UMEÅ UNIVERSITY

USING ENVIRONMENTAL DNA TO UNRAVEL AQUATIC ECOSYSTEM DYNAMICS

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To Freja and Frans, without whom this would have been finished much earlier.

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Abstract

Human-induced climate change has led to unprecedented declines in Earth's biodiversity and significant habitat loss. Aquatic ecosystems are especially at risk, facing pollution, overexploitation, and destruction. Consequently, monitoring biodiversity is critical. Traditional monitoring methods are often low in detection rates, time-consuming, invasive, and harmful to species, which hampers comprehensive biodiversity assessments. Environmental DNA (eDNA) offers a rapid alternative for taxonomic identification, extracting genetic material from soil, sediments, or water without capturing living organisms, proving useful where traditional methods fall short. However, its integration into aquatic ecology is hampered by unresolved methodological issues.

This thesis demonstrates how eDNA can help reconstruct fish colonization histories in lakes post-glacial retreat. I employed species-specific primers with digital droplet PCR and metagenomic shotgun sequencing on ancient DNA from Holocene lake sediments. My findings show the detectability of DNA from ancient fish populations. However, each method exhibited technical limitations that led to varying degrees of false negatives and false positive results. Additionally, I examined how Northern pike (*Esox Lucius*) affects ecological speciation in European whitefish (*Coregonus lavaretus*), promoting a shift from insectivorous to piscivorous states, enhancing predator biodiversity and biomass. Diet analyses of piscivorous birds through digital droplet PCR revealed that smaller whitefish support a larger, more diverse bird community. Finally, I compared two molecular techniques for quantifying bird diets from fecal DNA, finding that metabarcoding with a universal fish primer and digital droplet PCR yielded similar results. This research enhances our understanding of the potential and limitations of molecular tools for species identification and aids the integration of eDNA into aquatic ecology.

Keywords:

Environmental DNA, ancient DNA, colonization, apex predator, pike, whitefish, piscivorous birds, aquatic ecosystems, metabarcoding, digital droplet PCR

List of Chapters

This thesis provides a summary based on the following four manuscripts, which are referred to the text by their respective Roman numeral.

I. Estimating species colonization dates using DNA in lake sediment. **Olajos, F**., Bokma, F., Bartels, P., Myrstener, E., Rydberg, J., Öhlund, G., Englund G. (2018) Methods Ecol. Evol., 9 (2018), pp. 535-543.

II. Fish colonization patterns in central Sweden from droplet digital PCR and shotgun sequencing of sedimentary DNA. Manuscript. **Olajos, F**., Rouillard, A., Wang, Y., Pedersen, M.W., Capo, E., Öhlund, G., Englund G.

III. Apex predator induces predator-rich ecosystem state in northern lakes. Manuscript. Öhlund, G., **Olajos, F**., Öhlund, S-O., Nilsson, K., Jansson, J., Karlberg, Y., Söderlund, E., Foth, A., Peedu, M., Johansson, P., Lindberg, B., Finstad, A., Bartles, P., Hudson, A., Sjögren, E., Englund G.

IV. Consistent findings from ddPCR and metabarcoding analyses of piscivorous bird diets. Manuscript. **Olajos, F.**, Capo, E., Englund, G., Söderlund, E., Öhlund, G.

Author contributions

- **I.** G.E., G.Ö. and F.B. conceived the ideas; F.O., R.Z., G.E., E.M., R.B., J.R. and P.B. collected and sampled the cores; F.O. and X.-R.W. developed primers; F.O. performed all the laboratory analyses; F.B. developed the statistical model; F.O., F.B. and G.E. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.
- **II.** F.O. and G.E conceived the ideas; F.O., G.E. and E.C. collected and sampled the core. F.O developed the primers; F.O performed all ddPCR-related laboratory analyses; F.O and A.R performed all metagenomic-related laboratory analyses; A.R., Y.W., M.W.P., F.O. and E.C. performed bioinformatics and analyzed the data; G.E. and G.Ö. discovered the historical data; F.O wrote the first draft and finalized the manuscript after comments from co-authors.
- **III.** G.Ö. and G.E conceived the ideas; F.O., G.E., G.Ö., SO.Ö., J.J., M.P., and P.J. contributed to the surveys of fish and birds, F.O. developed the primers. F.O. and E.S. performed all ddPCR-related laboratory analyses.
- **IV.** F.O. conceived the ideas; G.Ö., G.E., F.O. and E.S. collected samples. F.O developed the primers; F.O and E.S performed all ddPCR-related laboratory analyses; F.O performed all metabarcoding-related laboratory analyses; F.O. and E.C. performed bioinformatics and analyzed the data; F.O wrote the first draft and finalized the manuscript after comments from co-authors.

Author abbreviations

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List of other published works by author

Kanbar H, Tran Le T, **Olajos F**, Englund G, Holmboe M (2023) Tracking mineral and geochemical characteristics of Holocene lake sediments: the case of Hotagen, west-central Sweden. *Journal of Soils and Sediments* 21(9)

Capo E, Giguet-Covex C, Rouillard A, Nota K, Heintzman PD, Vuillemin A, Ariztegui D, Arnaud F, Belle S, Bertilsson S, Bigler C, Bindler R, Brown AG, Clarke CL, Crump S, Debroas D, Englund G, Ficetola FG, Garner R, Gauthier J, Gregory-Eaves I, Heinecke L, Herzschuh U, Ibrahim A, Kisand V, Kjær KH, Lammers Y, Littlefair J, Messager E, Monchamp M-E, **Olajos F**, Orsi W, Pedersen MW, Rijal D, Rydberg J, Spanbauer T, Stoof-Leichsenring K, Taberlet P, Talas L, Thomas C, Walsh D, Wang Y, Willerslev E, Woerkom A, Zimmermann H, Coolen MJL, Epp L, Domaizon I, Alsos I, Parducci L. (2021) Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. *Quaternary*, 4(6).

Capo E, Spong G, Koizumi S, Puts I, **Olajos F**, Königsson H, Karlsson J, Byström P (2021). Droplet digital PCR applied to environmental DNA, a promising method to estimate fish population abundance from humic-rich aquatic ecosystems. *Environmental DNA*. 3(2):343-352.

Kanbar H, **Olajos F**, Englund G, Holmboe M (2020) Geochemical identification of potential DNA-hotspots and DNA-infrared fingerprints in lake sediments. *Applied Geochemistry* 122:104728.

Englund G, Öhlund G, **Olajos F**, Finstad A, Bellard C, Hugueny B (2020) Holocene extinctions of a top predator – Effects of time, habitat area and habitat subdivision. *Journal of Animal Ecology* 89(1).

Background

Understanding changing ecosystems with environmental DNA

The past century bears witness to a concerning decline in Earth's biodiversity, largely caused by human-induced disturbances (Butchart et al., 2010; Barnosky et al., 2011; Dirzo et al., 2014). This decline has triggered an acceleration in species extinction rates, now surpassing those observed before human industrialization (Pimm et al., 1995; Johnson et al., 2017). Beyond the immediate environmental consequences, such dramatic reductions in biodiversity may compromise the integrity of global ecosystems, but also, human well-being and the future sustainability of our planet (Diaz et al., 2006). In light of this, the monitoring of biodiversity has become increasingly important. Detailed and consistent screenings of biodiversity can provide valuable insights into the relationships, dynamics, and evolution of various organisms (Magurran, 2004; Tilman et al., 2014). In this era of rapid environmental change, continuous and rigorous assessments of how species are interconnected can be considered key in evaluating ecosystem health and identifying biodiversity hotspots (Myers et al., 2000).

The traditional monitoring techniques that have been used to build our current knowledge about the fauna and flora living in Earth's ecosystems often involve observation or capture of whole organisms. This requires intricate knowledge of physical species identification, which can be time-consuming and unreliable when the morphological variation within species is large compared to differences between species. This is especially true, for example, when closely related species have nearly indistinguishable juvenile stages, and when organisms exhibit significant phenotypic plasticity (Kelly et al., 2012). Traditional methods may also disturb habitats and have difficulty detecting small or elusive species, making comprehensive community surveys challenging. Lastly, these methods can be invasive, hazardous, and lethal to the organisms. This has prompted the search for innovative alternatives.

An alternative method, which has proven transformative in ecological research over the last decade, is the study of DNA present in environmental matrices—referred to as environmental DNA or eDNA (Taberlet et al., 2018). This DNA encompasses genetic material sourced directly from environmental samples such as soil, water, and air, allowing

for the identification and classification of species. The DNA, expelled into their surroundings by organisms, can originate from a variety of biological materials, ranging from excreted cells and tissues (like urine and feces) to larger sources, such as decaying organisms. These eDNA fragments can endure in the environment, with preservation periods varying widely depending on conditions—from days in temperate waters (Dejean et al., 2011) to millions of years in aquatic sediments (Armbrecht et al., 2022) and permafrost (Kjær et al., 2022). The application of eDNA has revolutionized biomonitoring and has become an instrumental tool in describing temporal changes in biodiversity (Bálint et al., 2018; Taberlet et al., 2018). It has been used to further enhance the understanding of different ecological states, help investigate environmental contamination, and even reliably detect invasive species (Goldberg et al., 2011; Darling & Mahon, 2011; Smart et al., 2015).

Conclusively, while challenges persist, eDNA offers a promising tool in the fight to reverse the global decline in biodiversity. Used complementary with traditional survey and inventorying methods, eDNA can provide an extra dimension and further enhance the potential in the realm of biodiversity conservation.

Monitoring fish communities from lakes with environmental DNA

Fishes, being relatively long-lived, mobile, and spanning diverse trophic levels, serve as excellent indicators of long-term habitat conditions and are sensitive to many human disturbances. Traditional fish monitoring methods, such as electrofishing, gillnetting, and acoustic surveys, often face challenges due to morphological identification, low detection rates, and gear selectivity issues. In contrast, DNA-based identification, especially when integrated with conventional survey methods, emerges as an important complement, enhancing species detection reliability and bolstering confidence in monitoring outcomes (Hinlo et al., 2017; Keck et al., 2022).

Molecular DNA methods used in aquatic monitoring range from the detection of specific species by classical PCR amplification to hybridization capture methods (Taberlet et al., 2018; Wilcox et al., 2018; Zinger et al., 2019; Capo et al., 2021). Firstly, single-species detection using PCR, qPCR, and ddPCR requires the development of primers that amplify a short region characteristic of the target taxon, typically mitochondrial in origin for fish populations (e.g., cytB, COI, 12S rRNA

genes). PCR assays require prior knowledge of the genetic diversity within the targeted biological groups and can only indicate the presence or absence of a target in a given sample. Both qPCR and ddPCR can provide some insight into the quantitative aspects of species abundance. Metabarcoding, another method, involves amplifying specific genic regions and sequencing them to characterize the diversity and composition of a taxonomic group in a sample (Taberlet et al., 2018). After sequencing, DNA sequences are assigned to organisms using reference databases that contain genetic sequences and corresponding taxonomic information. Depending on the resolution of the barcode, which can range from conserved to highly variable, assigned sequences allow for the identification of various taxonomic levels, from whole kingdoms to specific species. Overall, multispecies approaches generally use metabarcoding with universal primers and high-throughput sequencing. Metagenomics, in contrast, involves sequencing all DNA fragments present in a DNA extract. Metagenomic short DNA sequences can either be compared to reference databases or assembled into longer DNA fragments using k-mer clustering approaches for more accurate identification. Finally, hybridization capture is a viable alternative to metagenomics, capturing and sequencing low-abundant DNA sequences using baits created in-silico from a reference database.

As this research field is relatively new, eDNA monitoring time series at best span two decades (Bálint et al., 2018) and thus cannot provide data about pre-disturbance periods for most studied ecosystems. Fortunately, DNA traces preserved in sedimentary archives can be used to reconstruct past changes in both aquatic and terrestrial ecosystems (Capo et al., 2024). Historically, records of aquatic macro-organism abundance spanning significant periods are uncommon (Gregory-Eaves and Smol, 2024). However, the DNA preserved in sediments could bridge this informational gap, particularly as recent eDNA studies suggest a potential correlation between DNA copies in water and species abundance (Rourke et al., 2022). Additionally, the recovery of fish DNA from sediment has been documented (Huston et al., 2023), pointing toward sedimentary sequences as potential records of changes in fish DNA concentrations over time. Yet, more research is required to validate this theory for understanding historical fish abundance trends.

Methodological challenges

Like many scientific methods, eDNA-based approaches come with inherent challenges. One significant issue is the degradation of eDNA across various environments, which can reduce detection rates and the

accuracy of species identification. Macroorganisms shed DNA at varying rates, influenced by factors such as biomass, size, activity, and life stage (Goldberg et al., 2011; Maruyama et al., 2014; Pilloid et al., 2014; Klymus et al., 2015). Additionally, the persistence, degradation, and lifespan of deposited eDNA are affected by both biotic and abiotic factors, including microbial and enzymatic activity, pH, temperature, UV light, salinity, and flow rate (Barnes et al., 2014; Strickler et al., 2015; Eichmiller et al., 2016; Jane et al., 2015; Rourke et al., 2022; Brandão-Dias et al., 2023; Mauvisseau et al., 2022). Allochthonous DNA from external sources, such as piscivorous birds, can also skew results through feeding or fecal deposition in the ecosystem. Detecting a direct relationship between the abundance of eDNA and the corresponding abundance of study organisms is often challenging. eDNA concentrations can vary greatly across different environments, necessitating careful optimization of sampling methods to achieve consistent results, especially when exploring the relationship between eDNA, species abundance, and biomass. While some studies have established such relationships (Eichmiller et al., 2016; Lacoursière-Roussel et al., 2016; Capo et al., 2021), others have not (Rourke, 2023).

Our ability to analyze eDNA data relies heavily on the extent and quality of reference material stored in public databases. The most commonly used databases include the National Center for Biotechnology Information (NCBI), the European Molecular Biology Laboratory (EMBL), Barcode of Life (BOL), and MITOFISH. Although NCBI and EMBL house most published genetic sequences, they exhibit biases such as misassigned species, incomplete genome segments, and underrepresentation of many species or their genetic diversity. BOL and MITOFISH aim to standardize and enhance global usability for eDNA studies. In terms of molecular markers (primers), there are several essential trade-offs for effective fish eDNA detection. Mitochondrial markers are typically chosen due to their higher copy number abundance per cell compared to nuclear DNA. Generally, short barcodes are favored for their effectiveness in maintaining taxonomic precision, regardless of whether the study targets single or multiple species. Naturally, the degradation and fragmentation of DNA in the environment result in shorter fragments. In the future, ribosomal DNA markers might replace current molecular markers, offering higher specificity and accuracy in estimating biomass or population sizes.

Ecological context

Although the main focus of my studies was to develop eDNA methodologies, the specific ecological applications were inspired by previous work on speciation in whitefish. Öhlund et al. (2020) demonstrate how selective pressure from Northern pike (*Esox lucius*) causes European whitefish (*Coregonus lavaretus*) to diverge into distinct ecotypes to avoid predation, with traits such as size and habitat utilization evolving before genetically determined traits like gill rakers (**Fig. 1,2**). This thesis employs eDNA to explore the histories of fish and the diets of piscivorous birds, viewed through the lens of the pike-whitefish interaction. The study of fish history aims to extend the chronosequence beyond populations of known introduction age to discern the sequence of trait divergence and identify primary versus secondary selection mechanisms resulting from the initial divergence. The examination of bird diets is intended to describe how pike-driven whitefish divergence impacts the lake ecosystem.

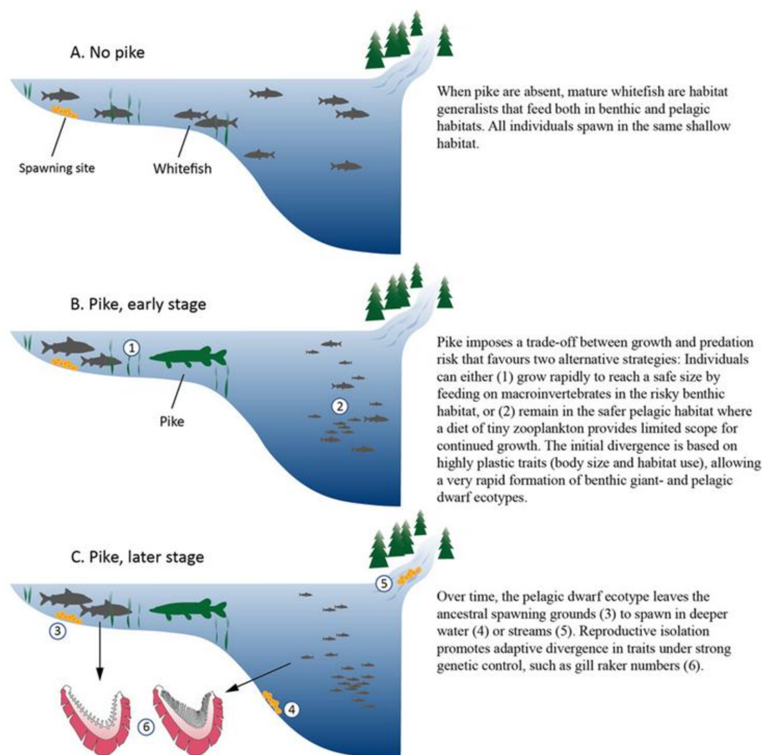

Figure 1. Illustration of the mechanism where pike causes phenotypic divergence in European whitefish (Öhlund et al., 2020).

Figure 2. Picture showing the two ecotypes that adhere to two distinct reproductively isolated populations. Both individuals are mature adults of comparable age. The top specimen is a benthic giant, the bottom specimen is a pelagic dwarf.

Objectives of the thesis

The overall objective of this thesis is to advance knowledge about aquatic ecosystems impacted by past and modern environmental change, using cutting-edge environmental DNA methods applied for biodiversity research. The specific methodological and ecological research questions addressed were:

1. Can we use the DNA preserved in lake sediments to reconstruct the history of fish populations? (chapter **I-II**)
2. When did fish populations colonize Swedish mountain lakes after the glacial retreat 9.000 years ago? (chapter **I-II**)
3. Can DNA extracted from faecal samples be used to reconstruct bird diets? (chapter **III-IV**)
4. How do apex predators regulate the composition and structure of aquatic food webs? (chapter **III-IV**)

Materials and Methods

Site locations

The two lakes targeted to study the history of fish populations are located in Jämtland and Västerbotten (Sweden) (**Fig. 3.A**, **I-II**). To study the diets of piscivorous birds, faecal samples were collected in Central to northern Sweden (**Fig 1.B**, **III-IV**).

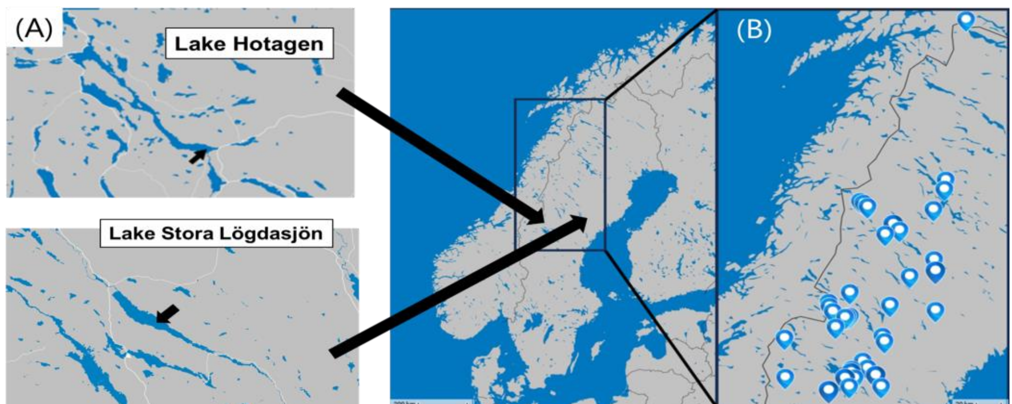

*Figure 3. (A) Map showing the sampling location of Lake Hotagen (**I-II**) and Lake Stora Lögdasjön (**I**). (B) Map showing the locations where feces from piscivorous birds were collected (**III-IV**).*

Sample collection

Sediment cores were collected from the lakes Hotagen and Stora Lögdasjön in September 2015 (for both, **I**) and in March 2018 (for Lake Hotagen, **II**), using a piston corer (86 mm internal diameter) at a water depth of 20 m (**Fig. 4**).

Bird faecal samples were collected from 2017 to 2022 in a total of 36 lakes situated across northern and central Sweden (**Fig. 5)** (**III-IV**). Six piscivorous bird species were studied: black-throated diver (*Gavia arctica*), red-throated diver (*Gavia stellata*), goosander (*Mergus merganser*), red-breasted merganser (*Mergus serrator*), arctic tern (*Sterna paradisaea*), and common tern (*Sterna hirundo*).

Figure 4. Sediment core sampling in Lake Hotagen. Left: Sampling from Ice - March 2018. Right: Sampling from boat - September 2015.

Figure 5. Examples of birds caught for diet analysis. Left: Juvenile tern spp. Middle: Adult common tern (Sterna hirundo). Right: Adult red-throated diver (Gavia stellata).

Primer design

In chapter **I**, three whitefish-specific primers were designed. For the analysis in chapters **II-IV**, a total of 8 primers were designed to target the following fish species: European whitefish (*Coregonus lavaretus*), common roach (*Rutilus rutilus*), Eurasian perch (*Perca fluviatilis*), brown trout (*Salmo trutta*), Eurasian minnow (*Phoxinus phoxinus*), Arctic char (*Salvelinus alpinus*), burbot (*Lota lota*) and northern pike (*Esox lucius*).

DNA extraction

DNA extraction was carried out for the molecular analysis performed in all chapters (**I-IV**). The protocols and reagents used for DNA extraction differ depending on the starting material. However, in general, the process of DNA extraction follows the same fundamental principles: 1. Lysis: Cells, tissues, proteins, and other structures are dissolved, releasing DNA into a solution. 2. Binding: The dissolved DNA is bound to a surface, such as beads or silica. 3. Washing: Impurities, proteins, and cellular debris are removed. 4. Elution: The purified DNA is released from its binding surface into a final liquid solution.

In Chapter **I**, DNA was extracted from sediment samples using the Poweroil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) as per the manufacturer's protocol. To increase the DNA yield, the extract was concentrated using a SpeedVac to a final volume of 25 µL. In Chapter **II**, two different DNA extraction methods were used for subsequent molecular analysis. For ddPCR analysis, DNA was extracted using the PowerSoil DNA Extraction Kit (Qiagen, Hilden, Germany) with a modified lysis step, and concentrated to a final volume of 50 µL using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). For shotgun sequencing, DNA was extracted using a custom method tailored for organic material, based on phenol-chloroform, and the samples were then concentrated to a final volume of 35 µL using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany).

In chapter **III** and **IV,** DNA was extracted from bird fecal matter using the DNeasy Blood and Tissue DNA Extraction Kit (Qiagen, Hilden, Germany) with a custom lysis step. Fecal samples were not concentrated.

All extractions, both sediment and fecal matter in all chapters **I-IV** were purified after elution using the OneStep PCR Inhibitor Removal Kit (Zymo Research, Freiburg, Germany).

PCR assay

Conventional PCR amplifications require molecular tools (i.e., primers) designed to bind and amplify a specific DNA fragment. This method has been largely used to detect a wide array of species (Taberlet et al., 2018) but is limited to presence or absence estimates. PCR is used in Chapter **I** to detect the presence of the European whitefish (*Coregonus lavaretus*) DNA in lake sedimentary archives.

ddPCR assay

The digital droplet PCR (ddPCR) is a molecular method that allows for the absolute quantification of specific DNA molecules from a given environmental sample. In ddPCR, the PCR reaction volume is partitioned into up to 20,000 small individual droplets; some contain the target molecule while others do not. Abundance estimates are based on the fluorescence emitted from each droplet, providing an indication of the success of PCR amplification. The relationship between positive and negative droplets allows for direct quantification of a sample without the need for standard curves or reference samples. ddPCR assays are less affected by PCR inhibitors, such as humic substances, because these are diluted when spread across droplets. This makes ddPCR more sensitive than other PCR-based methods (such as PCR and qPCR) when applied to environmental samples from organic-rich environments, including lake sediments or bird fecal matter. In chapter **II**, the ddPCR method is applied to assess the presence of fish DNA in ancient sediment samples. Furthermore, in chapters **III** and **IV**, ddPCR is performed to elucidate the dietary habits of piscivorous birds by analyzing the proportion of different fish DNA in avian fecal matter.

Metabarcoding assay

The metabarcoding approach utilizes universal primers that target conserved regions common across multiple species. These conserved regions are flanked by highly variable sequences, which allow for discrimination between taxa. Employing high-throughput sequencing, metabarcoding facilitates the identification of multiple taxa within a single environmental sample. PCR products from numerous samples can be processed and sequenced simultaneously, making metabarcoding a quick, efficient, and cost-effective method. In chapter **III**, this method is used to analyze the diet of piscivorous birds by amplifying fish DNA in avian fecal matter.

Metagenomic assays

Metagenomics is an umbrella term for molecular methods that enable the sequencing of all DNA fragments found in a given environmental sample. Unlike targeted approaches, metagenomics does not involve capture or enrichment processes, which allows it to sequence fragments that are often below the detection scope of PCR-based methods. This characteristic makes it particularly useful in environmental and ancient studies, where DNA tends to be highly fragmented. Additionally,

metagenomics does not require prior knowledge of the study system or the genetic composition of the species present; it sequences DNA molecules with equal probability. However, metagenomics is expensive, data-intensive, and relies on large-scale, taxonomically annotated databases for data processing. In chapter **II**, this method is used to analyze the presence of fish DNA in ancient lake sediments

Results and Discussion

This thesis provides new insights into the use of environmental DNA (eDNA) approaches to reconstruct past fish population histories and enhance our understanding of predator-prey interactions within aquatic food webs. My results demonstrate that fish DNA can be detected in lake sediments (**I-II**) and that fecal DNA can be used to ascertain the diets of piscivorous birds (**III-IV**). My work on lake sedimentary DNA offers additional information regarding fish colonization histories in both subglacial and postglacial lakes in Scandinavia following the glacial retreat 9,000-12,000 years ago (**I-II**). Additionally, the analysis of bird diets confirmed the significance of the pike-whitefish interactions in lake systems in determining the food sources of piscivorous birds (**III-IV**).

Lake sediments as a source for fish DNA

Most fish species currently found in Scandinavian lakes colonized soon after the last glaciation, 9,000-12,000 years ago (Huitfeldt-Kaas, 1918; Ekman, 1922; Svärdson, 1998; Lepiksaar, 2001; Berglund, 2004; Stroeven et al., 2016). Lakes situated below the highest post-glacial coastline were directly accessible to aquatic fauna colonizing from the Proto-Baltic Sea. Conversely, lakes above this coastline were inaccessible for many fish species due to waterfalls and steep rapids in connecting streams (Filipsson, 1994; Berglund, 2004; Hein et al., 2011; Stroeven et al., 2016), leading to varied and complex fish population histories influenced by both anthropogenic and natural factors (Englund et al., 2009; Hein et al., 2014; Henriksson et al., 2016). Lake sediments serve as a natural archive of genetic material from taxa within lakes and their catchments (Capo et al., 2024), where detection of DNA can be a valuable tool for reconstructing past ecosystems. However, challenges such as low DNA concentrations, DNA fragmentation, and PCR inhibitors can lead to high rates of false negative results. In this thesis, I present additional insights into the colonization histories of Scandinavian fish (**I-II**) and contribute to the advancement of methodologies for analyzing environmental DNA from fish in sediments (**I-II**). By employing a Bayesian algorithm to analyze DNA records from lake sediment cores, this approach helped identify false negatives and false positives, enabling me to infer colonization dates for European whitefish (*Coregonus lavaretus*) in two Swedish lakes, believed to have been colonized at different times post-glaciation. These PCR-based assays detected the presence of European whitefish 9,500 years ago in Stora Lögdasjön and 2,000 years ago in Lake Hotagen, situated above the highest post-glacial coastline (**I**). Furthermore, I applied cutting-edge

molecular methods, such as ddPCR and metagenomics, to sedimentary DNA obtained from Lake Hotagen to explore past fish histories in this lake (**II**). This second chapter pushes the boundaries in the detection of fish DNA from lake sediments with DNA for multiple species dating back to 7,000 years ago (whitefish, perch, pike, brown trout, roach, charr, burbot). These findings indicate that all currently native Swedish fish species have colonized the region, either naturally or through other means, for significantly longer periods than previously hypothesized. Moreover, metagenomic DNA analysis has proven more effective than PCR-based methods for reconstructing historical fish communities using DNA from lake sediments (**II**).

The food source of aquatic birds is determined by the predator-prey interactions between Northern pike and European whitefish

The European whitefish (*C. lavaretus*) forms ecotypes that are genetically and morphologically distinct in many Scandinavian lakes (Kahilainen and Østbye, 2006; Öhlund et al., 2020). It has been shown that the divergence process is primarily driven by a predatory fish species, the northern pike (*E. Lucius*). The pike induces a predominantly plastic differentiation in whitefish, leading to the emergence of benthic giants and pelagic dwarfs as distinct ecotypes. This pike-induced divergence gives rise to a new food-web structure, changing from an insect dominated state to a piscivorous state that increases the body size and growth rates of most piscivorous fish (**III**). Analysis of stomach contents reveals that the diet of piscivorous fish primarily consists of small pelagic dwarf whitefish, suggesting that these prey fish are a key factor in altering the structure of the food web (**III**). Additionally, increases in both the abundance and diversity of piscivorous birds suggest that the evolution of whitefish dwarfs change the structure of the bird community (**III**). To test this hypothesis, I used digital droplet PCR to analyze fish DNA in fecal samples of piscivorous birds. This method allowed me to quantify the proportion of whitefish in their diets, providing insights into how the availability of this fish species influences lacustrine bird communities. I discovered that the bird diet is heavily influenced by the composition of fish communities varying from one type of lake to another (**III**). In systems with pronounced whitefish divergence, the diets of piscivorous birds (terns, loons, and mergansers) were dominated by whitefish dwarfs. Conversely, in lakes without pike and monomorphic whitefish, the diets of these birds primarily include other species like perch, minnows, and roach (**III-IV**).

Methodological Comparisons in eDNA Diet Analysis

Metabarcoding, a common method in environmental DNA (eDNA) research, utilizes universal primers to target conserved regions across species, enabling the parallel processing of numerous samples (Zinger et al., 2019; Taberlet et al., 2018). However, this technique can introduce biases that affect quantitative abundance estimates, primarily due to the stochastic nature of PCR (Lahr and Katz, 2009). The intentional nucleotide ambiguity in metabarcoding primers may lead to uneven amplification, and inhibitors from environmental samples can result in false negatives (Eriksson et al., 2017; Monchamp et al., 2023). Comparing results against known DNA quantities can help elucidate these potential issues. In this thesis, I utilized both digital droplet PCR (ddPCR) and metabarcoding approaches to determine the diets of various bird species in boreal ecosystems. The DNA of a total of 11 fish genera was detected in fecal samples, including *Coregonus*, *Lota*, *Perca*, *Phoxinus*, *Rutilus*, *Salmo*, *Salvelinus*, *Cottus*, *Esox*, *Pungitius*, and *Thymallus* (**IV**). The comparison between ddPCR and metabarcoding assays revealed that metabarcoding outperformed the ddPCR method in determining the richness of fish communities, as ddPCR assays are limited by the number of primers designed for the study. In terms of the composition of the bird diets, both methods provided overall similar results, though there were discrepancies for certain individuals.

Conclusions and Perspectives

This thesis demonstrates the potential of environmental DNA (eDNA) analysis in reconstructing historical aquatic biodiversity and ecosystem dynamics. The application of molecular tools for species identification provided insight into the colonization patterns of fish in Scandinavian lakes and enhanced our understanding of the intricate connections within food webs and underscore the impact of key fish species on broader ecosystem dynamics.

Utilization of eDNA and the relevant molecular tools will have a significant impact on the description, modeling, and prediction of fish biodiversity in a myriad of environments. Non-PCR based molecular diagnostic tools such as CRISPR or DNA capture through non-PCR based shotgun sequencing can be seen as the next logical step in eDNA studies to distance the results from some of the inherent biases introduced by PCR. It offers a more precise and impartial approach to species identification over conventional aquatic monitoring procedures. It can be considered key to further develop and standardize procedures for sampling, laboratory work and bioinformatics. It will also be important to increase the knowledge of how environmental conditions influence DNA in different ways, so that tailored solutions for specific studies can be developed. As eDNA methods continue to evolve, they promise to become even more refined and accessible, opening up new possibilities for non-invasive, comprehensive, and efficient biodiversity assessments. Such insights are invaluable in predicting the outcomes of current and future impacts on biodiversity driven by climate change, habitat loss, and other anthropogenic factors.

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Reflections and Acknowledgements

On a brisk and misty autumn morning, many years ago, I arrived at my office to discover a small, white cardboard box perched on my desk. The box, slightly dented from hours of laborious field work, bore faint writing in blue marker: 'Bird-poo for Fredrik,' accompanied by an obnoxious smiley face. Reflecting on a recent discussion—one I had forgotten—I had failed to anticipate the level of enthusiasm regarding this particular subject matter from the other members of the group. Bemused, I recalled some cliché career advice: 'Stay open to unexpected opportunities and embrace the unknown.' Well, if life gives you lemons, make lemonade. Unfortunately, this particular 'lemon' smelled awful, and the 'lemonade' was even worse. Knowing nothing about the intricate and magical world of avian toilet habits, and being merely a humble sediment wrangler, I discovered that, from an analytical perspective, sediments and bird poo are surprisingly similar. It seems that fate, indeed, is not without a sense of irony....

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