Trends in aquatic environmental DNA research in Alaska

Brandi Kamermans[1], Maggie Harings[2], Rachel Lekanoff[2], Laura E. Timm[2,3], Erik Schoen[1], J. Andrés López[2,4], Jessica R. Glass[2]

1 International Arctic Research Center, University of Alaska Fairbanks
2 College of Fisheries and Ocean Sciences, University of Alaska Fairbanks
3 National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Ecology and Fisheries Management
4 University of Alaska Museum of the North, University of Alaska Fairbanks

Contact information: Brandi Cron (brkamermans@alaska.edu) Maggie Harings (maharings@alaska.edu), Rachel Lekanoff (rmlekanoff@alaska.edu), Laura E. Timm (letimm@alaska.edu), Erik Schoen (eschoen@alaska.edu), J. Andrés López (jalopez2@alaska.edu), Jessica R. Glass (jrglass@alaska.edu)

1. Abstract

Environmental DNA (eDNA) analysis is an emerging tool with significant potential to advance biomonitoring, particularly in remote and logistically challenging environments. To evaluate the state of eDNA research in Alaska, we conducted a literature review and a regional survey. The review identified 22 peer-reviewed studies published between 2010 and 2025, while the survey of 54 individuals representing state, federal, academic, tribal, and nonprofit organizations (46 responses) captured information on ongoing and unpublished projects. Our literature review and survey results reveal that most published and ongoing studies in Alaska employ eDNA metabarcoding to assess community assemblages, species distributions, and biodiversity patterns. However, respondents reported several barriers to implementation, including limited funding, infrastructure, and assay availability, as well as uncertainty in laboratory selection, sampling protocols, and data analysis. Despite these challenges, cross-sector collaborations are developing. Within the growing effort to harness eDNA as a management tool, collaborations with subsistence harvesters are in the forefront of using eDNA for management purposes. This study provides the first comprehensive overview of eDNA research in Alaska, identifies key data gaps, and offers examples of co-production of knowledge currently underway in the state. Frameworks developed in Alaska may inform the advancement of remote biomonitoring programs globally.

Keywords: management, biomonitoring, biodiversity, Arctic, subarctic, co-production

2. Introduction

Small- and large-scale drivers, like shifts in temperature and transformations in land-use, are affecting biodiversity worldwide (Genet *et al.,* 2018; Rasmus *et al*., 2024; Wägele *et al.,* 2022). Assessments of species distribution, abundance, and biodiversity are pivotal to understanding aquatic ecosystems, but aquatic biomonitoring programs are frequently hampered by limitations of scale, throughput, and cost (Carrizo *et al*., 2017; Dolman *et al*., 2012; Johnston *et al.,* 2023). These limitations are particularly acute in remote regions, including high-latitude and oceanic systems, where difficulty of access, inclement weather, and access to laboratory facilities pose additional challenges (Cardenas *et al.,* 2024; Chen *et al.,* 2022; Reddy, 2021; Wang *et al*., 2024). Environmental DNA (eDNA) analysis is emerging as a powerful and cost-effective tool for assessing and monitoring biodiversity across diverse aquatic environments (Blackman *et al.,* 2024; Deiner *et al*., 2017; Rourke *et al.,* 2022).

Alaska is the largest, most sparsely populated, and northernmost U.S. state (census.gov) includes vast freshwater and marine ecosystems. The opportunities and challenges of applying eDNA approaches in remote aquatic environments are exemplified by an expanding body of research in Alaska and its use as a monitoring and management tool. Alaska is home to some of the most valuable fisheries in the world and boasts an extensive 66,640 miles (107,247 km) of coastline (Beaudreau *et al.,* 2019) and approximately 3 million lakes, >12,000 rivers, and 34,000 miles of tidal shoreline (Milner, 1997). Researchers in Alaska have already demonstrated that eDNA methods can match or complement traditional monitoring approaches like weir counts (Levi et al., 2019) and net sampling (Deeg *et al.,* 2023) when appropriately calibrated for environmental factors such as stream flow and DNA degradation (Matter *et al.,* 2018; Levi *et al*., 2019; Pochardt *et al*., 2020). Multiple collaborations with agencies and subsistence harvesters are leveraged for monitoring programs. For instance, eDNA monitoring has been proven to be a sustainable option for long-term monitoring, especially in remote areas (Pochardt *et al.,* 2020).

Prior survey results indicate that funding constraints are significant for the initial investment in eDNA research due to specialized equipment, reagents, and the need for trained personnel for sample collection and laboratory and data analysis (Hirsch *et al.,* 2024; Capurso *et al.,* 2023; Stein *et al.,* 2023). Effective eDNA sampling often requires large volumes of water or soil and repeated visits to sites, which can be time-consuming and costly. Additionally, perceived lack of laboratory capacity and infrastructure for eDNA processing is universal barrier faced by researchers working in remote locations. Here, we review and synthesize the rapidly growing applications of eDNA analysis for ecosystem monitoring in the freshwater and marine environments of Alaska for a breadth of target taxa. We identified current challenges and limitations faced by eDNA researchers. We identified current salient challenges and limitations faced by eDNA researchers. Our findings underscore that while Alaska-based studies show strong alignment with national and international best practices, several persistent barriers remain. To explore the interconnectedness of agencies and funding to overcome perceived limitations we examine the prevalence and overlap of barriers by funding type. Collaboration among state, federal, academic, tribal, and nonprofit organizations are essential to realizing the full potential for eDNA for natural resource management. Researchers in Alaska are already building capacity with collective action to support training, mentorship, and infrastructure development at local, regional, and international scales.

3. Materials and methods

3.1 Literature review and survey development and distribution

To characterize past and present eDNA methods used for aquatic ecosystems in Alaska, we conducted a literature review in November 2023. Scientific articles and reviews of aquatic eDNA science in Alaska were retrieved from Google Scholar, PubMed, JStor, Research Gate, Semantic Scholar (Table A1). We designed a survey to document published and unpublished research. The survey captured perspectives on communicating eDNA study results and perceived barriers to conducting eDNA research in Alaska (See survey in the Appendix). Survey participants who chose to self-identify are listed in the Acknowledgements.

3.2 Data encoding

Raw survey responses and data from publications were compiled for analyses. For multiple choice questions and short answers, each response from each participant was treated as a separate data point. For example, respondents reported testing several different filter pore sizes during a single research project. Each unique size was considered a separate observation during data analysis. If a participant's response to a question was ambiguous, we categorized their answer as "unknown."

Study regions included Bering Sea, Chukchi Sea, Beaufort Sea, Gulf of Alaska, Interior and North-northwest, Southcentral, Southeast, Statewide, West-southwest, and Aleutian Islands. In addition to finding locations in the published literature, the survey included a question asking the latitude and longitude of studies. Funding sources were categorized as federal grant funding, state grant funding, Tribal grant funding, private funding, and/or other funding sources. Aquatic ecosystems sampled included ocean, lake, wetland, river/stream, tidewater, and/or estuary.

Survey responses identified several additional taxa, and some responses selected all taxa. For data analysis, we categorized the target taxa as: mammals, fishes, pathogens, invertebrates (macroinvertebrates, crustaceans, mollusks, and cephalopods), plants (plants and microalgae), and all species.

Filter pore sizes in micrometers (µm) included 0.1, 0.22, 0.4, 0.45-1.5, 1, 1.2, 1.5, 3, 5, 7, 10, unknown and none (e.g., no filter was used and gauze and ethanol precipitation methods were utilized for capturing and concentrating eDNA in the field). Filter types included unknown, none (ethanol precipitation), glass microfiber (GMF), sterile gauze, polyethersulfone (PES), cellulose acetate, nylon, polycarbonate (PC), nylon net filter, and cellulose nitrate (CN). Contamination control implementation step(s) at which a sample blank was used included sample collection, filter subsampling, DNA extraction, DNA sequencing, other, or none.

Project types included presence/non-detection, species quantification, rare species assessment, invasive species detection, eDNA ecology, field-based method-comparison, lab-based method-comparison, and/or assay development/validation. Rare species assessments and invasive species detection are special cases of presence/non-detection, and we considered them separate types to capture. Participants could select both "presence/non-detection" and "rare species assessment." These definitions were chosen based on the eDNA literature. For example, presence/non-detection studies use eDNA to test for the presence or absence of an organism (Perl *et al.,* 2022) and species quantification studies use eDNA to measure species abundance (Baker *et al.,* 2018; Wang *et al.,* 2020; Yates *et al.,* 2021). Presence/non-detection and species quantification studies can target rare organisms (Carim *et al*., 2015; Wilcox *et al.,* 2016; McKelvey *et al.,* 2016), and detrimental non-native species (Morrisette *et al*., 2022). Metabarcoding provides community-level data that informs biodiversity and population assessments (Kelly *et al*., 2014a, b; Thomsen *et al.,* 2015). Field-based method-comparisons compare different environmental factors and sampling techniques on eDNA results (Larson *et al.,* 2022; Matter *et al*., 2017), while laboratory-based method-comparison studies compare detection of eDNA with other sampling methods and other biodiversity measures (Dunker *et al.,* 2016). eDNA ecology studies focus on the range of biotic and abiotic environmental factors that contribute to eDNA persistence and the limits of detection (Dejean *et al.,* 2011; Barnes and Turner, 2016; Hansen *et al*., 2018). Assay development/validation studies develop and assess new assays for species-specific detection (Thalinger *et al.,* 2021).

Publication status included completed, published or ongoing, unpublished, and other. Collaborators included state agency, federal agency, Tribal entity, academic institution, nonprofit, consultant, subsistence harvesters, recreational fishermen, commercial fishermen, and/or other. Barriers included insufficient funding, lack of agency/organization support, lack of laboratory access / lack of funding for sample analyses, uncertainty about how to analyze data, none, and/or other. When a response to this question could not be parsed from literature, we recorded "unknown."

Seven publications became available after the survey closed in January 2024. Although they are included here for completeness, they were excluded from the figures. The figures presented in this article represent the state of eDNA research in Alaska based on our literature review and regional survey to gather perspective on barriers and collaborations. Some of the authors of these studies participated in the survey and indicated that their work was in progress or unpublished at the time. As such, including these studies in our analyses after the survey closed could result in duplication of survey responses. However, we aim to provide the readers with a complete list of publications which are summarized in Table A2.

3.3 Data analysis

When summarizing the results of the multiple-choice questions, we treated each answer as a unique entry. Therefore, if participants selected more than one answer (e.g., they were targeting two unique taxa or were affiliated with two types of institutions), the total number of entries could exceed the number of participants. In several cases, short-answer responses provided under "other" required category revision, including grouping options and/or creating new options. Data categories are detailed below:

Study region: Bering / Chukchi Sea and Arctic Ocean (Bering, Chukchi, and Beaufort Sea), Gulf of Alaska, North of the Alaska Range (Interior and North-northwest), Southcentral, Southeast, Statewide, and/or West-Southwest (West-southwest and Aleutian Islands).

Targeted taxa: survey responses identified several additional taxa, and some responses selected all taxa. For data analysis, we categorized the target taxa as: mammals, fishes, pathogens, invertebrates (macroinvertebrates, crustaceans, mollusks, and cephalopods), plants (plants and microalgae), and/or all species.

Funding: We included the following funding categories on the survey: federal, state, tribal, and/or other (international, private, pursuing funding, and unknown).

Aquatic ecosystems sampled: ocean, lentic systems (lake and wetland), lotic systems (river/stream), and/or coastal aquatic ecosystems (tidewater and estuary).

Barriers: participants reported "lack of access to laboratory equipment", "wait time for results," "variability in sampling protocols," "access to remote locations for sampling," "uncertainty regarding lab selection," "skepticism from end-users," "limited capacity of labs within organization," "lack of existing assay," "lack of access to field sampling equipment", "inconclusive results," and "facility infrastructure." We classified barriers as: uncertainty and skepticism (uncertainty regarding lab selection, uncertainty about how to analyze data, inconclusive results, variability in sampling protocols, and skepticism from end-users); lack of access (access to remote locations for sampling, lack of access to laboratory equipment, lack of personnel; lack of existing assays, lack of access to field sampling equipment, and lack of agency/organization support); insufficient funding; and unknown/ none/ facilities and capacity/

wait time for results. The lack of access category functionally included the least commonly cited barriers.

Filter pore size: Survey responses were collected as short answers and identified a range of filter pore sizes used, as well as categories for unknown and none.

Filter type: Participants added options such as “NA (ethanol precipitation)”, “nylon”, and “nylon net filter” in the survey. We categorized filter types into the following groups: none (ethanol precipitation), nylon, nylon net filter, glass microfiber (GMF), sterile gauze, polyethylene sulfone (PES), cellulose acetate, polycarbonate (PC), and cellulose nitrate (CN).

Negative control use: the use of negative controls within each study workflow was assessed by separating the workflow into the following: sample collection, filter subsetting, DNA extractions, DNA analyses (plate blanks used for qPCR and/or metabarcoding analyses). Some survey participants chose none and/or unknown for these steps.

Collaborations: participants chose from state agency, federal agency, tribal entity, academic institution, nonprofit, consultant, subsistence harvesters, recreational fishermen, commercial fishermen, and other.

We compiled and analyzed data survey responses and the information parsed from the literature review in RStudio (R version 4.3.2) (R Core Team, 2024). We generated figures and tables using R packages ggplot2 (Wickham 2016) and iGraph (Csárdi *et al.,* 2024). To understand the historical use of eDNA in the state, we focused on eDNA analyses over space and time. Then, given the diverse interests in aquatic biomonitoring in Alaska and the associated differences in publication cultures, we sought to characterize studies in our data by targeted taxa, aquatic ecosystems sampled, and publication status. We mapped the locations of studies across Alaska, including surrounding waters, distinguished by regional boundaries (e.g., Southcentral, North-Northwest, Gulf of Alaska) that are used for natural resource management. We used the reported latitude and longitude from surveys and published studies to guide our understanding of where research has been conducted and for specific target groups of organisms. Moreover, to understand research interest across these regional boundaries and identify project funding sources (e.g., federal, state), we assessed the funding origins for projects in each region. Respondents to a recent survey (Capurso *et al.,* 2023) suggested that better collaboration and sharing of knowledge and data among managers could improve monitoring strategies in the future; here we investigate the interconnectedness of the Alaska eDNA research teams.

4. Results

All figures are compiled from published and unpublished results. Our dataset included 15 published studies (Fig.1 and Table A3-4). The application of eDNA in Alaska to monitor species has increased overall since 2010, with a more rapid increase since 2019 (Fig.1). Of authors corresponding to the 15 published studies in Alaska, 80% participated in the survey. In addition, we compiled a noncomprehensive list of companies offering eDNA analyses throughout the world (Table S5).

Spatial data from published and unpublished studies reveal that projects mainly occurred along accessible locations (e.g., road systems, shorelines, and locations associated with scientific research surveys, such as the Northern Gulf of Alaska Long-Term Ecological Research program) (Fig.2). Figure 3 brings together photographs from the contributing survey participants, offering readers a visual overview of the diverse environments, taxa, and sampling devices featured across studies. Unpublished results revealed that the breadth of eDNA research in Alaska extends well beyond that which is represented in the published literature, which primarily focused on fishes in lentic and lotic systems, with some inclusion of mammals, invertebrates, and pathogens (Fig.4). The aquatic ecosystems sampled with the greatest taxonomic diversity targeted was the ocean (Galaska *et al.,* 2023; Larson *et al.,* 2022; Parsons *et al.,* 2018; Menning *et al.,* 2021; Menning *et al.,* 2020b; Deeg *et al.,* 2023).

Methodological studies focused on assay development and validation (n=8), field methods (n=12), and laboratory methods (n=3) were also published throughout the study period (Fig.5). Invasive species studies in Alaska began in 2013 (Dunker *et al.,* 2016) and nine more have been implemented between 2013–2023 (Fig. 5). During 2010–2025, eDNA studies in Alaska used 14 unique filter pore sizes and 11 unique filter types (Fig.6-8 and Table A4).

The three most used filter pore sizes were 0.45 µm, 1 µm, and 5 µm, though the use of 5 µm filters did not begin until 2021. The diversity in filter pore sizes used across studies increased from 1 in 2010 to 9 in 2022 (Fig.6). Cellulose nitrate (n=12), polyethersulfone (n=10), and glass microfiber (n=7) were the most frequently used filter types (Fig.6A).

We identified four stages throughout the process of eDNA application when practitioners used negative controls during 2010–2023: sample collection, filter subsetting, DNA extraction, and DNA analyses (plate and library preparation; Fig.6C). During 2021–2023, negative controls were implemented evenly across all four stages. Negative control use has increased over time and only a small number of studies report no negative controls or unknown implementation of negative controls. Initially, negative controls were used solely during eDNA sequencing. Negative control implementation at sample collection was first reported in 2013 and has since increased in use, while filter subsetting and DNA extraction blanks first were reported in 2014 and 2016, respectively. Both practices were unreported between 2017–2020, only reappearing in studies in 2021.

Most studies assessed presence/non-detection of species (n=26), species richness (n=20) or species quantification (n=16). Methodological studies focused on assay development and validation (n=8), field methods (n=12), and laboratory methods (n=3) were also published throughout the study period. (Fig.5). Invasive species studies in Alaska began in 2013 (Dunker *et al.,* 2016) and nine more have been implemented between 2013–2023 (Fig.5).

The eDNA based research and monitoring studies using eDNA in Alaska were funded by federal and state agencies, private entities, tribal entities, universities, international, and unknown funding sources (Fig.9). Federal funding (40 of the 46 responses) was the most frequently reported, followed by state (29 of 46 responses) and university funding (11 of 46 responses). Federal, state, tribal, and university sources provided funding related to eDNA biomonitoring in southeast Alaska (Figure 6B). The fewest studies were conducted in the Bering Sea/Chukchi Sea (1 of 46 responses) and the Gulf of Alaska (1 of 46 responses; Fig.9B). Tribally funded projects (4 out of 46 responses) were less frequently reported than state funded studies (29 out of 46 responses) in our dataset and focused on regions that included North of the Alaska Range, Southcentral, and West-Southwest (Fig.9B).

Survey participants identified 16 unique barriers they faced while conducting eDNA research in Alaska (Fig.9). Insufficient funding, lack of access, uncertainty and skepticism about protocols, and unknown, none, and wait time for results were the top barriers faced in descending order of mentions (Fig.10A). Lack of access included responses such as the following: access to remote locations for sampling, lack of facility infrastructure, lack of access to laboratory equipment, lack of agency/organization support, lack of personnel, and limited capacity of laboratory within organization (Fig.9A). Uncertainty and skepticism were a common barrier for federal, state, university, and tribally funded projects (Fig. 9A). In addition, barriers associated with "uncertainty and skepticism" included uncertainty about how to analyze data, uncertainty regarding lab selection, and variability in sampling protocols were perceived by survey participants that were university funded studies (Fig.10B). Federally funded projects identified inconclusive results, skepticism from end-users, uncertainty about how to analyze data, uncertainty regarding lab selection, and variability in sampling protocols as barriers (Fig.10B). State funded projects identified inconclusive results and uncertainty about how to analyze data (Fig.10B). Tribally funded projects identified lack of access to remote locations, lack of facility infrastructure, and lack of laboratory equipment (Fig.10A). The tribally funded project also identified barriers associated with uncertainty and skepticism that included only uncertainty about how to analyze data as a barrier (Fig.10B). University funded projects identified lack of access to remote locations for sampling, lack of access to laboratory equipment, and uncertainty about how to analyze data (Fig.10).

Survey participants were asked to identify their collaborators in project development and implementation. The provided options included ten options: (1) state and (2) federal agencies, (3) tribal entities, (4) academic institutions, (5) nonprofits, (6) consultants, (7) subsistence harvesters, (8) recreational fishermen, (8) commercial fishermen, and (10) an "other" category. The federal entity option (33 of 46 responses), academic institutions option (27 of 46 responses), and state entity collaborations (27 of 46 responses) were the most mentioned collaborators. One survey participant chose "other" and added "foundation."

As Figure 11 shows, federal and academic entities are the central hubs of collaboration, with state and tribal entities also strongly connected. Other groups (e.g., nonprofits, private entities, subsistence harvesters) appear less frequently but still form part of the broader network. Inclusion of subsistence harvesters, and commercial and recreational fishermen provided additional insight into partnerships with local communities.

5. Discussion

This review highlights past and current applications of eDNA analysis for aquatic biomonitoring in Alaska. Our review represents 53 studies, published and unpublished, spanning aquatic ecosystems across Alaska and associated state and federal waters, dating back to 2010.

5.1 *Application of eDNA in Alaskan aquatic biomonitoring*

The increase in eDNA studies across the state over time is likely fueled by advancements in next-generation sequencing and eDNA collection technologies, lower sequencing and data processing costs, and computational advancements in analyzing large -omics datasets. Comparable growth patterns have been documented in recent eDNA reviews (Sahu *et al.,* 2025; Takahashi *et al.,* 2023). Notably, the application of metabarcoding is increasing in Alaska. Mirrored globally by an increase in metabarcoding as an emerging and most widely used eDNA technique (Nørgaard *et al.,* 2021; Sahu *et al.,* 2021, 2025; Takahashi *et al.,* 2023).

Globally and in the United States, fishes and freshwater habitats are among the most frequently studied using metabarcoding (Capurso *et al.,* 2023). Studies in Alaska indicate a strong research focus on fishes. The aquatic ecosystem sampled with the greatest taxonomic diversity targeted was the ocean (Galaska *et al*., 2023; Larson *et al.,* 2022; Parsons *et al.,* 2018; Menning *et al.,* 2021; Menning *et al.,* 2020b; Deeg *et al.,* 2023). Unpublished results revealed that the breadth of eDNA research in Alaska extends well beyond that which is represented in the published literature, which primarily focused on fishes in lentic and lotic systems, with some inclusion of mammals, invertebrates, and pathogens.

5.2 *Using eDNA for fisheries management*

The emphasis of studies on fishes likely reflects the ecological and economic significance fisheries have in Alaska. The value of state and commercial fisheries in Alaska and its surrounding federal waters was estimated at roughly 5 billion dollars in 2023 (NOAA 2024). Alaska's vast and remote geography, coupled with its highly diverse habitats, makes representative sampling logistically difficult. These data gaps carry serious implications. Population declines in some Western Alaska salmon stocks have resulted in fishery closures, heightened user conflicts, and profound cultural and food security impacts for Indigenous communities (Schoen *et al.,* 2023). Researchers in Alaska have already demonstrated that eDNA methods can match or complement traditional monitoring approaches like weir counts (Levi *et al.*, 2019). One of the strongest examples of eDNA applied to long-term fisheries monitoring comes from the Chilkoot Indian Association, which has pioneered the use of eDNA for tracking eulachon (Saak) since 2014 and has since expanded efforts to monitor other fish species. The Chilkoot Indian Association's leadership demonstrates how eDNA can support community-driven fisheries management.

5.3 *Data gaps and biomonitoring of taxa beyond fishes*

Notably, our dataset indicates a paucity of eDNA studies targeting aquatic bacteria and fungi in both published and unpublished literature in Alaska, which may have been due to the fisheries-oriented audiences targeted by our survey. The unpublished studies uncovered in our survey results indicate an equal emphasis on invertebrate and non-mammalian vertebrate taxa including crustaceans and mollusks. Given the essential roles these taxa hold in nutrient cycling, trophic webs, and ecosystem stability, this taxonomic bias limits our understanding of ecological systems (Sahu *et al.,* 2025). Applying eDNA methods to these groups presents specific challenges, including the difficulty of detecting intracellular microbes and the need for customized sampling strategies in highly turbid or structurally complex environments (Marques *et al.,* 2021). Prior reviews of eDNA research suggest that microbial communities, microalgae, crustaceans, and mollusks are consistently underrepresented in published literature compared to other taxonomic groups like fishes and mammals (Sahu *et al.,* 2025).

5.4 *Insufficient funding and constraints on eDNA research design*

Published literature and survey responses indicate that research efforts in Alaska have begun to address the diversity of study designs, ecosystems, and target species, laying important groundwork for developing regionally appropriate methodological standards. Despite this progress, researchers funded by federal, state, academic, and tribal entities consistently identified three major barriers to advancing eDNA work in Alaska: (1) insufficient funding, (2) limited access to resources, and (3) uncertainty or skepticism around protocols. These challenges are particularly pressing in eDNA, a field shaped by diverse interests and priorities across stakeholders.

The most recurrent perceived limitations identified by survey participants were funding constraints. Results are similar those from eDNA survey results in 2023 (Capurso *et al.,* 2023). Costs can be significant for the initial investment in eDNA research due to specialized equipment, reagents, and the need for trained personnel for sample collection and laboratory and data analysis (Stein *et al.,* 2023). Additionally, survey results highlight a perceived lack of laboratory capacity and infrastructure for eDNA processing in Alaska. Respondents perceive lack access to remote locations, facility infrastructure, sampling equipment, agency and organizational support, species-specific assays, personnel, and have limited capacity laboratories (Fig. 10A). Another major barrier is the absence of sequencing infrastructure within the state. There is a significant lack of laboratory equipment available to eDNA practitioners in Alaska who do not have access to in-house laboratories. Due in part to minimal in-state sequencing facilities, many eDNA projects rely on private laboratories outside of Alaska to perform these essential laboratory methods (Table A5).

Only seven institutions in Alaska have qPCR capabilities. These include the University of Alaska Fairbanks (UAF) and Anchorage (UAA) campuses (academic); USGS, NOAA, and USFWS (federal); ADFG (state); and the Alutiiq Pride Marine Institute (tribal). Metabarcoding, which requires high-throughput short-read sequencing, can only be accomplished at five in-state institutions and, of these, only the UAF Genomics Core Lab operates as a contract facility capable of conducting DNA extractions, amplification, and sequencing on an Illumina MiSeq. While federal collaborators generally have access to the necessary equipment and facilities, the other nine collaborators in the network reported limited or no access (Fig.10).

Hirsch and others (2024) note that shipping can be a major constraint on access and research capacity; a similar dynamic is observed in Alaska. Laboratory supplies, when obtainable, can take months to arrive due to lack of regional suppliers. and statewide transportation infrastructure This barrier makes it difficult, and sometimes impossible, for researchers to meet stringent standards or produce results in a timely manner. While sending samples out for processing may, in some cases, be less costly than acquiring materials and establishing local processing capacity, this approach raises additional challenges, including reduced control over experimental procedures and data handling. Moreover, only a subset of companies advertises data interpretation services, which remains one of the primary barriers for eDNA researchers in Alaska. Even when interpretation support is available, the cost of these services may be prohibitive for user groups already constrained by limited funding. This highlights a critical need for additional private or contract laboratories within Alaska that can support eDNA analysis and methods development.

5.6 *Region-specific assays and invasive species management*

Recent literature highlights that many locations worldwide lack local species DNA reference libraries. This is a challenge echoed by researchers in this study and documented elsewhere (Perry *et al.,* 2022; von der Heyden, 2022; Schenekar, 2023). Survey respondents identified the absence of reference libraries as a fundamental barrier. Relying on non-Alaska-specific assays introduces the risk of false positives or erroneously identifying a species. It can also lead to false negatives that fail to detect a present species. Species-specific assays are especially valuable for the early detection of climate-driven ecological changes, including harmful algal blooms and invasive species. For example, *Alexandrium* and *Pseudo-nitzschia* algae show considerable genetic variation across environments and regions, spanning national and international boundaries (Brunson *et al.,* 2024). Similarly, monitoring invasive species requires sensitivity to genetic variation within populations. Capturing resolution through eDNA assays would not only support detection but also provide insight into population structure, invasion pathways, and adaptive differences across environments (Coyle *et al.,* 2019). Improving the sensitivity and accuracy of eDNA detection strengthens the reliability of ecological monitoring and management outcomes (Bohmann *et al.,* 2022; Johnson *et al.,* 2024; Rishan *et al.,* 2023). In metabarcoding, a reference library is a collection of specific genetic sequences used for taxonomic identification. For example, CALeDNA hosts a metabarcoding reference database (https://ucedna.com/reference-databases-for-metabarcoding). While a reference database is a broader term that can encompass these libraries as well as other related genomic information, such as functional annotations (Keck *et al.,* 2023; Mendoza *et al*., 2015).

To-date, the Borealis Biodesign (https://www.borealisbiodesign.com/reference-databases) is the only publicly available annotated reference library that is annotated for Alaska-specific assays. Reference databases are essential for metabarcoding because they act as a "lookup table" to identify the species present in a sample by matching DNA sequences to known organisms. This step is crucial for assigning taxonomic identity and understanding the biodiversity of a sample, and the accuracy of the results depends directly on the quality, completeness, and accessibility of the database (Mugnai *et al.,* 2023). Included in this review is a noncomprehensive list of primer and locus that have been for eDNA analysis in Alaska (Table S6).

5.7 *Uncertainty as a perceived barrier*

Some respondents of our survey mentioned uncertainty about variable sampling protocols as a barrier and, indeed, we observed wide ranges of pore sizes and filter types used across studies. Development and sharing of citable sampling protocols (e.g. Harings *et al.,* 2024) has been initiated by some of the authors of this article to help to demystify and standardize sampling methods across organizations and research teams. Perceived limitations of eDNA include inconclusive results, skepticism around protocols, variability in sampling approaches, and uncertainty regarding data analysis. A lack of standardized communication and training practices among scientists, managers, and policymakers further complicates interpretation of results. Additionally, limited access to computational resources and the specialized training required for bioinformatics present practical barriers, making eDNA technologies difficult to implement for many researchers (Hirsch *et al.,* 2024). Researchers in Alaska have implemented co-production research and knowledge exchange networks to mitigate barriers faced by partners (see section 5.9)

5.8 *Solutions to barriers through standardization*

As we embark on the potential use of eDNA across ecosystems and research collaborations, an emerging need to standardize field and laboratory methods has become evident. Although a statewide genetics policy exists to protect wild populations in Alaska, primarily salmon (Davis, 1989), no formal accreditation currently exists for eDNA analyses under recognized organizations such as the International Organization for Standardization (Trujillo-González *et al.,* 2021). Furthermore, there is a lack of national oversight through agency-specific guidelines. Despite this, Alaska-based studies conducted in recent years are aligning well with current eDNA standards and guidelines, particularly in their consistent use of negative controls. These studies demonstrate thoughtful implementation of quality assurance measures and are contributing valuable data to broader reviews. We summarized guidelines in publications from USDA, USFWS, and USGS to the methods used by eDNA researchers in Alaska, noting the absence of guidelines by many organizations (Table A7). Our survey results can be used to generate guiding documentation, for specific ecosystems, regions, and taxa that could serve as a blueprint for designing defensible eDNA research designs from sample collection to funding opportunities.

5.9 *Training and promoting co-production research and knowledge exchange*

Co-production of knowledge is a collaborative process that brings together diverse perspectives from researchers, agencies, and community partners to achieve shared research goals or products (Rudolf *et al.,* 2025). In our evaluation of agencies and networks, we uncovered multiple collaborations with subsistence harvesters (Fig.11). eDNA methods on the Yukon and Kuskokwim rivers, initiated by Tribal organizations and involving agency and academic partners are underway (Fig.3D). Similarly, the Alutiiq Pride Marine Institute and researchers at the University of Alaska Fairbanks have developed a project-specific research and data management plan that incorporates the CARE principles (Carroll *et al*., 2020). Knowledge sharing about eDNA collection and analytical methods has already begun between organizations and agencies in Alaska. Training opportunities are written into funding opportunities, increase the sustainability of long-term monitoring, and ensure continuity in data collection. Sovereign Autonomy for Monitoring Non-human genes (SALMONg, LLC), conducts molecular-based genetics education for environmental monitoring with tribal communities in Alaska (Fig.3I). Co-production approaches, rooted in local priorities for natural resources, can provide equitable pathways to expand and sustain community-led eDNA research.

6. Conclusions

We present the first comprehensive synthesis of aquatic eDNA research in Alaska. Globally, researchers face barriers to deploying eDNA, and Alaska is no exception. Our survey revealed that practitioners often struggle to identify appropriate sampling protocols and analysis methods due to the wide variety of approaches available. Developing regional guiding documents support regulatory and management applications but also help protect Tribal resource interests in legal contexts. By identifying barriers and opportunities, we aim to ensure that future applications advance equitably, ethically, and effectively, contributing to robust biomonitoring programs in remote regions and informing global responses to the biodiversity crisis.

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Figure legends:

Figure 1: The stacked plot displays the number of combined unpublished and published eDNA studies initiated in 2010–2023. The studies are plotted based on the project start date. We examined the number of projects in the dataset by each year they were initiated and by project type.

Figure 2: Map of Alaska with the location of eDNA projects that were initiated between 2010–2023. The colors represent the taxa targeted in each study type. Regional boundaries are outlined in black, except for the Gulf of Alaska (GOA; Northern Gulf of Alaska LTER (long term ecological research). Samples collected in the Bering, Chukchi, and Beaufort Seas are co-located with long-term EcoFOCI moored sites and the established Distributed Biological Observatories and are not shown on this map as these locations were not in the published report (Galaska *et al.,* 2023).

Figure 3: Jessica Glass in Kachemak Bay (credit: Lindsey Stadler/UAF) (B) Dustin Carl at Lowell Point in Resurrection Bay collects eDNA samples to detect fishes (credit: Allison Carl/Alutiiq Prime Marine Institute); (C) Markus Horning (WTF) and Amy Bishop with PESCA sampler (UAA) (credit: Jessica Glass/UAF) (D) Richie Wachter collects an eDNA sample at Takotna River to detect fishes (credit: Andrew Magel/Kuskokwim River Intertribal Fish Commission); (E) Steve Hoekwater collects an eDNA sample at Berg Lake to survey for fishes (credit: Nathan Davis/USFWS); (F) Nathan Davis collects an eDNA sample at Dolly Varden Lake to survey for fishes (credit: Matt Bowser/USFWS); (G) Matt Bowser filters an eDNA water sample from Barabara Lake to survey for fishes (credit: Dom Watts/USFWS); (H) UAF trains ADF&G personnel in eDNA sampling techniques to prepare for a field season in Northwestern Alaska (credit: Maggie Harings/UAF); (I) Brandi Kamermans (SALMONg, LLC/UAF) teaches a field-based eDNA course on the Kuskokwim River (credit: Erik Schoen/UAF); (J) Sebastian Zavoico at Whale Mountain on the Kongakut River collects eDNA to detect mammals, aquatic invertebrate communities, and fishes (credit: Ken Tape/UAF); (K) Maggie Harings collects an eDNA sample from the Chena River to detect and fishes (credit: Erik Schoen/UAF); (L) Katie Drew (BLM) collects samples at Harry Potter Lake; (M) Allison Carl at Lowell Point in Resurrection Bay (credit:Dustin Carl/Alutiiq Pride Marine Institute

Alluvial plots comparing ecosystems to target taxa in A) published and B) unpublished studies.

Figure 4: The bubble plot displays the number of combined published and unpublished eDNA studies as the proportion of eDNA project types applied between 2010–2023. For each category, the bubbles represent the proportion of projects started in each year.

Figure 5: The bubble plot displays the number of combined published and unpublished eDNA studies as the proportion of eDNA project types applied between 2010–2023.

Figure 6: We assessed temporal trends in filter pore size, filter type, as well as the implementation of negative controls throughout the duration of the study (e.g., field negative, extraction negative). Bubble plots for A) study types; B) filter types; and C) the use of negative controls at various steps during the sampling period between 2010-2023.

Figure 7: Bubble plots showing the distribution of filter pore sizes used across different waterbody types. Bubble size represents the frequency with which a given filter pore size was reported for each waterbody type. The "Total mentions" column indicates the number of times each filter pore size was reported across all studies for the ecosystem type.

Fig. 8: Bubble plots showing the distribution of filter pore sizes used across different target taxa. Bubble size represents the frequency with which a given filter pore size was reported for each target taxa. The "Total mentions" column indicates the number of times each filter pore size was reported across all studies for target taxa

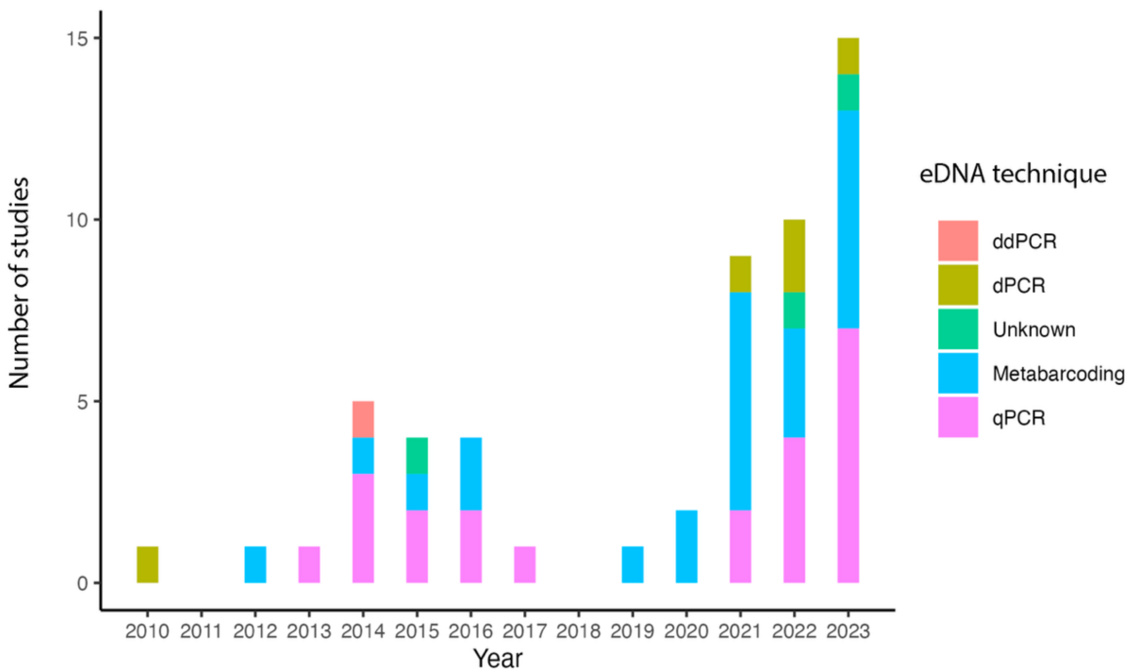
Figure 9: Alluvial plots illustrating the funding sources associated with (A) barriers faced while conducting eDNA research; B) the Alaskan study regions from Figure 3. Other includes funding agencies that are international, private, pursuing funding, and unknown.

Figure 10: Alluvial plots illustrating the funding sources associated with two categories of barriers identified in Figure 6: (A) lack of access and (B) uncertainty and skepticism.

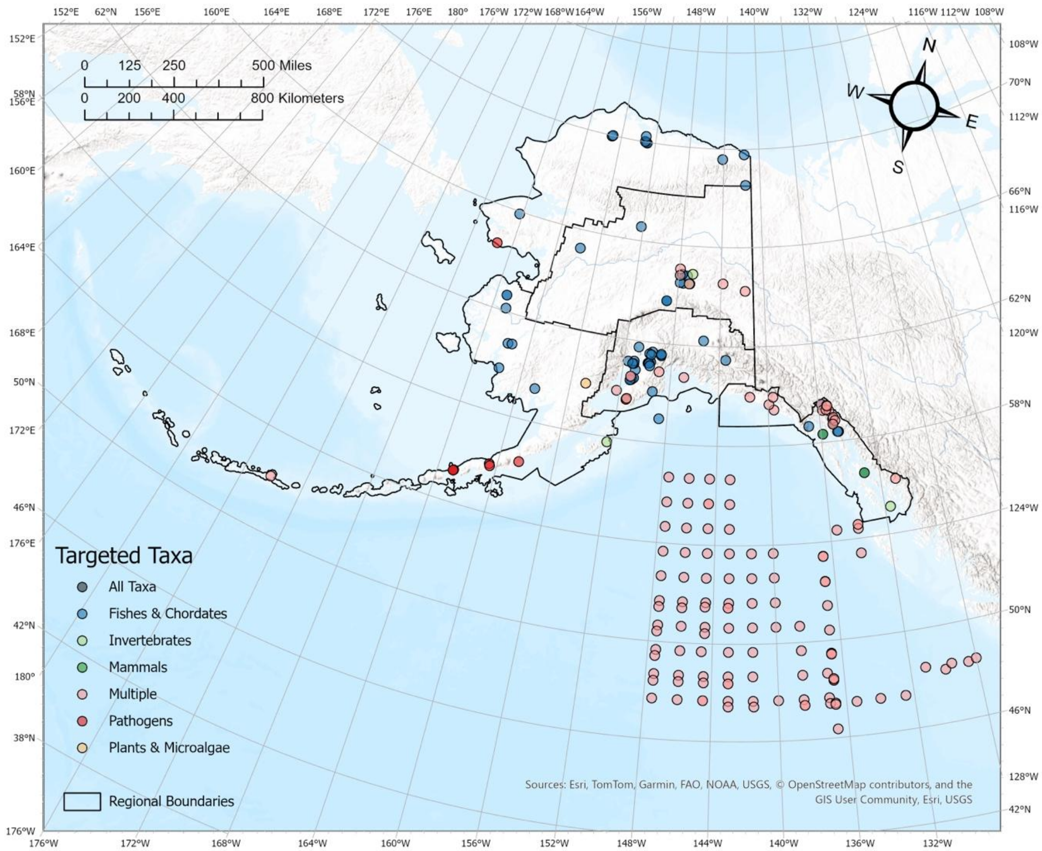
Figure 11: Network diagram for collaborations among eDNA researchers identified from the literature review and survey responses. Gray lines indicate a collaboration between entities, with line thickness representing the number of occurrences. Collaboration network among entities involved in eDNA research in Alaska, based on the literature review and survey responses.

Figures:

Fig. 1



995 Fig.2

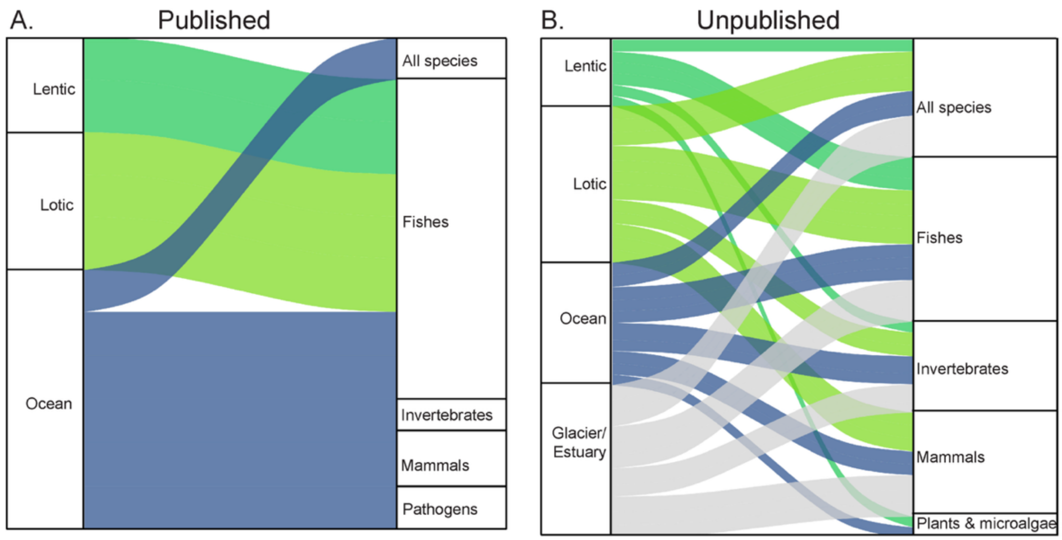


996

997

Fig.3

999 Fig. 4



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1001

1002 Fig.5

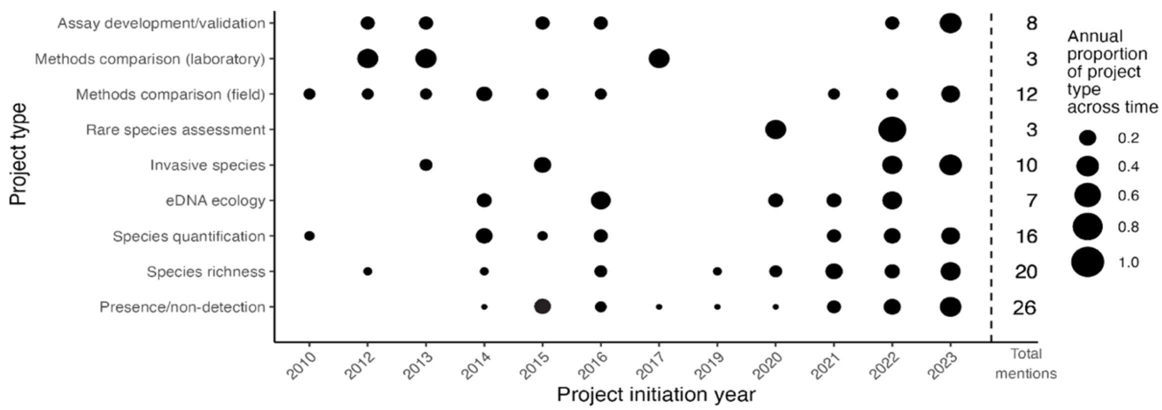

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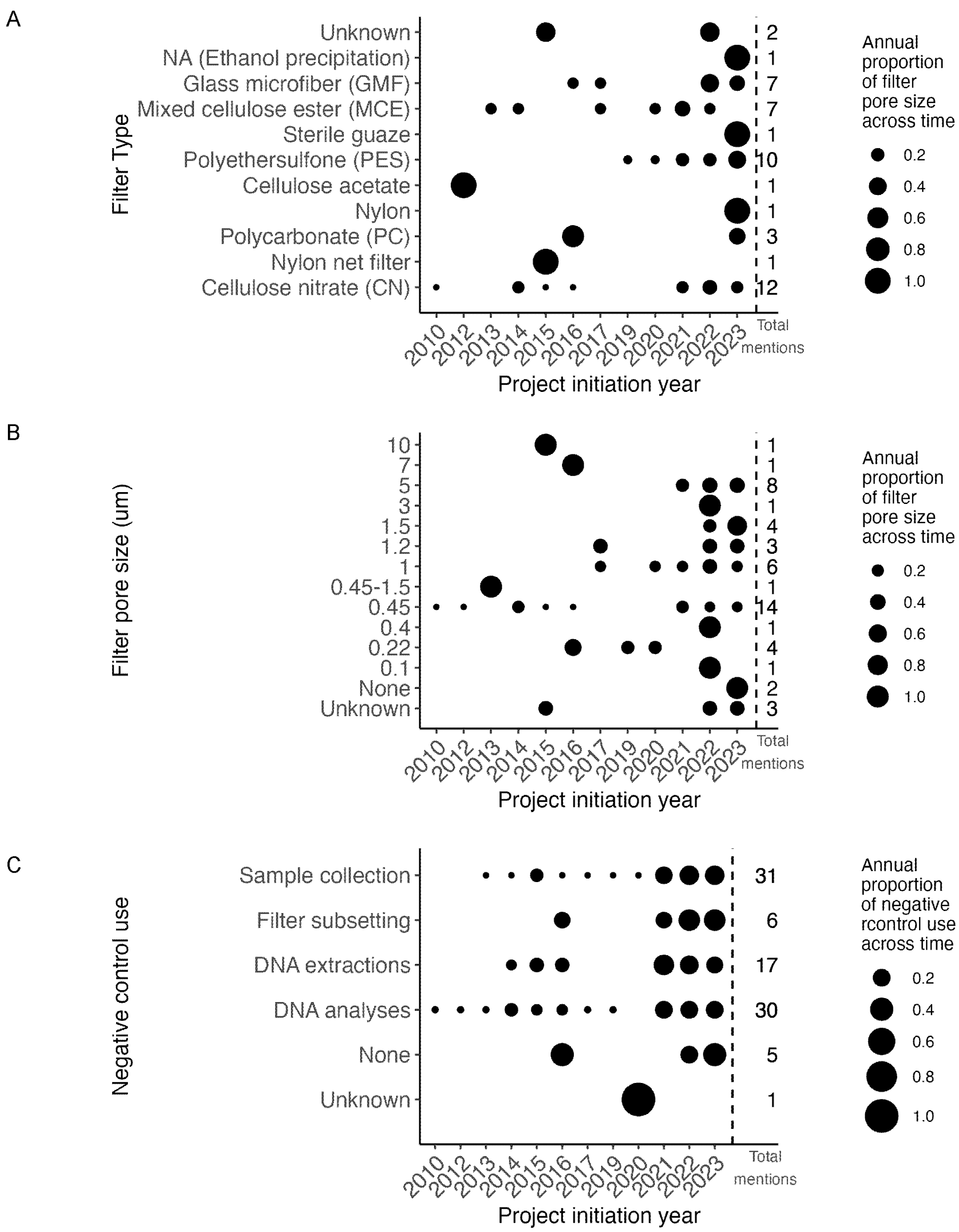
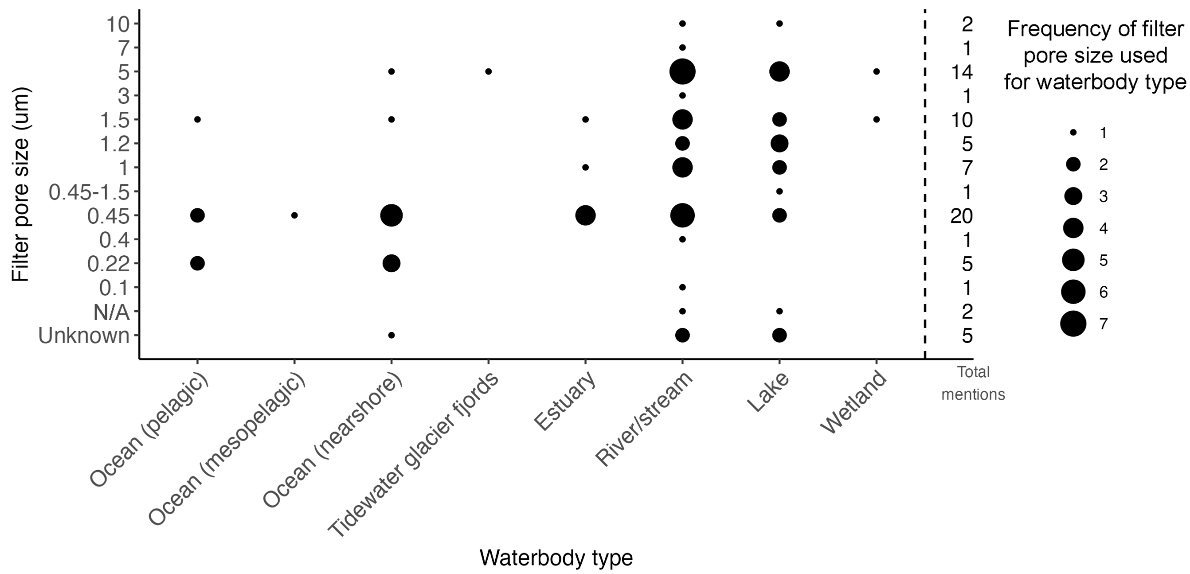
Fig. 6



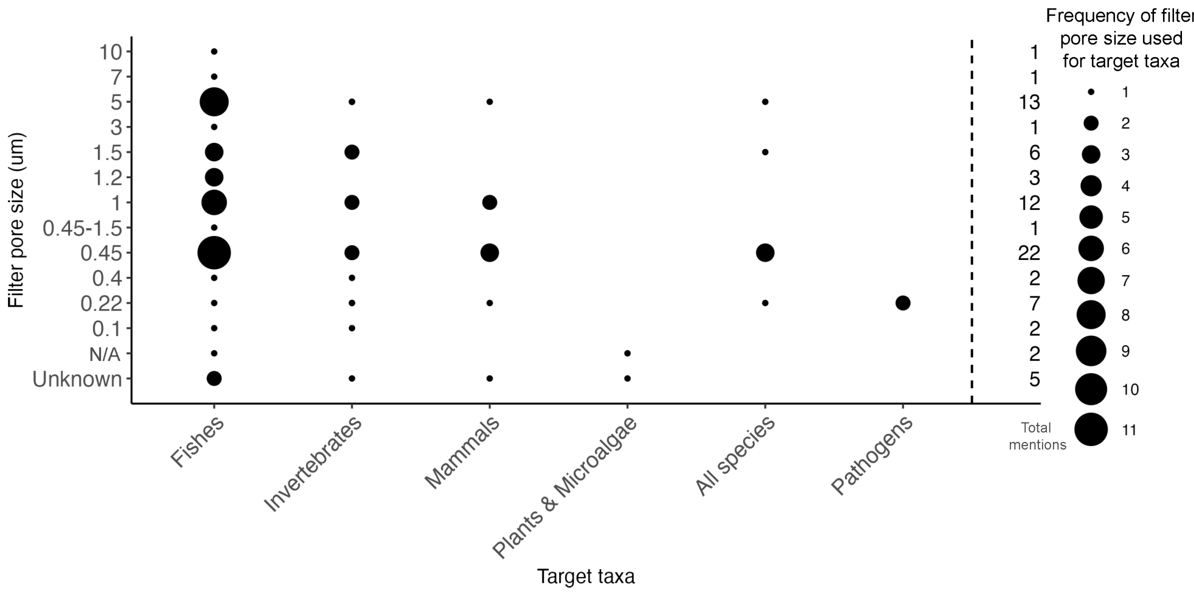
A
Filter Type
Unknown
NA (Ethanol precipitation)
Glass microfiber (GMF)
Mixed cellulose ester (MCE)
Sterile guaze
Polyethersulfone (PES)
Cellulose acetate
Nylon
Polycarbonate (PC)
Nylon net filter
Cellulose nitrate (CN)
2 1 7 7 1 10 1 1 3 1 12
2010 2012 2013 2014 2015 2016 2017 2019 2020 2021 2022 2023
Total mentions
Project initiation year
Annual proportion of filter pore size across time
0.2 0.4 0.6 0.8 1.0
B
Filter pore size (um)
10
7
5
3
1.5
1.2
1
0.45-1.5
0.45
0.4
0.22
0.1
None
Unknown
1 1 8 1 4 3 6 1 14 1 4 1 2 3
2010 2012 2013 2014 2015 2016 2017 2019 2020 2021 2022 2023
Total mentions
Project initiation year
Annual proportion of filter pore size across time
0.2 0.4 0.6 0.8 1.0
C
Negative control use
Sample collection
Filter subsetting
DNA extractions
DNA analyses
None
Unknown
31 6 17 30 5 1
2010 2012 2013 2014 2015 2016 2017 2019 2020 2021 2022 2023
Total mentions
Project initiation year
Annual proportion of negative rcontrol use across time
0.2 0.4 0.6 0.8 1.0

1011 Fig. 7



1012

Fig.8



Frequency of filter pore size used for target taxa
Filter pore size (um)
10
7
5
3
1.5
1.2
1
0.45-1.5
0.45
0.4
0.22
0.1
N/A
Unknown
1
1
13
1
6
3
12
1
22
2
7
2
2
5
Total mentions
1
2
3
4
5
6
7
8
9
10
11
Fishes
Invertebrates
Mammals
Plants & Microalgae
All species
Pathogens
Target taxa

1016 Fig. 9

1017

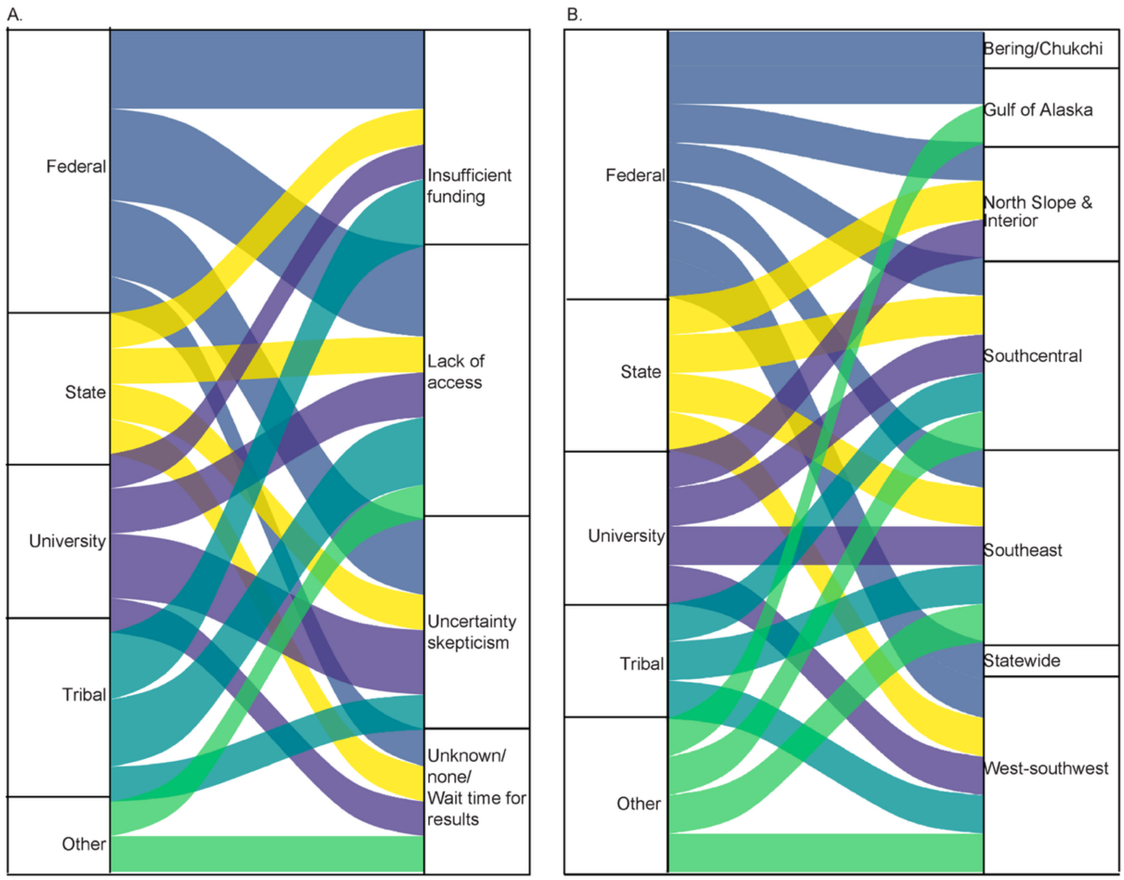


Fig.10

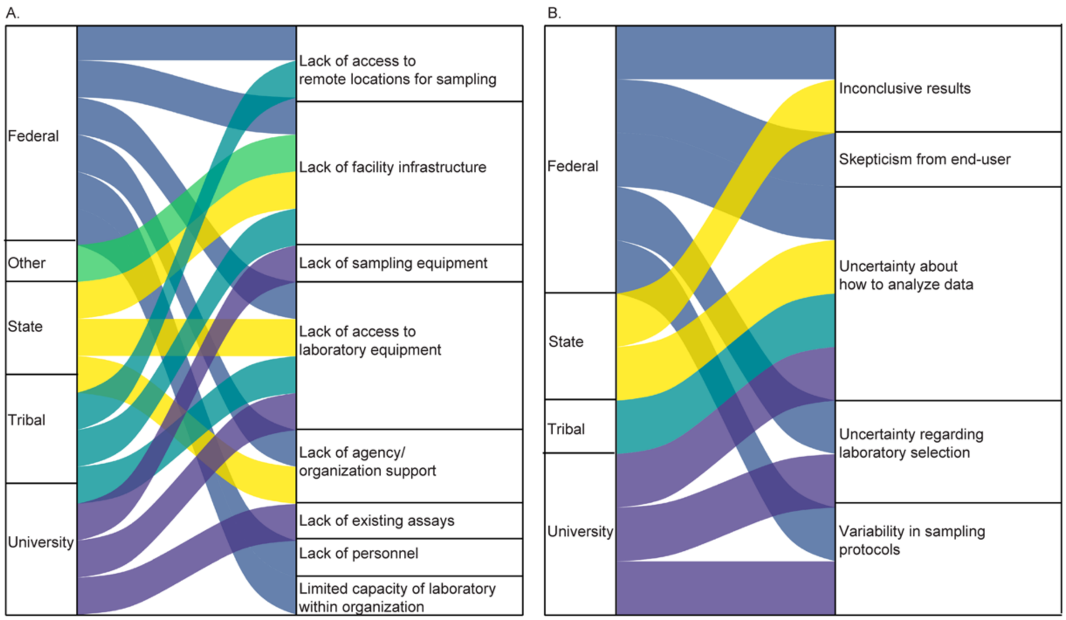

A.
Federal
Other
State
Tribal
University
Lack of access to remote locations for sampling
Lack of facility infrastructure
Lack of sampling equipment
Lack of access to laboratory equipment
Lack of agency/ organization support
Lack of existing assays
Lack of personnel
Limited capacity of laboratory within organization
B.
Federal
State
Tribal
University
Inconclusive results
Skepticism from end-user
Uncertainty about how to analyze data
Uncertainty regarding laboratory selection
Variability in sampling protocols

Fig.11

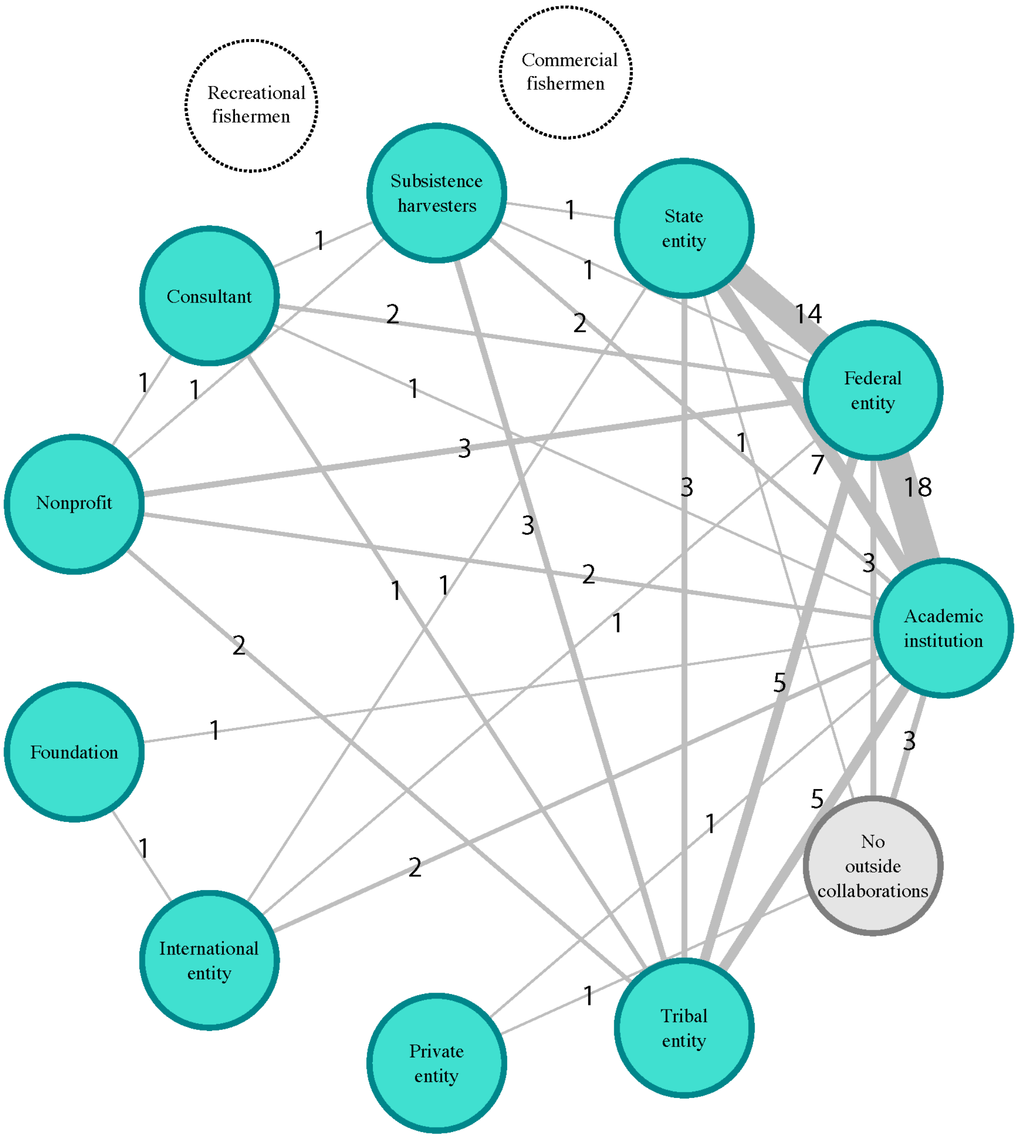

Recreational fishermen
Commercial fishermen
Subsistence harvesters
State entity
Consultant
Federal entity
Nonprofit
Academic institution
Foundation
No outside collaborations
International entity
Private entity
Tribal entity

Table A1: Keywords used to identify and studies published using environmental DNA methods in Alaska.

Search engine	Keywords
Connected papers	Alaska, environmental, DNA
Google Scholar, PubMed, JStor, Research Gate, Semantic Scholar	Arctic, Alaska, environmental, DNA, quantitative PCR, qPCR, metagenomics, Taqman, digital droplet PCR, ddPCR, digital PCR, dPCR, metabarcoding, primers, high-throughput, illumina, sequence, biodiversity, monitoring, invasive, endangered, salmon

Table A2: Publications from 2024-2025 that are not included in the data analyses and figures.

Reference	**Project Start year**	**Study type**	**Eco-system**	**Target taxa**	**Region**	**Filter Type**	**Filter pore size**	**DNA analyses**
Baetscher *et al.*, 2024	2021	Quantification	Ocean	Chum salmon (*Oncorhynchus keta*)	Southeast Alaska	Cellulose nitrate (CN)	0.45	qPCR
Benson *et al.,* 2024	2018	Presence/ non-detection, Species quantification	Lake	Plants (*waterweed Elodea canadensis and western waterweed E. nuttallii*)	NA	Glass microfiber (GMF)	1.2	qPCR
Deeg *et al.*, 2024	2022	Presence/ non-detection	Ocean	*Oncorhynchus*	Northeast Pacific	0.45	PES	Metabarcoding
Gillespie *et al.*, 2024	2023	NA	Ocean	NA	Aleutian Islands, Gulf of Alaska, Unalaska	0.45	NA	NA
Ledger *et al.,* 2025	NA	Presence/ non-detection	Ocean	Fishes (*Sebastes* species)	AFSC Groundfish Assessment Program 2022 Aleutian Islands	Cellulose nitrate (CN)	0.45	Metabarcoding

					Bottom Trawl Survey			
Ledger *et al.*, 2024	2022	Quantification	NA	Walleye pollock *(Gadus chalcogrammus),* Pacific cod *(Gadus macrocephalus), and* Arctic cod *(Boreogadus saida)*	NA	NA	NA	qPCR and Metabarcoding
Parsons *et al.,* 2025	2016	Presence/ non-detection	Ocean	Mammals (harbour porpoise (*Phocoena phoc-oena*)	Inshore waters of Southeast Alaska and Western Gulf of Alaska	Mixed cellulose ester (MCE)	0.45	Metabarcoding

Baetscher, D. S., Pochardt, M. R., Barry, P. D., & Larson, W. A. (2024). Tide impacts the dispersion of eDNA from nearshore net pens in a dynamic high-latitude marine environment. *Environmental DNA*, 6, e533. https://doi.org/10.1002/edn3.533

Benson,A., Russ,O.L, Withers, J.H., Olsen,J.B., Fox, J.L., Lance, R.F., Guan, X., Wenburg, J.K. (2024) Use of Environmental DNA for Early Detection of Invasive *Elodea* Species in Interior Alaska. *Journal of Fish and Wildlife Management*; 15 (1): 195–202. doi: https://doi.org/10.3996/JFWM-23-047

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Gillespie, Treyson et al. (2024). Expedition Report : EX2303, Seascape Alaska 2: Aleutians Deepwater Mapping (Mapping). https://doi.org/10.25923/mg6x-cn64

Ledger, K. J., M. Everett, K. M. Nichols, W. A. Larson, and D. S. Baetscher (2025) " Development and Validation of an eDNA Primer Set for Rockfishes in the Alaska Current Provides a New Tool for Fisheries Management." *Environmental DNA* 7, no. 4: e70176. https://doi.org/10.1002/edn3.70176.

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Table A3: Survey responses from 15 published articles. Responses were recorded for barriers, project region, funding, project collaborators.

Reference	**Barriers**	**Project region**	**Funding**	**Project collaborators**
Matter *et al.,* 2018	None	Interior	Federal, State	State entity, Federal entity, Academic institution
Khalsa *et al.,* 2020	None	Interior	Federal, State	State entity, Federal entity, Academic institution
Levi *et al., 2*019	Unknown	Southeast	Federal, State, International	State entity, Federal entity, Academic institution, International entity
Dunker *et al.,* 2016	Insufficient funding	Southcentral	Federal	State entity, Federal entity
Rodgers *et al.*, 2017	Unknown	North-northwest	State, Federal, University	Federal entity, Academic institution
Menning *et al.,* 2020a	Insufficient funding, Lack of personnel	Southcentral, Interior, North-northwest	Federal	State entity, Federal entity, Academic institution
Menning *et. al.*, 2021	Insufficient funding, Lack of personnel	North-northwest, West-southwest	Federal	Federal entity
Menning *et al.,* 2020b	Insufficient funding, Lack of personnel	West-southwest	Federal	Federal entity
Deeg *et al.*, 2023	Unknown	Gulf of Alaska	International, Federal	Academic institution, Foundation, International entity

Pochardt *et al.,* 2020	Unknown	Southeast	State, Federal	Federal entity, Academic institution
Larson *et al.,* 2022	None	Southeast	Federal, State	State entity, Federal entity, Academic institution
Parson *et al.,* 2018	None	Southeast	Federal	Federal entity, Nonprofit, Consultant
Galaska *et al.*, 2023	Access to remote locations for sampling	Bering Chukchi, Beaufort Sea	Federal	Federal entity
Sepulveda *et al.*, 2018	Skepticism from end-users	Southcentral	Federal	State entity, Federal entity
Tillotson *et al.*, 2018	Uncertainty about how to analyze data	West-southwest	Federal, University	Species quantification, eDNA ecology

Deeg, C. M., Li, S., Esenkulova, S., Hunt, B. P. v, Schulze, A. D., & Miller, K. M. (2023). Environmental DNA survey of the Winter Salmonosphere in the Gulf of Alaska. *Environmental DNA*, *5*(3), 519–539. https://doi.org/https://doi.org/10.1002/edn3.404

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Levi, T., Allen, J. M., Bell, D., Joyce, J., Russell, J. R., Tallmon, D. A., Vulstek, S. C., Yang, C., & Yu, D. W. (2019). Environmental DNA for the enumeration and management of Pacific salmon. *Molecular Ecology Resources*, 19(3), 597–608. https://doi.org/10.1111/1755-0998.12987

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Table A4: Survey responses from 15 published articles. Responses are recorded for study type, ecosystem, target taxa, filter type, filter pore size, and DNA analyses.

Reference	**Study type**	**Eco-system**	**Target taxa**	**Filter Type**	**Filter pore size**	**DNA analyses**
Matter *et al.,* 2018	Presence/non-detection, Species quantification, eDNA ecology, Methods comparison (field)	River/ stream	Fish (including lampreys)	Cellulose nitrate (CN)	0.45	qPCR
Khalsa *et al.*, 2020	Presence/non-detection, Species quantification, eDNA ecology, Methods comparison (field)	River/ stream	Fish (including lampreys)	Glass microfiber (GMF)	7	qPCR
Levi *et al.,* 2019	Species quantification, Methods comparison (field)	River/ stream	Fish (including lampreys)	Cellulose nitrate (CN)	0.45	qPCR
Dunker *et al.,* 2016	Invasive species, Methods comparison (laboratory), Assay development/validation, Methods comparison (field)	Lake	Fish (including lampreys)	Mixed cellulose ester (MCE)	0.45-1.5	qPCR

Rodgers *et al.*, 2017	Assay development/validation, Species quantification, Invasive species, Presence/non-detection	River/ stream, Lake	Fish (including lampreys)	Nylon net filter	10	qPCR
Menning *et al.*, 2020a	Species richness, Assay development/validation, Methods comparison (field), Methods comparison (laboratory)	Lake, River/ stream	Fish (including lampreys)	Cellulose acetate	0.45	Metabarcoding
Menning *et. al.*, 2021	Species richness	Ocean (nearshore)	Pathogens	Polycarbonate (PC)	0.22	Metabarcoding
Menning *et al.*, 2020b	Species richness, Assay development/validation, Presence/non-detection	Ocean (nearshore)	Pathogens	Polycarbonate (PC)	0.22	Metabarcoding
Deeg *et al.*, 2023	Species richness, Presence/non-detection	Ocean (pelagic)	Fish (including lampreys), Macroinvertebrates, Cephalopods, Chordates	Polyethene sulfone (PES)	0.22	Metabarcoding
Pochardt *et*	Species quantification,	River/ stream	Fish (including lampreys)	Cellulose nitrate (CN)	0.45	dPCR

al., 2020	Methods comparison (field)					
Larson *et al.*, 2022	Presence/non-detection, eDNA ecology	Ocean (nearshore)	Fish (including lampreys)	Cellulose nitrate (CN)	0.45	Metabarcoding
Parson *et al.*, 2018	Presence/non-detection, Sequencing	Ocean (nearshore)	Mammals (marine)	Cellulose nitrate (CN)	0.45	Metabarcoding, qPCR
Galaska *et al.*, 2023	Species richness	Ocean (nearshore), Ocean (pelagic)	All species	Polyethene sulfone (PES)	0.22	Metabarcoding
Sepulveda *et al.*, 2018	Presence/non-detection, Methods comparison (laboratory)	Lake	Fish (including lampreys)	Glass microfiber (GMF), Mixed cellulose ester (MCE)	1, 1.2	qPCR
Tillotson *et al.*, 2018	Species quantification, eDNA ecology	River/stream, Lake	Fish (including lampreys)	Cellulose nitrate (CN)	0.45	qPCR

Deeg, C. M., Li, S., Esenkulova, S., Hunt, B. P. v, Schulze, A. D., & Miller, K. M. (2023). Environmental DNA survey of the Winter Salmonosphere in the Gulf of Alaska. *Environmental DNA*, *5*(3), 519–539. https://doi.org/https://doi.org/10.1002/edn3.404

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Menning, D., Simmons, T., & Talbot, S. (2020a). Using redundant primer sets to detect multiple native Alaskan fish species from environmental DNA. *Conservation Genetics Resources, 12*(1), 109–123. https://doi.org/10.1007/s12686-018-1071-7

Menning, D. M., Ward, D. H., Wyllie-Echeverria, S., Sage, G. K., Gravley, M. C., Gravley, H. A., & Talbot, S. L. (2020b). Are migratory waterfowl vectors of seagrass pathogens? *Ecology and Evolution, 10*(4), 2062–2073. https://doi.org/10.1002/ece3.6039

Pochardt, M., Allen, J. M., Hart, T., Miller, S. D. L., Yu, D. W., & Levi, T. (2020). Environmental DNA facilitates accurate, inexpensive, and multiyear population estimates of millions of anadromous fish. *Molecular Ecology Resources*, *20*(2), 457–467. https://doi.org/10.1111/1755-0998.13123

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Sepulveda, A., Hutchins, P., Massengill, R., & Dunker, K. (2018). Tradeoffs of a portable, field-based environmental DNA platform for detecting invasive northern pike (Esox lucius) in Alaska. *Management of Biological Invasions*, *9*, 253–258. https://doi.org/10.3391/mbi.2018.9.3.07

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, *220*, 1–11. https://doi.org/https://doi.org/10.1016/j.biocon.2018.01.030

Table A5: A noncomprehensive list of companies offering eDNA analyses throughout the world, as well as the services they offer based on content appearing on their website. Note that many of these companies likely offer additional services beyond what was noted on their websites.

Company	Location	Field kits	Extractions	Library prep	Metabarcoding	qPCR	dPCR	ddPCR	Data analysis	Training
AllGenetics	Spain	x	x		x					
Applied DNA Sciences	United States									
Applied Genomics LTD	United Kingdom	x	x		x					
AquaBiota	Sweden				x	x				
Azenta/Genewiz	United States					x	x			
Borealis Biodesign	United States				x				x	
Bureau Veritas	North America ?					x				
Dante Genomics					x					
CALeDNA	United States	x	x	x	x	x		x		x
eDNA Labs	Croatia		x		x	x		x		
eDNAtech	Canada	x	x		x					
EnviroDNA	Australia				x	x				
Environmental Genomics and Conservation Genetics Laboratory	United States				x	x			x	
Eurofins Genomics					x	x				
Fera	United Kingdom	x								
Genidaqs	United	x	x		x	x			x	

	States									
ID-Gene	Europe?	x	x		x				x	
identifica	Portugal	x	x		x	?				
Jonah Ventures	United States	x	x		x	x				
Measurlabs	Finland				x					
National Genomics Center for Wildlife and Fish Conservation	United States									
Pacific Northwest Environmental DNA Laboratory	United States		x			x			x	
SGS	Portugal		x		x					
SimplexDNA	USA, Europe	x	x		x					
Sinsoma	Austria	x	x		x					
SPYGEN	France, Canada	x			x					
SureScreen Scientifics	England	x				x				
Sylphium Molecular Ecology	Netherlands	x	x		x	x				
Taxus Medio Ambiente	Spain				x					
TropWATER	Australia		x		x				x	x
Wilder Lab	New Zealand	x			x					

Table A6: Primer sets and probes for eDNA analysis used in Alaska.

Target taxa	5’ to 3’ end	Target region; taxa	Citation
Vertebrate specific; *Ophiodon elongatus, Stenobrachius leucotensis, Oxylebius pictus, Lipolagus ochotensis, Diaphus, Trichodon trichodon, Sebastinae, Atheresthes, Liparidea, Oligocottus maculosus, Gasterosteidae, Artedius, Anoplopomatidae, Hexagrammos, Osmeriformes, Ammodytes, Gadidea, Clinocottus Ccuticeps, Leptocottus armatus, Peuronectidae, Clupea pallasii, Cottiodidei, Salmoninae*	12S-V5-F CGACAGGTTCAGA GTTCTACAGTCCGA CGATCACTGGGATT AGATACCCC 12S-V5-R GTGACTGGAGTTCA GACGTGTGCTCTTC CGATCTTAGAACAG GCTCCTCTAG	12S mitochondrial gene	Larson *et al.,* 2022
Pleuronectidae, Onchorynchus nerka, Stenobrachius, Lipolagus ochetensis, Sebastes, Tarletonbeania, Squalus acanthias, Onchorynchus kisutsh, Oncorynchus keta, Symbolophorus californiensis, Onchorynchus gorbusha, Clupea pallasii, Lycodinae,		Cytochrome oxidase subunit 1 (COI)	Deeg *et al.,* 2023

Diaphus theta, Nansenia			
Esox lucius	EluCOI-F CCTTCCCCCGCATA AATAATATAA EluCOI-R GTACCAGCACCAGC TTCAACAC EluCOI probe 6FAM-CTTCTGACTTCTCC CC-MBG-NFQ	Cytochrome oxidase subunit 1 (COI)	Dunker *et al.,* 2016
Esox lucius	EluCOI-F CCTTCCCCCGCATA AATAATATAA EluCOI-R GTACCAGCACCAGC TTCAACAC EluCOI probe 6FAM-CTTCTGACTTCTCC CC-MBG-NFQ	Cytochrome oxidase subunit 1 (COI)	Sepulveda *et al.,* 2018
Oncorhynchus nerka	*F GGAAACCTTGCCCA CGCG R AAAAGTGGGGTCTG GTACTGAG probe FAM-CTCTGTTGACTTAAC CATC-MGB*	Cytochrome oxidase subunit 1 (COI)	Levi *et al.,* 2018

Oncorhynchus kisutch	*F CGCTCTTCTAGGGGA TGATC* *R CTCCGATCATAATCG GCATG* *probe FAM-ATTTACAACGTAATC GTC-MGB*	Cytochrome oxidase subunit 1 (COI)	Levi *et al*., 2018
Oncorhynchus tshawytscha	F CTGGCACMGGGTG AACAGTCTACC R AAT GAA GGG AGA AGA TCG TYA GAT CA probe 6FAM-CTCCTGCGTGGGCT AG-MBG-NFQ	Cytochrome oxidase subunit 1 (COI)	Khalsa *et al.*, 2020
Labyrinthula *sp.*	F CAATGAATATCTTG GTTTCCG R GAGTGCTCGTTTGT GGACG	5.8	Menning *et al.,* 2021
Labyrinthula *sp.*	F ACCACATCCAAGGA AGGC R AATATACGCTACTG GAGC	18S	Menning *et al.,* 2021
Halo/Phytophthora spp.	F AACTTTCCACGTGA ACCG R TAAAAGCAGAGAC TTTCG	ITS	Menning *et al.,* 2021
Phytophthora sp.	F TCDTCDHTATTAGG TGC	Cytochrome oxidase subunit 1 (COI)	Menning *et al.,* 2020b

	R GTRTTWAARTTTCT ATC		
Oncorhynchus sp.	AK16SF CGA GAA GAC CCT ATG GAG C AK16SR GCG CTG TTA TCC CTA GGG T	16S	Menning *et al.,* 2020a
Oncorhynchus sp.	AK12S-F CTC GTG CCA GCC ACC GCG GTTA AK12S-R GGG TAT CTA ATC CCR GTT TG	12S	Menning *et al.,* 2020a
Oncorhynchus sp.	AKCOISal-F TAG TAT TTG GTG CCT GAG C AKCOISal-R ATY ATA ACG AAG GCA TGG GC	Cytochrome oxidase subunit 1 (COI)	Menning *et al.*, 2020a
Coregonus sp.	AKCOICor-F GCT GCT AGG ACA GGA AGG GA AKCOICor-R GCT GCT AGG ACA GGA AGG GA	Cytochrome oxidase subunit 1 (COI)	Menning *et al.*, 2020a
Prosopium coulterii	AKCOIProR ATC ATA ACG AAG GCG TGG GC	Cytochrome oxidase subunit 1 (COI)	Menning *et al.*, 2020a
Oncorhynchus nerka	SECO3_861-930-F TCTGCCCTTCTCCT TACGATTTT SECO3_861-930-R GTTCGACCTAGAAA TCGCCCTT SECO3_861-930-probe	Cytochrome c oxidase subunit III gene	Tillotson *et al.*, 2018

	6FAM-5′-CCATCCTGTTCCTCCT-3′-MGBNFQ		
Eukaryotes, Bacteria and Archaea	F GTGYCAGCMGCCGCGGTAA R CCGYCAATTYMTTTRAGTTT	16 rRNA mitochondrial locus	Galaska, Brown, McAllister, 2023
Eukaryotes and bacteria	F CTGGTGCCAGCAGCCGCGGYAA R TCCGTCAATTYCTTTAAGTT	18S nuclear RNA	Galaska, Brown, McAllister,
Thaleichthys pacificus	Euc_COI_R (5′- Euc_COI_R (5′- Euc_COI_R-F CTCCCTCCTTCCTTCTCCTT Euc_COI_R-R GGTCTGGTACTGGGAAATGG Euc_COI_R-Probe 6FAM-AGCGGGAGCCGGGACTGGCT-MGBNFQ	Cytochrome oxidase subunit 1 (COI)	Pochardt *et al.,* 2020
Lota lota	F GCCGTAATACTCCTTGGCCTT R CAATCGGGTTAGCGGGTGTA probe FAM-TGCCCTTGCCCTCTTCT-	Cytochrome oxidase subunit 1 (COI)	Rodgers *et al.,* 2017

Cottus cognatus	F GGAGGCGTCCTAGCCCTC R GAGTCCAAAATAGGAATTGGGTCAC Probe FAM-CATCCATCCTGGTGCTCAT-MGB-NFQ	Cytochrome b (cytb)	Rodgers *et al.*, 2017
Salvelinus alpinus/Salvelinus malma	F CCGCCACAGTACTTCACCTTCTA R AGGCCAAGCAATATAGCTACGAAA Probe FAM-CCGACAAAATCTCATTCC -MGB-NFQ	Cytochrome oxidase subunit 1 (COI)	Rodgers *et al.*, 2017
Thymallus arcticus	F TGTGGGCTGTTCTGATTACCG R TGCTGGGTCAAAGAAAGTGGTATTA Probe FAM-CTTGCAGCAGGTATC- MGB-NFQ	Cytochrome oxidase subunit 1 (COI)	Rodgers *et al.*, 2017
Elodea spp.	F GAAGCGGCAGAAATCAGTGG R TCTTGGGGTTTTMGTTATTTTGACCR Probe FAM-TCATAGTAAACTAAGTTCCTCACAC-MGB-NFQ	Elod-1	Benson *et al.*, 2024

Elodea spp.	F- TATCCAAAARGGTC CCGCCC R- TGCATCATGTTGAA ACGCGA FAM- TGCYTGCGTTACGT GAAAGTAATACGT - MGB-NFQ	Elod-2	Benson *et al.,* 2024
Elodea canadensis	F CCTATTGGCCAAGA ATTCATTAAG R CTCTGATMGAATTG GAAAGCATTAAA Probe FAM- CTTTTATGAAGTAG GGATATTCCATCG- MGB-NFQ	*ELCA7-1*	Benson *et al.,* 2024
Elodea nuttallii	F CYTTGATTGGGCCC TCTCAG R GGTTGAAGATCACA GGGCGA Probe FAM- CCAAGAACCGAATT AAAAATAGAGTGG- MGB-NFQ	*ELNU2-1*	Benson *et al.,* 2024
Sebastes	SebDLoop_F ATNACCATATCTAG GNTTNAACC SebDLoop_R TGRRCTTGTTGGTC GGYT		Ledger *et al.,* 2025

Sebastes	Thr-RF GAGGAYAAAGCAC TTGAATGAGC D-RF CCTGAAAATAGGAA CCAAATGCCAG		Ledger *et al.,* 2025

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Table A7: Published guidelines used and published by resource management with summaries from each resource for what sampling methods are recommended. Methods include when and if to use negative controls, which filter pore size and filter type to use, and year of publication for eDNA-based methods.

	Sampling method	**Negative controls**	**Filter pore size**	**Filter type**	**Cite**
United State Department of Agriculture [1]	Vacuum filtration	Laboratory	1.5 µm	Glass microfiber	Carim *et al.,* 2016
United States Fish and Wildlife Service [2]	Vacuum filtration, centrifugation, precipitation, flocculation	Field and laboratory	0.2 - 5 µm	Glass microfiber, cellulose nitrate, or mixed cellulose ester filters	Bockrath *et al.,* 2022
United States Geological Survey [3,4]	Vacuum filtration	N/A	N/A	N/A	Laramie *et al.,* 2015
Alaska Invasive Species Partnership[5]	N/A	N/A	N/A	N/A	Dunker *et al.,* 2022

Alaska Department of Fish and Game Division of Sport Fish[7]	Vacuum filtration	N/A	1.0–1.2 µm	Whatman glass	Massengill *et al.,* 2022
United States Geological Survey, Nonindigenous Aquatic Species Database[8]	Vacuum filtration	Field and laboratory	N/A	N/A	Ferrante *et al.,* 2023

Carim, Kellie J.; McKelvey, Kevin S.; Young, Michael K.; Wilcox, Taylor M.; Schwartz, Michael K. 2016. A protocol for collecting environmental DNA samples from streams. Gen. Tech. Rep. RMRS-GTR-355. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 18 p.

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Survey Questions:

University of Alaska Fairbanks (UAF) staff and graduate students in the College of Fisheries and Ocean Sciences and the International Arctic Research Center are investigating how environmental DNA (eDNA) science is being applied to address research questions in the state. Specifically, they are interested in projects in **Alaska** that use **eDNA from water samples specifically (no soil samples, ancient DNA, stomach content samples, fecal samples, etc...).** Many eDNA studies in the state are ongoing and have not yet been published, making a literature review limiting. In turn, UAF researchers developed this survey to gather additional data on unpublished projects. **If your project is published, you can use this survey to provide links to these documents (see "Survey guidelines").** The long-term goal of this effort is to foster discussions of possible **standardization of eDNA methods and applications** to maximize the power of this ecological tool. Findings from this project will be presented at AMSS and AFS-AK 2024 and will aid in the development of a review paper.

Survey guidelines

- **Submit a single survey** if your projects have been published (peer-reviewed or technical reports). When you begin the survey, you'll reach a question that allows you to share links or titles to all publications in a single entry, thus avoiding a submission for each study. **Time commitment: 5 minutes**
- **Submit one survey per study** if your projects are unpublished (the survey goes faster than you may anticipate!). **Time commitment: 10 minutes**

Feel free to forward this to other project leads that might be interested in contributing to this statewide project. **We look forward to sharing our findings with you!**

Statement of consent

I understand the information presented to me. My questions have been answered to my satisfaction, and I agree to participate in this study. I am 18 years old or older and I have been offered a copy of the consent to participate form (click HERE to download). By clicking the 'submit' button at the end of this survey I agree to participate.

Survey questions:

Project status

1. Project status*

a. Completed, published (peer-reviewed or technical report)
b. Completed, unpublished
c. Ongoing, unpublished
d. Other:

2. Publication link(s)

3. Please provide a link to your publication(s) and/or report(s) corresponding to your eDNA project(s) - we'll take care of the rest!

4. If you're willing, please provide a general description of your sampling location below (e.g. lake name, section of river, ocean zone, single lat/long in center of sampling locations, link to metadata with location(s), etc...). If you've submitted DNA sequence data to NCBI with metadata that contained sampling locations, you can include the corresponding links below.

Project development

5. When did you collect your first sample (MM-YY, for example if you started in April 2023 you would answer 04-23)?

6. When did you collect your last sample (MM-YY)?

7. In what region of Alaska did your project take place? If marine-based, please select "other" and enter the general location (e.g. Cook Inlet). If your study area lands on the border of two regions, select both regions.

a. 1 (north-northwest)
b. 2 (interior)
c. 3 (west-southwest)
d. 4 (southcentral)
e. 5 (southeast)

8. Which type(s) of collaborator(s) were involved with project development and/or implementation (select all that apply)?

a. State agency
b. Federal agency
c. Tribal entity
d. Academic institution
e. Nonprofit
f. Consultant
g. Subsistence harvesters
h. Recreational fishermen
i. Commercial fishermen
j. Other:

9. What was the primary funding source(s) for your study (select all that apply)?

a. Federal grant funding
b. State grant funding
c. Tribal grant funding
d. Private funding
e. Other:
f.

10. What best describes your project (select all that apply)?

a. Biodiversity/species richness assessment
b. Invasive species assessment
c. Rare species assessment
d. Presence, non-detection of specific species
e. Species quantification
f. eDNA ecology (distribution, persistence, etc)
g. Field sampling methods comparison
h. Laboratory methods comparison

i. Assay development, validation
j. Other:

Sample collection

11. What time of water bodies did you collect your samples from (select all that apply)?*
a. River/stream
b. Lake
c. Wetland
d. Estuary
e. Ocean (nearshore)
f. Ocean (pelagic)
g. Other:

12. What species were you targeting?*
a. All species found in sample (biodiversity assessment)
b. Fish (including lampreys)
c. Crustaceans
d. Reptiles
e. Mammals (marine)
f. Mammals (land-based)
g. Mollusks
h. Macroinvertebrates
i. Plants (macrophytes)
j. Microalgae
k. Other:

13. What technique(s) did you use to analyze your DNA extractions (select all that apply)? *
a. Metabarcoding
b. qPCR (i.e. real-time PCR)
c. Digital PCR (dPCR)
d. Droplet digital PCR (ddPCR)
e. CRISPR
f. Other:

14. What type of filter did you use during filtration (select all that apply)? *
a. Mixed cellulose ester (MCE)
b. Polyethene sulfone (PES)
c. Polycarbonate (PC)
d. Cellulose nitrate (CN)
e. Polyvinylidene fluoride (PVDF)
f. Glass microfiber (GMF)
g. Other:

15. What was the filter pore size?

Laboratory + data analyses

16. At what steps did you implement biological and/or technical replicates (select all that apply)?*
a. Field sampling
b. Filter subsetting (i.e. cutting filters in half - one half archived, the other used for DNA extractions)
c. DNA extractions
d. DNA analyses (metabarcoding, qPCR, etc...)
e. Other:

17. At what steps did you implement sample blanks (i.e. field blanks, qPCR blanks; select all that apply)?*
a. Sample collection
b. Filter subsetting (i.e. cutting filters in half - one half archived, the other used for DNA extractions)
c. DNA extractions
d. DNA analyses (metabarcoding, qPCR, etc...)
e. Other:

18. Which of the following techniques did you use (select all that apply)?*
a. Metabarcoding
b. qPCR (i.e. real-time PCR)
c. Digital PCR (dPCR)
d. Droplet digital PCR (ddPCR)
e. CRISPR
f. Other:

19. What type of statistical approaches did you use to analyze your results (select all that apply)?
a. Graphical analyses
b. Descriptive statistics
c. Generalized linear models
d. Site occupancy model
e. Other:

Dissemination of finding + results

20. How did you communicate your findings with others (select all that apply)?*
a. Professional conferences
b. Direct communication with state/federal managers, researchers
c. Direct communication with user groups (e.g. subsistence, commercial, recreational harvesters)
d. Public meetings or outreach events
e. Peer-reviewed article
f. Results have not been communicated to those outside of the core research group
g. Other:

Barriers in eDNA research

21. What kinds of barriers have you faced when conducting eDNA research? *

a. Insufficient funding
b. Lack of agency/organization support
c. Lack of laboratory access / lack of funding for sample analyses
d. Uncertainty about how to analyze data
e. None. I am well-versed in eDNA methods and applications.
f. Other:

22. Did you hire anyone outside of your agency to complete any of the following steps (select all that apply)?*

a. Field sample collection
b. DNA extractions
c. DNA quantification
d. Metabarcoding
e. Sequencing efforts
f. Computational analyses (i.e. bioinformatics)
g. We did not hire anyone outside of our agency
h. Other: