionally, the success of ZQM establishment is contingent upon water quality parameters, such as dissolved calcium, pH, water temperature, salinity, and to a degree, dissolved oxygen (Sprung 1987; Hincks and Mackie 1997). Because dissolved calcium concentration and pH are the most limiting environmental parameters to ZQM establishment in the Columbia River Basin and the greater Northwest (McMahon 1996; Hincks and Mackie 1997), the same conditions are assumed to apply in Alaska.

Once ZQM are established, water temperatures in Alaska are not expected to limit growth because they inhabit a wide range of temperatures in North America (Depew et al. 2021). They have been found to survive at temperatures between zero and 31°C (McMahon 1996; Karatayev et al. 1998; Texas Parks and Wildlife Department 2010). Although there is variability in thermal tolerance among adult ZQM, water temperatures of 9°C or less and 18°C or greater are unsuitable to sustain ZQM veligers (Churchill 2013; Locklin et al. 2020). ZQM are susceptible to hypoxia-induced mortality. Dissolved oxygen greater than 2 mg/L is necessary for adult survival, but 50–90% dissolved oxygen saturation is optimal for all life stages (Texas Parks and Wildlife Department 2010). Waters with pH greater than 6.9 are necessary for adult survival, and pH greater than 7.1 and 7.4 are optimal for juveniles and veligers, respectively (Claudi et al. 2012). Salinity values greater than 14 ppt and turbidities greater than 80 nephelometric turbidity units are limiting to all life stages of ZQM (Texas Parks and Wildlife Department 2010).

Adult ZQM are not expected to reproduce or survive in waters with dissolved calcium concentrations below 12 mg/L (Hincks and Mackie 1997). Freshwater systems with calcium concentrations 12 mg/L to 15 mg/L provide suitable conditions for juvenile and adult ZQM survival. Reproduction will not occur in these concentrations because young veligers cannot survive. However, if late-stage veligers were introduced into these conditions, they may settle and persist. Once calcium concentrations exceed 15 mg/L, some successful reproduction is expected, if all other conditions are suitable. As calcium concentrations increase, the rate of successful reproduction and veliger survival increases. In waters with calcium concentrations above 24 mg/L, calcium is not a limiting factor for any life stage.

OBJECTIVES

This project will provide information about presence or absence of ZQM in Southcentral Alaska fresh waters, characterize lakes by vulnerability for ZQM infestation based on site specific variables that include lake access, infrastructure presence, public use patterns, and select water quality parameters including dissolved calcium, dissolve oxygen, pH, temperature, salinity and water clarity.

All protocols implemented to meet the objectives of this project will be done as staff capacity allows.

PRIMARY OBJECTIVES

1) Inventory and characterize public and private access infrastructure (docks, piers, platforms) at all monitoring locations, particularly the number and type of boat launches present.
2) Document water quality parameters at surface and depth for all monitored waters, including pH, salinity, temperature, and water clarity, and collect water samples for dissolved calcium.
3) Deploy artificial substrates in all monitoring locations to detect juvenile and adult ZQM.
4) Evaluate water chemistry and water quality to categorize water bodies by suitability for ZQM.

SECONDARY OBJECTIVES

1) Generate location maps of sampled water bodies that include water quality data to inform planning for long-term monitoring.
2) Upload all monitoring data into the Alaska Aquatic Nonindigenous Species Clearinghouse and share with databases at other entities (i.e., Environmental Protection Agency, U. S. Geological Survey).
3) Inventory existing invasive species signage present at monitoring locations.

METHODS

STUDY AREA

This project will monitor at least 20 lakes in Southcentral Alaska for ZQM (e.g., Figure 3 and Table 2). These locations were chosen using the Alaska Lakes Prioritization Database (ALPD): https://accsmaps.maps.arcgis.com/apps/instant/sidebar/index.html?appid=d7b31e186b48463c844bfc88f15c6476 (Figure 4). This tool was developed by University of Alaska, Anchorage in collaboration with ADF&G to prioritize water bodies based on parameters that allow for the invasion of Elodea (*Elodea sp*.) and northern pike (*Esox lucius*). Database inputs include boat launch presence, float plane accessibility, distance to roads, and other variables that are used to rank waters based on vulnerability to introduction of AIS. Because these same variables are relevant to ZQM introductions, the 2023 sample locations were selected based on the most vulnerable lakes in this database. In addition, the lakes that appeared vulnerable to as few as 1 life stage of ZQM based on data collected in the 2022 feasibility monitoring were also added to this list (Appendices A1 and A2). Secondarily, consideration was given to the feasibility of staff implementing monitoring protocols at prioritized waters.

STUDY DESIGN

A minimum of 20 high priority lakes were selected for monitoring in 2023 (Figures 3 and 4). All lakes will be surveyed for infrastructure presence (Objective 1), water quality and dissolved calcium (Objective 2), and settlement of juvenile and adult mussels (Objective 3). The lakes chosen for monitoring are within easy travel distance from ADF&G area offices and are either high-risk according to the ALPD or were identified as suitable for survival for at least 1 life stage of ZQM during 2022 feasibility work. Following implementation of protocols described below for Objectives 1–3, data will be evaluated after the season is over to determine suitability for ZQM survival (Objective 4).

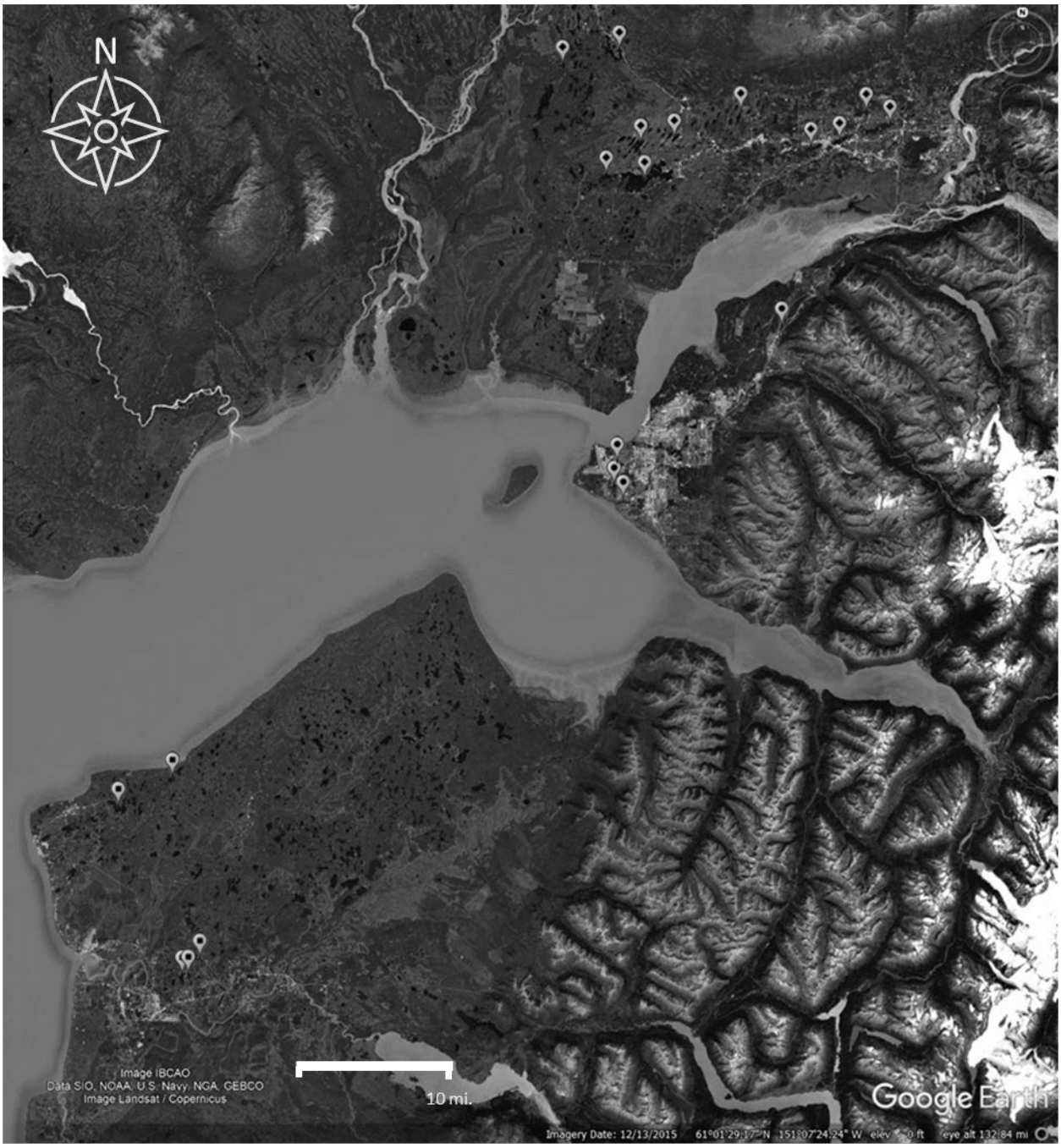

Figure 3.–ZQM sample locations in the Matanuska–Susitna Valley, Anchorage, and the Kenai Peninsula for 2023, marked with pins.

Table 1.–List of ZQM monitoring locations prioritized for 2023.

Region	Locations for 2023	Secondary list (as capacity allows)
Matanuska-Susitna	Anderson Lake	Alexander Lake
	Big Beaver Lake	Fish Lake
	Big Lake	Kashwitna Lake
	Finger Lake	Long Lake
	Flat Lake	Sucker Lake
	Horsehoe Lake	Trapper Lake
	Lucille Lake	
	Nancy Lake	
	Seymour Lake	
	South Rolly Lake	
	Wasilla Lake	
Anchorage	Campbell Lake	Beach Lake
	Lake Hood	Cheney Lake
	Lower Fire Lake	Mirror Lake
	Sand Lake	Sixmile Lake
		Take Lake
		West Chester Lagoon
Kenai Peninsula	Daniels Lake	Hidden Lake
	East Mackey Lake	Kenai Lake
	Sevena Lake	Skilak Lake
	Stormy Lake	
	West Mackey Lake	
Prince William Sound and Copper River		Bering Lake
		Eyak Lake
		McKinley Lake
		Martin Lake

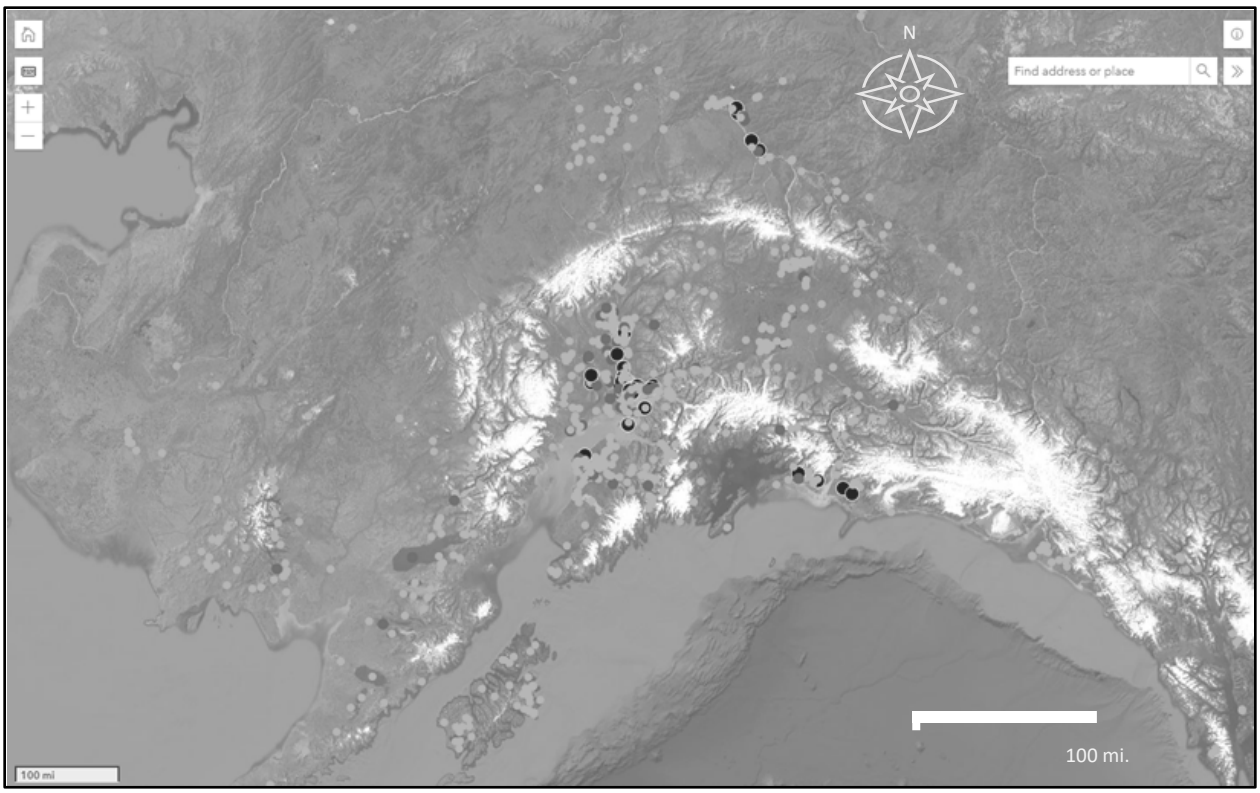

Figure 4.–Screenshot from the AK Lakes Prioritization Database (accessed March 29, 2023, 5:35:00 PM).

Source: https://accsmaps.maps.arcgis.com/apps/instant/sidebar/index.html?appid=d7b31e186b48463c844bfc88f15c6476.
Note: Based on access, dark locations are vulnerable to AIS incursions.

DATA COLLECTION

Primary Objective 1: Document infrastructure

The risk of exposure to invasive ZQM for individual water bodies is gauged by multiple factors that involve access and user activities. The ADF&G Alaska Lakes Database and other agency resources do not always document the presence of infrastructure. A primary objective of this project is to document the number and types of infrastructure available for watercraft, such as presence of developed or primitive boat launches and docks, both public and private. This objective includes observation of absence or presence, quantity and type of access infrastructure, and a description and location of any aquatic invasive species signage posted at the water body. Based on the 2022 feasibility work, it was determined that quantifying infrastructure prior to visiting the site via online mapping sources such as Google Earth, Google Maps, or online Borough Maps was highly efficient. Maps of each monitoring location will be saved as a PDF and archived with the monitoring data. Printed or digital copies of the maps will be taken into the field to verify the current infrastructure. Verified infrastructure data will be recorded (Appendix B1).

Primary Objective 2: Document water quality parameters

Water Quality Sampling

1) Water sampling for dissolved calcium concentration

Water samples will be collected twice a year in the spring and fall by ADF&G and then analyzed for dissolved calcium by the University of Alaska, Applied Science, Engineering, and Technology Lab under supervision by Dr. Patrick Tomco.

Based on a usable compromise between extensive sampling and limited staff capacity, the number of sampling sites per water body will be determined by lake size as follows[1]:

- lakes ≤ 100 surface acres 1 sample
- lakes 101–200 surface acres 2 samples
- lakes 201–400 surface acres 3 samples
- lakes 401–800 surface acres 4 samples
- lakes >800 surface acres 1 additional sample for each 400 acres.

The size of the lake will be determined using either an online mapping tool such as Google Earth, or if the lake has been mapped for bathymetry by ADF&G, from the acreage estimates produced by mapping. For lakes over 800 surface acres, 1 additional sampling site will be added for each additional 400 acres. For example, lakes between 801 and 1,200 acres will have 5 sample sites, a 1,201 lake will have 6 sample sites, etc. Big Lake, which is approximately 3,000 acres, will have 10 sample sites. For lakes requiring more than 1 sample, sites will be evenly distributed around the water body to maximize capture of spatial variability. Sites will be proximal to boat launches or infrastructure at all water bodies, where possible.

In addition to lake water sampling, dissolved calcium sampling includes collecting 2 types of blanks (filtered blanks and preservation blanks) to serve as controls for the analysis of water samples (Appendix C1). Blanks only need to be collected at the first sampling site at each lake per sampling event.

All samples and blanks will be preserved with 0.5 ml of ultrapure nitric acid solution using a pipette. Preservation of samples can occur on site or later in the day after all samples have been collected, at a location where handling small amounts of nitric acid solution is safe. One pipette will be used to add nitric acid solution to filtered blanks, preservation blanks, and water samples collected the same day, unless the pipette comes into contact with lake water, in which case the pipette will be discarded. Each pipette will be discarded after one day of use. Once preserved with nitric acid, samples and blanks are shelf stable without refrigeration for about 6 months and will be submitted for analysis before then.

Filtered blank samples will be prepared using distilled or deionized water (DI), which is preferred. The DI water will be used to rinse a 60 ml syringes 3 times, and then 60 ml of DI water will be drawn in and a new 0.45 µm filter will be screwed onto the syringe. Next, the 60 ml of DI water will be filtered from the syringe into a labeled sample bottle. The bottle will be placed in a cooler. Filtered blank samples will be preserved using a pipette to add 0.5 ml of ultrapure nitric acid solution to the sample.

[1] Note that sampling for Objectives 2 and 3 will occur at the same monitoring locations.

Preservation blank samples will be prepared using the same syringe as was used for the filtered blank sample to draw 60 ml of DI water. This time, without filtering it, the DI water will be syringed into a labeled sample bottle. The sample will be placed in a cooler. Preservation blank samples will be preserved by using the same pipette as used for the filtered blanks to add 0.5 ml of ultrapure nitric acid solution to the sample.

After both blanks have been collected and stored in a cooler, collection of water samples will begin. A 60 ml syringe will first be rinsed with lake water 3 times. Then, 60 ml of lake water will be drawn into the syringe from the sampling site and a new 0.45 µm filter will be screwed onto the syringe. All the water in the syringe will be filtered into a labeled sample bottle. If the filter clogs before all water has been filtered, the filter will be replaced with a new one and filtering will continue until all water has been filtered into the sample bottle. The sample will be placed in a cooler. Samples will be preserved by using a pipette to add 0.5 ml of ultrapure nitric acid solution to the sample bottle when at a location where the nitric acid is safe for handling.

2) Water quality sampling: pH, dissolved oxygen, temperature, salinity and turbidity

Water quality sampling will occur concurrently and at all sites where dissolved calcium samples are collected and artificial substrates are deployed. Staff will determine a unique identifier for the site, collect GPS coordinates in decimal degrees (World Geodetic System 84 standard) and then follow water quality sampling protocols (Appendix C2).

Discrete values for salinity, pH, dissolved oxygen, and temperature will be collected using a Hydrolab or YSI sonde (Appendix C2). The sonde will be lowered to 1 meter below the surface to collect surface measurements and then 1 meter above the bottom to collect bottom measurements. The sonde will be held at depth for as long as is needed for values to stabilize. If the site where the dissolved calcium sample was collected is less than 2 m deep, the water quality sample will be taken in deeper water as close to the site as possible.

To measure water clarity, a Secchi disk will be lowered until it is no longer visible, then brought back up until visible again, noting depth. This will be repeated twice for a total of 3 measurements. The three depths will be denoted and used to calculate an average depth for the sample site (to the nearest 0.1 meter). If the sampling site is too shallow to measure water clarity, the Secchi disk will be moved into deeper water as close to the site as possible.

Primary Objective 3: Monitor for settlement of ZQM by deploying artificial substrates

Artificial substrate (AS) deployment sites will co-occur where calcium samples and water quality measurements are taken (see Objective 2 above). AS will be deployed in the spring in nearshore locations where in-water structures are available to tether to or in open water tethered to a buoy (Appendix C3). If only 1 site is needed, the AS will be attached at the site nearest to the infrastructure of greatest recreational use. If infrastructure is not present, a site near the middle of the lake will be selected, and the AS will be tethered to an ADF&G-marked buoy. For lakes requiring more than 1 sample site, at least 1 site will have an AS tethered to the in-water structure or tethered to a marked buoy in close proximity to the structure. Other sites at the water body will be distributed evenly throughout the lake and tethered to ADF&G-marked buoys. Sites will be selected to minimize vandalism. When private infrastructure is the best location for AS, permission from the infrastructure owners will be received prior to deployment. The invasive species program coordinator can initiate communication with owners at the request of staff.

AS will be secured to in-water infrastructure, floating structures, or buoys using parachute cord, line or chain at approximately 1 meter from the bottom at each site. If AS sampling sites are deep (>20 m), an additional AS will be hung on the same line 1 meter below the surface. AS will be deployed when water quality samples are collected, as early in the open-water season as staff time allows. The AS will be retrieved at the end of the open water season in October.

A colored cable tie will be attached to the AS parachute cord or line at the water surface for deployments from stationary infrastructure, and the area will be marked by flagging if tied to nearshore structures (Figure 5). The cable tie will distinguish ADF&G ownership. AS will be placed in locations where water level variability does not affect depth of AS, so sites will be chosen to allow immersion of AS throughout the field season. ADF&G tags will be attached at the bottom of the AS. If AS deployed above the lake bottom are not heavy enough to remain at target depth, a small weight may be attached to keep it in place.

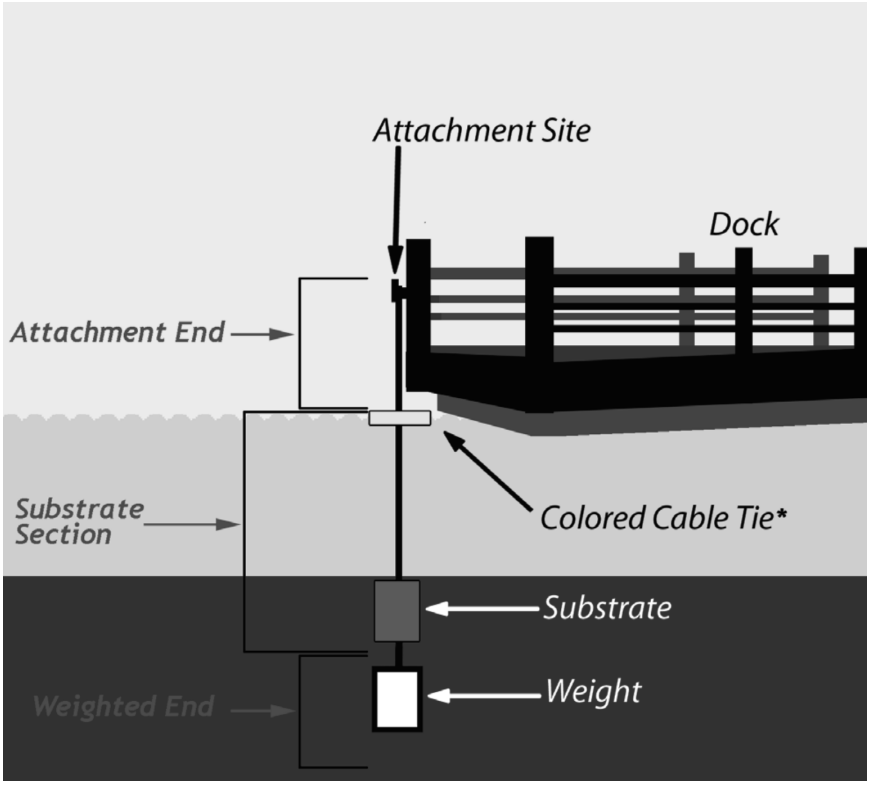

Figure 5.–Example of artificial substrate sampler deployment.

Note: Colored cable tie* denotes the location of the surface of the water. This mark ensures that the substrates are attached at appropriate locations on the line and the line is deployed correctly to sample the intended water depth(s). Illustrations by California Department of Fish and Wildlife.

At the end of the open-water season, AS will be retrieved, placed in a plastic tub, and visually examined for settled juvenile and adult mussels. Tactile examination will be used to detect organisms smaller than visible to the eye. Recently settled post-veliger mussels will be very small so if the AS surfaces have bumps that feel gritty like sandpaper, have seed-like bumps, or are heavily fouled with suspicious organisms, the AS will be placed in a plastic bag to be examined thoroughly later in a well-lit indoor location. If larger organisms can be removed, they will be

placed in a labeled container and preserved with ethanol if they cannot be identified to species. Photos will be taken of surfaces and collected samples of unknown or suspicious organisms.

The morphology of the native freshwater mussel, *A. beringiana,* is notably different than dreissenid mussels; thus, distinguishing between the two should not be problematic. One key difference is that adult dreissenid mussels attach to surfaces with byssal threads whereas native mussels lack them. However, any mussels that cannot be identified to species observed during surveys will be collected and analyzed by the University of Alaska, Anchorage.

Primary Objective 4: Categorize water bodies by ZQM suitability and invasion risk.

Dissolved calcium samples will be analyzed in the lab after the season is over. Once results are received from the lab, dissolved calcium values will be added to the monitoring records for each lake in the data file. Based on these data, lakes will be categorized according to suitability for ZQM, which will determine future sampling plans. Suitability parameters will be identified as follows:

- < 12 mg/L Ca2+ = Unsuitable
- 12–14 mg/L Ca2+ = Low Suitability
- 15–23 mg/L Ca2+ = Moderate Suitability
- ≥ 24 mg/L Ca2+ = High Suitability

DATA REDUCTION

For all survey protocols, either a sample site data sheet (Appendix B2) will be completed in the field or the data will be entered directly into the project data file (Figure 6) using field tablets. Upon completion of surveys all field data will be entered into the project data file. Data consistency and accuracy is important for collating information across different collectors, and for providing these data to databases such as the Alaska Aquatic Nonindigenous Invasive Species Clearinghouse.

For UAA	**Sample Number**	Sequential number of samples (#1-X) to easily assign calcium values in the lab
(For lab purposes)	**Location**	Lake name
	Calcium	Fill in with lab results
	Filtered Blank	Fill in with lab results
	Preservation Blank	Fill in with lab results
2023 Survey Locations	**Location**	Lake name
	Region	Area lakes is in (Anchorage, Kenai, Mat-su, JBER, West Cook Inlet, etc.)
	Surface Acreage	Record surface acreage if known
	# of Survey Sites	Number of survey sites in the lake
	Public Boat Launches/ Docks	Liist the number of public boat launches or docks
	Private Boat Launches/ Docks	List the number of private boat launches or docks
	Other Infrastructure	Fill in if applicable
	Comments	Fill in if applicable
Dreissinid Survey Data	**Sample Number**	Sequential number of samples to easily assign calcium values in the lab
	Location	Lake name
	Section ID	Unique section ID (i.e., XXL_Year_Site#)
	Date	Record the date
	Collectors	Record initials of the field crew
	Latitude	Record GPS coordinates
	Longitude	Record GPS coordinates
Substrate Data	**Depth (m)**	List the depth the substrate is suspended at
	Attachment	List what the substrate is attached to
	Condition	When setting out substrates, record "Deployed"; When retrieving substrate, record "Retrieved, Stolen", or whatever is most applicable
	Surface Description	invertebrates, biofilm, etc.); If the substrate is being deployed, record "N/A"; Provide more detail in the comments if needed
	Detection	Recored "ND" for non-detect. "Detected" if ZQM are found, or N/A if no substrate is associated with the sample
	Time	Record the time
	Temp. (Surface)	YSI Data
	DO mg/L (Surface)	YSI Data
	DO % (Surface)	YSI Data
	Salinity (Surface)	YSI Data
	pH (Surface)	YSI Data
Water Quality Data	**Temp. (Bottom)**	YSI Data
	DO mg/L (Bottom)	YSI Data
	DO % (Bottom)	YSI Data
	Salinity (Bottom)	YSI Data
	pH (Botttom)	YSI Data
	Visibility (M)	Secchi Disk Data

Figure 6.– Variables and metadata for ZQM monitoring data entry.

SCHEDULE AND DELIVERABLES

Dates	Activity
Spring 2023	Operational planning
Summer 2023	Survey Southcentral water bodies
Fall–Winter 2023	Data compiled
Winter 2023–Spring 2024	Prepare project report for funding entities

RESPONSIBILITIES

Tammy Davis, Fishery Biologist IV, ADF&G
Duties: Primary project biologist; coordinate planning, purchase equipment; provide oversight on project plans; enter and manage data; prepare project reports; coordinate and manage completion of project deliverables.

Kristine Dunker, Fishery Biologist III, ADF&G
Duties: Secondary project biologist; make recommendations on project plans; assist with data management; review and provide recommendations on project report.

Rob Massengill/ Eric Wood (in transition to position) Fishery Biologist II, ADF&G
Duties: Assist with planning, implementing field protocols; review and provide recommendations on project report.

Parker Bradley, Fishery Biologist II, ADF&G
Duties: Assist with planning, implementing field protocols; review and provide recommendations on project report.

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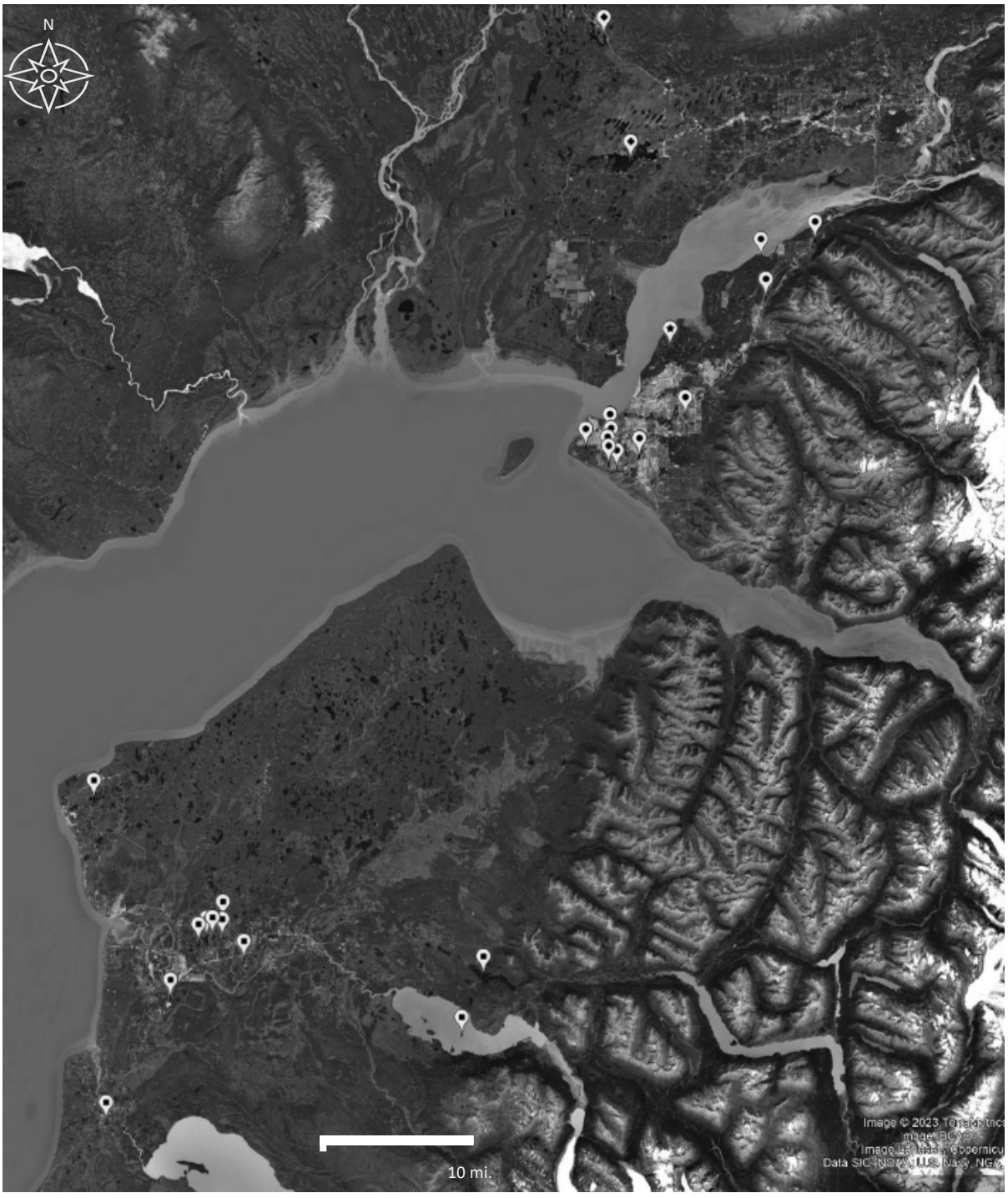
APPENDIX A: FEASIBILITY STUDY LOCATIONS

Appendix A1.–Feasibility study locations, 2022.

Area[a]	Waterbody	Calcium level[b]	Infrastructure[c]	Native mussels[d]
Matanuska-Susitna)	Nancy Lake	≥ 12 mg/L	Y	P
	Big Lake	≥ 24 mg/L	Y	N
Anchorage	Beach Lake	≥ 12 mg/L	N	N
	Campbell Lake	≥ 12 mg/L	N	N
	Cheney Lake	≥ 24 mg/L	N	P
	DeLong Lake		Y	N
	Jewel Lake		Y	P
	Lake Hood	≥ 12 mg/L	Y	N
	Little Campbell Lake		Y	N
	Lower Fire Lake	≥ 24 mg/L	N	P
	Mirror Lake	≥ 24 mg/L	Y	N
	Sand Lake		Y	N
	Sixmile Lake	≥ 24 mg/L	Y	N
	Taku Lake	≥ 24 mg/L	N	N
	West Chester Lagoon	≥ 12 mg/L	Y	N
Kenai Peninsula	Daniels Lake	≥ 12 mg/L	Y	N
	Derks Lake		Y	N
	East Mackey Lake	≥ 24 mg/L	Y	P
	Hidden Lake	≥ 24 mg/L	Y	N
	Island Lake		Y	P
	Johnson Lake		Y	N
	Longmere Lake		Y	N
	North Vogel Lake		N	N
	Sandpiper Lake		N	N
	Sevena Lake	≥ 12 mg/L	Y	N
	Skilak Lake	≥ 12 mg/L	Y	N
	Sport Lake		Y	N
	Tote Road Lakes		Y	N
	Vogel Lake		N	N
	West Mackey Lake		Y	N

Appendix A2.–Map of feasibility study locations in 2022.



APPENDIX B: DATA SHEETS

Appendix B1.–Infrastructure field data sheet.

Alaska Department of Fish and Game

In-Water Infrastructure Survey Datasheet

Water body: _____ **Collector:** _____

Date: _____ **Time:** _____

SITE Identifier	Launch/ Dock	Location		Type Primitive/ Improved	Type Public/ Private
		Lat	Lon		

AIS SIGNAGE

Identity (logos)	Name/desc.	Location		Species/Topic
		Lat	Lon	

Note: GPS coordinates do not need to be collected for private docks.

Appendix B2.–Sample site data sheet.

Water Quality/ Chemistry/AS Field Datasheet

Water body: _____ **Site Number:** _____

Collector: _____ **Date/Time:** _____

GPS Coordinates: _____

Water Quality

	Temp °C	pH	DO mg/L	DO %	Conductivity (specify units)	Salinity (PPT)	Secchi Depth
Surface							
Bottom							

Calcium Samples (mark if collected)

Dissolved Calcium _____ Preservation Blank_____ Filtered Blank _____

Artificial Substrates

Location description:	**Substrate depth (m):**
Deployment Date:	**Retrieval Date:**
Substrate (Circle one): Present Missing	**Substrate Condition** (Circle one): Intact Damaged
Mussels (Circle one): Present Absent	**Surface Description** (Circle one): Bio-film Gritty Seed-like
If present, approximate number of mussels (Circle one)	1-10 11-100 100+
Comments:	

APPENDIX C: PROTOCOLS

- Wear gloves and eye protection when handling acid.
- Empty the 60 ml syringe into the sample bottle.
- Samples should be kept in a cool and dark environment when possible until preserved with acid.
- If nitric acid is added to all samples at the end of the day, one pipette can be used. If a pipette comes into contact with lake water, discard it and use a new one.
- Use a new syringe at each site.
- All samples must be labeled: Date, location, unique sample identification.

1. **Dissolved Calcium Sampling Blanks**.
   Prepare one of each type of blank sample for each water body, if sampling more than one site per water body, blank samples to be prepared only at the first site visited.

   a. Equipment:

   i. Black Sharpie
   ii. Labeled filtered blank sample bottle, 60 ml
   iii. Labeled preservation blank sample bottle, 60 ml
   iv. Syringe, 60 ml
   v. Syringe filter, 0.45 um
   vi. Ultrapure nitric acid solution
   vii. DI water
   viii. Pipette
   ix. GPS
   x. Nitrile Gloves
   xi. Eye Protection (for adding nitric acid solution)

   b. Filtered Blank protocol.

   i. Rinse 60 ml syringe with DI water 3 times
   ii. Draw 60 ml of Ultrapure water
   iii. Screw new filter on syringe
   iv. Filter 60 ml water into sample bottle
   v. Place sample into cooler
   vi. Add 0.5 ml of ultrapure nitric acid solution to sample using a pipette, at end of sampling event or end of the day.

-continued-

c. Preservation Blank protocol.

i. Using same syringe as filtered blank sample draw 60 ml of DI water
ii. Syringe 60 ml water into sample bottle (no filtering)
iii. Place sample into cooler
iv. Using a pipette, add 0.5 ml of ultrapure nitric acid solution to sample, at end of sampling event or end of the day.

2. Dissolved Calcium: Water samples

a. Equipment

i. Black Sharpie
ii. Sample bottle
iii. Syringe 60 ml
iv. Syringe filter 0.45 um
v. Ultrapure nitric acid solution
vi. Pipette

b. Water Sample collection protocol

i. Rinse 60 ml syringe with site water 3 times
ii. Rinse sample bottle and cap with site water 3 times
iii. Draw 60 ml of water directly from water at the site
iv. Screw new filter onto syringe
v. Fill sample bottle with 60 ml filtered water
vi. Replace filter with new one if it becomes clogged to complete filtering all water
vii. Use one pipette to add 0.5 ml of ultrapure nitric acid solution to each sample using one pipette
viii. Discard pipette after one day of use
ix. Place sample into cooler

You should have enough sampling equipment to use a new syringe for each water body; thus, avoiding the need to decontaminate bottles, syringes, etc.

Unless there is contamination (e.g., touching the water in a sample bottle), one pipette can be used per day to add preservative (nitric acid) to blanks and water samples.

Appendix C2.–Protocol for surveying water quality parameters: pH, temperature, dissolved oxygen, salinity, and turbidity.

1. **Water Quality Sampling**
    a. **Equipment**
        i. Hydrolab or YSI sonde water sampling instrument
        ii. Instrument charger or extra batteries
        iii. Storage solution
        iv. Secchi disk with 25 ft./7.5m line marked every 0.1 meter
        v. GPS
    b. **Conduct water quality sampling at location of artificial substrate**
        i. Remove sonde and pH sensor covers
        ii. Slowly lower sonde to AS
        iii. Record measurements twice, once one meter above the bottom and another one meter below the surface. If water is less than 2 m, move into deeper water.
        iv. Replace sonde and pH sensor covers
        v. Clean, drain and dry instrument.
    c. **Secchi Disk**
        i. Slowly lower secchi disk until no longer visible
        ii. Slowly raise the secchi disk until it just becomes visible
        iii. Note this depth
        iv. Repeat i.- iii. three times for average depth
    d. **Tidbit Temperature Logger**
        i. Tether the logger midway through the water column to the line holding the AS at the first sample site/ lake.
        ii. Following the instructions provided by Onset™, offload the data during AS retrieval in the fall (Appendix 5)

Where artificial substrates are deployed, water quality sampling should occur at the same location and depth.

1. **Artificial substrate (AS) surveys**
    a. Equipment
        i. Artificial substrates; 1 per site
        ii. Parachute cord
        iii. Zip ties
        iv. Knife
        v. Ziploc bags
        vi. Lighter
        vii. ADF&G Identification tag
        viii. Hole punch
        ix. Plastic tub large enough to hold AS
        x. GPS
        xi. Ethanol

    b. Deploy artificial substrates.
        i. Tie parachute cord to AS using a bowline knot, then a half hitch or other secure knot, zip tie the tag end.
        ii. Add ADF&G identification tag to bottom of the AS
            1. Tie opposite end of parachute cord to the infrastructure to deploy AS.
            2. Hang AS in the deepest water, about 1 meter from the lake bottom
            3. If the lake exceeds 20 m in depth, add an additional AS to the line one meter below the surface.
            4. Conceal the appearance of the AS, as best as possible.
            5. Take photo of location of deployed AS

    c. Retrieve artificial substrates
        i. Reel in AS
        ii. Place AS in tub
        iii. Inspect AS visually and with tactile examination
        iv. Take pictures of the sampling plates if organism visible
        v. Collect samples of any unknown or suspicious organisms established on the plate, if they can be scraped off.
        vi. If AS appears to be heavily fouled, place it in a bag. If extra AS are available, deploy a different AS.
        vii. Note known native species present on AS, particularly mussels.