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Cover: Northern pike releasing DNA (Image by Erik Karlsson using DALL-E 3)

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Abstract

Environmental DNA (eDNA) has emerged as a useful tool for species detection and assessment of biodiversity and hold promise for quantitative estimates. This development is of particular interest for species such as northern pike (*Esox lucius*), an ecologically and socio-economically important apex predator in freshwater and coastal ecosystems, for which suitable monitoring techniques is lacking due to low catchability with traditional monitoring methods.

In this thesis I explore the potential of eDNA to enhance the understanding of pike ecology and provide reliable data on abundance needed for informed management decisions. Relationships between eDNA and biomass of pike were first established under controlled conditions, revealing strong positive linear relationships between eDNA and biomass of juvenile and adult pike. Subsequent field experiments investigating eDNA relationship with pike further found that abundance of pike together with temperature and size of sampled area could explain considerable variation in eDNA concentrations. The temporal eDNA dynamics of spawning run was investigated in an additional field study, demonstrating strong positive linear relationship between eDNA levels and amount and biomass of migrating pike and eDNA levels. Furthermore, I evaluated eDNA methodologies and developed a genetic marker targeting highly repetitive nuclear DNA, providing a stronger eDNA signal compared to mitochondrial assay.

The results from this thesis provide important knowledge and advances towards achieving quantitative estimates of fish populations through eDNA-based monitoring. In addition, the method developments achieved within the thesis through improved sensitivity of eDNA assays enhances detection accuracy of aquatic species. Together, the findings of this thesis will have significant implications for conservation and management of aquatic ecosystems.

Keywords: real-time PCR, chelex, Baltic Sea, abundance, biomass, spawning, temperature, COI, 5S rDNA

Utvärdering av potentialen hos miljö-DNA för kvantitativ övervakning av populationer av gädda (*Esox lucius*)

Sammanfattning

Miljö-DNA (eDNA) har visat sig vara ett användbart verktyg för detektion av arter och bedömning av biologisk mångfald och har påvisat potential för kvantitativa uppskattningar. Denna utveckling är av särskilt intresse för arter som gädda (*Esox lucius*), en ekologiskt och socio-ekonomiskt viktig toppredator i sötvattens- och kustekosystem, för vilken lämpliga övervakningstekniker saknas till följd av låg fångstbarhet i traditionella redskap inom övervakning.

I denna avhandling undersökte jag potentialen hos eDNA för att förbättra förståelsen av gäddans ekologi och tillhandahålla tillförlitlig data som behövs för välgrundade förvaltningsbeslut. Samband mellan eDNA och gäddbiomassa etablerades först under kontrollerade förhållanden, vilket visade starka positiva linjära samband mellan eDNA och biomassa hos både juvenil och vuxen gädda. Fältförsök som undersökte sambanden mellan eDNA och mängd gädda visade vidare att mängd gädda tillsammans med temperatur och storleken på det undersökta området kunde förklara variationen i eDNA-koncentrationer. Den temporala eDNA dynamiken undersöktes i ytterligare en fältstudie, vilken visade starka positiva linjära samband mellan mängden och biomassan av vandrande gäddor och eDNA-nivåer. Vidare utvärderade jag eDNA-metoder och utvecklade en genetisk markör som riktar in sig på mycket repetitivt nukleärt DNA, som visade sig ha potential för känsligare eDNA-signaler.

Resultaten från denna avhandling ger viktig kunskap och framsteg mot möjligheten att uppnå kvantitativa uppskattningar av fiskpopulationer genom eDNA-baserad övervakning. Dessutom innebär metodutvecklingen med förbättrad känslighet hos eDNA-analyser en ökad detektionsnoggrannhet för akvatiska organismer. Tillsammans kommer resultaten i denna avhandling att få betydande konsekvenser för bevarande och förvaltning av akvatiska ekosystem.

Keywords: real-time PCR, chelex, Östersjön, abundans, biomassa, lek, temperatur, COI, 5S rDNA

Dedication

For Edda and Arna

So, I started to walk into the water. I won't lie to you boys. I was terrified, but I pressed on. And as I made my way past the breakers, a strange calm came over me. I don't know if it was divine intervention or the kinship of all living things, but I tell you Jerry, at that moment, I was a marine biologist.

- George Costanza

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Karlsson E., Ogonowski M., Sundblad G., Sundin J., Svensson O., Nousiainen I., Vasemägi A. (2022). Strong positive relationships between eDNA concentrations and biomass in juvenile and adult pike (*Esox lucius*) under controlled conditions: Implications for monitoring. Environmental DNA, 4(4), pp. 881–893.

II. Ogonowski M., Karlsson E., Vasemägi A., Sundin J., Bohman P., Sundblad G. (2023). Temperature moderates eDNA–biomass relationships in northern pike. Environmental DNA, 5(4), pp. 750–765.

III. Karlsson E., Pukk L., Ogonowski M., Sundblad G., Sundin J., Vasemägi A. Increased power of repetitive nuclear DNA for species detection and eDNA-biomass estimation – performance of an ultra-high copy marker of northern pike (submitted manuscript).

IV. Karlsson E., Ogonowski M., Sundblad G., Sundin J., Bohman P., Vasemägi A. Environmental DNA reflects spawning migration dynamics of northern pike (manuscript).

1. Introduction

It is a well-established fact that sustainable management and conservation of fish populations rely on robust and reliable assessments on the health of populations. Ideally, assessments are based on reliable fisheries-independent data attained through monitoring programmes. For aquatic monitoring of coastal and freshwater species, this is conventionally achieved through passive monitoring methods such as multi-mesh gillnets. These have produced long, valuable and comparable time series for most freshwater species of economic and ecological interest whilst being comparatively easy to use and applicable to a range of species and sizes (Appelberg et al. 1995). Yet, whilst providing valuable and reliable data these methods are invasive and result in substantial fish mortality for targeted species and by-catch alike, of special concern for monitoring of rare or endangered species (Uhlmann & Broadhurst 2015) and can have harmful impact on certain environments (Shester & Micheli 2011). In addition, the passive nature of gillnets has the effect that not all species and sizes are necessarily caught in a representative manner, as the gillnets rely on fish being active swimmers (Hubert et al. 2012) and are dependent on behaviours and morphology of species (Reis & Pawson 1999; Villegas-Ríos et al. 2014). The result is a negative bias of this monitoring method towards many sedentary species (Olsson 2019). Furthermore, repeated exposure to methods such as gillnets can induce shifts in behaviour that can result in avoidance, causing under-estimation of catches (Arlinghaus et al. 2017).

1.1 Northern pike

The northern pike (*Esox lucius*, Linnaeus 1758), hereafter pike, is an apex predator native to fresh- and brackish water across the northern hemisphere

and fills an important ecological and regulatory role in the ecosystems it inhabits (Craig 1996; Donadi et al. 2017). Large predatory fish in general play fundamental controlling and shaping roles in aquatic ecosystems (Pauly et al. 1998) and healthy populations of predatory fish can help thwart trophic cascades and eutrophication symptoms (Eriksson et al. 2011; Östman et al. 2016).

Commercial fisheries targeting pike is relatively low in Sweden with catches and effort in decline, partly explained by a decrease in demand and price. The bulk of catches instead originate from recreational rod-and-reel fishing (Skov & Nilsson 2018; Bergström et al. 2022). Among recreational fishers, pike is a popular game fish and a substantial effort is directed towards catching, especially large, individuals (Paukert et al. 2001; Arlinghaus et al. 2018). Therefore, pike is vulnerable to overexploitation (Arlinghaus et al. 2010). The overexploitation is to a certain degree counteracted by the practice of catch-and-release, which has proven to be effective for pike (Arlinghaus et al. 2008) and generally employed by pike anglers (Bergström et al. 2022).

Interest in recreational fishing is high in Sweden, compared to other European countries (Arlinghaus et al. 2015), with an estimated 1.2 million anglers generating an annual turnover of close to 6 billion SEK in 2022 with pike being one of the most popular species (Havs- och Vattenmyndigheten 2024).

Pike in the Baltic Sea exhibit two different reproductive strategies by either spawning in the brackish water of shallow coastal bays or by migrating into adjacent freshwater habitats, such as wetlands or shallow lakes. A strong natal homing behaviour (Engstedt et al. 2014; Larsson et al. 2015) has resulted in genetically isolated populations of Baltic Sea pike (Laikre et al. 2005; Wennerström et al. 2017) exhibiting stable genetic differentiation on a fine spatial scale (Nordahl et al. 2019; Diaz-Suarez et al. 2022) and putative local adaptations in relation to salinity tolerance (Sunde et al. 2018), temperature (Sunde et al. 2019) and growth (Tibblin et al. 2015).

The current status and health of Baltic Sea pike populations remain largely unknown, as no adequate monitoring methodologies currently exist. The data available does however indicate that populations have been declining since the mid 1990's, in particular regarding large individuals (Bergström et al. 2022; Olsson et al. 2023). A range of possible causes have been presented, including recreational fisheries, loss of spawning habitat,

eutrophication and increasing predation pressure (Sundblad & Bergström 2014; Hansson et al. 2018; Donadi et al. 2020; Bergström et al. 2022). Recent changes in the Baltic Sea has resulted in pike being subject to increased predation pressure from both sides of the food web. With steadily increasing populations of grey seals (*Halichoerus grypus*, O. Fabricius, 1791) and great cormorants (*Phalacrocorax carbo sinensis*, Linnaeus, 1758) there has been an increasing predation pressure on pike from the top of the food web (Hansson et al. 2018). On the other end of the food web, pike are subject to an increasing abundance of three-spined stickleback (*Gasterosteus aculeatus*, Linnaeus 1758) which readily forage on pike egg and larvae, with a resulting regime shift in dominance from large predatory fish to smaller prey fish (Nilsson et al. 2019; Donadi et al. 2020; Eklöf et al. 2020). Furthermore, coastal development has caused a considerable loss of suitable spawning habitats, such as wetlands. The loss of coastal spawning grounds has been identified as a potential contributing factor to the declining pike populations (Sundblad & Bergström 2014). To counteract this loss, considerable efforts has recently been directed towards the restoration of wetlands to support and stimulate Swedish pike populations (Tibblin et al. 2023).

Despite harbouring both great ecological value by occupying an essential regulatory role in the food web as top predator, and socio-economic value by being the focus of many recreational fishers, pike has historically been largely overlooked in Swedish monitoring. Swedish monitoring of fish populations is primarily performed through gill-nets and other passive gears, that have garnered long and valuable time series for most species of economic interest (Appelberg et al. 1995). These were established in an era when pike was seen as nuisance that would not warrant any form of monitoring in the foreseeable future (Svärdson & Molin 1968). This together with the species sedentary behaviour, making passive monitoring methods unsuitable, can at least in part explain the lack of sufficient data for reliable assessment of health of Swedish pike stocks. Whilst native to the Nordic countries, pike is predicted to expand its distribution and colonize new lakes and river systems (Spens et al. 2007), facilitated by increasing temperatures from climate change with potential adverse effects on these ecosystems (Hein et al. 2012), thus adding another important aspect as to why an effective monitoring of the distribution of pike is needed.

The lack of availability of data impedes our understanding of coastal and littoral food web dynamics, essential to ecosystem-based management. To enable governmental bodies to make informed decisions regarding population management and conservation, it is imperative to develop novel reliable monitoring methods of pike populations.

1.2 Environmental DNA

Environmental DNA, eDNA, is commonly defined as the mixture of all genomic DNA left in an environment by the organisms that inhabit it. The environment can be water, soil, or sediment and the DNA can have any type of origin, be it from mucus, faeces, epithelial cells, hair or scales; the defining part of eDNA, that distinguishes it from other types of DNA, is that it is a mixture from various species and that it is sourced from an environment, and not an organism.

First termed in 1987 by Ogram et al. (1987), the potential of eDNA for macro-organism monitoring purposes was demonstrated by Ficetola et al. (2008), where the method was applied for detection of the invasive American bullfrog (*Lithobates catesbeianus,* Shaw 1802) in Europe. After which the method received increasing attention with initial studies establishing eDNA as a suitable tool for detection of metazoans in aquatic environments, particularly detection of invasive species in small waterbodies (Goldberg et al. 2011; Jerde et al. 2011; Minamoto et al. 2012; Thomsen et al. 2012a; Pilliod et al. 2013; Takahara et al. 2013). Further advances revealed the quantitative potential of eDNA and how it could be adopted towards monitoring of population sizes (Elbrecht & Leese 2015; Lacoursière-Roussel et al. 2016; Doi et al. 2017; Itakura et al. 2019; Rourke et al. 2021; Spear et al. 2021).

eDNA is shed to the environment through a range of processes such as egestion, secretion, exfoliation and reproduction (Barnes & Turner 2016). These processes vary between species and over time (Sassoubre et al. 2016) and are affected by abiotic factors such as temperature and pH (Lacoursière-Roussel et al. 2016; Jo et al. 2022) and are subject to inter-species variation from body size, life stage and behaviour (Pilliod et al. 2013; Klymus et al. 2015; Thalinger et al. 2021). Establishing mechanisms and dynamics of eDNA therefore becomes necessary on a species specific level.

Persistence of eDNA in natural environments is central to its utility as a monitoring tool, as it determines the longevity of measured eDNA signals. Degradation of eDNA, through microbial consumption has been shown to initially occur rapidly (Maruyama et al. 2014; Nevers et al. 2018) yet eDNA can remain detectable for as long as 60 days after fish has been removed (Thomsen et al. 2012b). Persistence is additionally influenced by length of target DNA, with longer fragments degrading at a faster rate (Jo et al. 2017). It is also affected by temperature and pH, with DNA persisting longer in colder, more alkaline environments (Strickler et al. 2015).

Further consideration needs to be taken regarding spatial dispersal of eDNA across aquatic environments (Taberlet et al. 2018), as eDNA can become undetectable mere meters from its source in lentic waters (Brys et al. 2021) and remain detectable over 100 km from its origin in lotic waters (Pont et al. 2018). Understanding the dispersal and spatial variability of eDNA across environments can be vital when designing monitoring programs and experiments.

There is a growing body of proof for positive relationships between fish abundance, biomass and eDNA concentrations (Rourke et al. 2021), shown in both controlled (Lacoursière-Roussel et al. 2016; Benoit et al. 2023) and natural environments (Salter et al. 2019; Spear et al. 2021; Rourke et al. 2024). However, these relationships vary greatly between species (Rourke et al. 2021) and are affected by environmental factors (Jo 2023), with greater variance introduced in natural environments (Yates et al. 2019).

Given the growing interest for eDNA, a great diversity of methodologies have been applied to the various steps of data collection, including capture, isolation and quantification of eDNA (Majaneva et al. 2018; Loeza-Quintana et al. 2020; Wang et al. 2021). With each method entailing its own inherent benefits and disadvantages, careful evaluation and consideration is necessary to assure that robust and reliable data is generated for the purpose of long-term monitoring.

2. Aim of this thesis

The overall aim of this thesis was to assess the quantitative aspects of eDNA through empirical experiments and evaluate the prospect of eDNA to provide quantitative estimates of fish abundance. By doing so, this thesis would contribute to better, non-lethal and more cost-effective monitoring of pike populations as well as for other species. To achieve this I investigated the following questions:

Does measured pike eDNA concentrations reflect abundance and biomass of pike under controlled conditions? (**Paper I**)

What eDNA methods are most suited for the aim of quantitative monitoring of pike? (**Papers I** and **III**)

How does eDNA concentrations relate to observed abundance and biomass of pike in natural environments? (**Papers II** and **IV**)

Can eDNA be used to infer relative abundance of populations for monitoring purposes? (**Papers I, II** and **IV**)

3. Material and methods

To assess the quantitative aspect of eDNA and evaluate the potential of eDNA to infer relative abundance estimates of pike populations, an empirical approach was taken. This was achieved through a series of experiments starting from controlled conditions continuing into natural environments with increasing complexity. Method development followed hand-in-hand with the experiments.

3.1 eDNA-abundance/biomass relationships under controlled conditions

As an initial first step of the thesis the relationship between eDNA concentration and abundance/biomass of pike needed to be established to determine if the method can achieve relative abundance estimates under conditions with minimal complexity and interfering factors. To empirically establish these relationships, two experiments under controlled conditions were performed (**Paper I**).

To start, juvenile pike (young-of-the-year) were collected from a wetland in the vicinity of Oxelösund and transported to the Institute of Freshwater Research, Drottningholm. At the laboratory, pike of similar sizes were selected and kept in four different densities, replicated three times, in aquariums with constant temperature and a 12 hour light:dark cycle. Water samples for eDNA quantification were collected at three times after pike had been introduced to the aquariums. Pike where then removed, euthanized and weighed and an additional four water samples were taken to investigate eDNA degradation rates. Water samples were filtered onto cellulose nitrate filters with eDNA extracted using DNEasy PowerWater kit (Qiagen, Hilden, Germany). Real-time PCR (qPCR) was used to estimate eDNA

concentrations using a mitochondrial eDNA marker targeting the cytochrome oxidase I gene (COI) (Olsen et al. 2015, 2016), proven efficient for detection of pike (Dunker et al. 2016). Estimated eDNA concentration was compared to abundance and biomass of pike through linear regression.

To further establish how individual biomass of pike related to eDNA, an experiment on adult pike was conducted at the Institute of Freshwater Research, Drottningholm. Adult pike of varying size was collected by a commercial fisher in Lake Mälaren and transported to the laboratory where they were kept individually under semi-natural conditions in outdoor mesocosms filled with water from the adjacent Lake Mälaren. This allowed for replication of natural conditions in terms of temperature and sunlight, whilst other parameters could be kept constant and controlled. Pike were kept in mesocosms for a week with eDNA samples taken at three occasions after which pike were removed, euthanized and weighed. Water samples of 1L were taken and filtered onto a combination of cellulose nitrate and glass microfiber filters, which allowed for a larger volume of water to be processed as the larger pore-sized glass microfiber filter acted as a pre-filter. DNA was extracted using a Chelex 100 (BioRad, Hercules, USA) protocol and was quantified through qPCR. Measured eDNA levels were then compared to individual biomass of pike through linear regression

3.2 Optimization of eDNA sampling techniques

In order to identify the most suitable methods and further develop eDNA towards monitoring, different approaches for extracting (**Paper I**) and quantifying eDNA (**Paper III**) was empirically compared.

To assess the effect on eDNA yield from choice of extraction method, a small scale experiment was conducted at the Institute of Freshwater Research, Drottningholm where pike in two different densities (low and high) were kept in outdoor mesocosms under flow-through water from the adjacent Lake Mälaren. DNA from samples taken from these mesocosms was extracted using three different methods; DNEasy PowerWater (Qiagen), DNeasy Blood & Tissue (Qiagen) and Chelex 100 (BioRad) and quantified using qPCR and an assay targeting the mitochondrial COI gene. Estimated eDNA concentration was compared between type of extraction method and pike density.

In the initial experiments, a previously published assay targeting the mitochondrial gene Cytochrome Oxidase I (Olsen et al. 2015, 2016), was used to quantify pike eDNA, with good results. To investigate whether other markers could improve detection and quantification capabilities, a new marker targeting highly repetitive nuclear DNA was developed and tested against the mitochondrial marker. The 5S rDNA region of the pike genome was identified as being repeated in extreme numbers (>20 000 copies per genome, Symonová et al. 2017) and therefore of interest and suitable for development. Five initial markers were designed using Primer3 (Untergasser et al. 2012). As the region occur in tandem repeats within the genome the primers were initially tested on pike tissue using PCR with varying temperatures and annealing, and elongation times to identify a protocol that would provide amplification products of singular length. Species specificity of primers was verified through PCR on tissue samples of a range of species. Based on results from initial tests, one primer was selected as suitable for development of a TaqMan assay. The species specificity of the assay was further tested through qPCR on tissue from 36 co-occurring species as well as limits of detection (LOD) and quantification (LOQ) being determined according to Klymus et al., (2020). The assays performance was compared to that of the COI assay on different pike tissues as well as eDNA samples from previous experiments (aquarium and mesocosm).

3.3 eDNA relationships in natural environments

As strong positive correlations between eDNA and pike biomass had been demonstrated under controlled conditions, the logical next step became to investigate how these relationships transferred to natural environments. This was empirically explored through two field studies. Furthermore, the effect of spatial and temporal variation in the eDNA signal as well as effect from abiotic variables such as water temperature and size of sampling area was investigated (**Papers II** and **IV**).

To evaluate the ability to infer abundance of pike through eDNA samples taken in natural environments, a field survey was conducted in 22 shallow bays along the Swedish east coast in 2020. Each of these bays were first sampled for eDNA, with eight samples taken from each bay. Samples were taken by dividing each bay into four transects: three shallow along the shoreline and one deeper in the middle of the bay. Subsamples were taken

every 50 m along the transect, based on reported eDNA detection range from caged pike (Dunker et al. 2016), and pooled. From the pooled water two replicate samples were taken. Water was filtered onto two membrane filters (CN and GMF) loaded into a Swinnex filter holder using a plastic syringe (Figure 1).

Figure 1. Equipment used for eDNA sampling consisting of plastic syringe, Swinnex filter holder, membrane filters (CN and GMF), zip-lock bags and forceps. Photo: Göran Sundblad/Erik Karlsson (**Paper II**).

Filters were transported to the Institute of Freshwater Research, Drottningholm, where DNA was extracted by Chelex and quantified by qPCR targeting COI. Inhibition of qPCR was evaluated by addition of an internal positive control (IPC), a synthetic strand of DNA added to each sample and quantified by running duplex qPCR reactions. Samples where IPC amplified later than expected were considered inhibited and excluded from analysis.

After eDNA sampling, each bay was sampled using rod-and-reel fishing, enabling for calculation of catch per unit effort (CPUE), an estimation of population size, and comparison between the estimates gained through

eDNA and rod-and-reel sampling. Each bay was fished by two fishers for four hours two days in a row, except in a few cases were weather or distance between bays instead led to fishing a full day in each bay. Each caught pike was weighed and measured for length and maturity and spawning stage was visually observed and noted, before releasing the pike. To account for varying effort across bays and influence of outside angler presence on CPUE, CPUE was standardized. This allowed a fairer comparison of standardized CPUE and eDNA concentrations.

The relationship between eDNA concentration and standardized CPUE of pike was estimated by modelling eDNA concentration as a function of variables known to influence DNA levels in water, such as water temperature and bay size.

The previous parts of the thesis had demonstrated that eDNA can be used to describe spatial differences in relative population sizes. To further understand how eDNA relate to known absolute abundance and biomass and the temporal variation around the relationship between eDNA and abundance and biomass in natural environments an empirical field study was conducted.

The study was carried out in Hemmesta wetland, to which pike annually migrate from the Baltic Sea to spawn. To test the hypothesis that eDNA can be used to monitor the spawning migration, a visual fish counter (VAKI Aquaculture Systems Ltd, Iceland) was installed at the entrance of the wetland. The fish counter was composed of a photo tunnel housing technology that allowed for detailed registration of everything that passes through it and with AI, and human quality control, accurately identifies species and measure size of individual fish passing through, with high accuracy (±2 cm). By placing the fish counter with guiding arms at the entrance to the wetland, blocking alternative paths, migration of pike could be closely monitored and thereby provided known absolute numbers of pike within the wetland at any given point in time (Figure 2).

Figure 2. Fish counter prior to installation (top left), pike migrating into the wetland (top right) fish counter being installed at the mouth of the wetland seen beyond (bottom). Photo: Göran Sundblad (**Paper IV**).

Sampling of eDNA was performed using the same methodologies as in the previous study with samples taken in the immediate vicinity of the fish counter on 12 different occasions during the spawning migration. On each occasion six biological replicates were taken. Filtration and extraction of DNA followed the practices of **Paper II**. Quantification of eDNA was performed via qPCR using the two TaqMan assays; the mitochondrial assay used previously as well as the newly developed nuclear assay described in **Paper III**.

4. Results and Discussion

4.1 eDNA-abundance/biomass relationships under controlled conditions

Through experiments conducted in aquaria and mesocosms, I show strong linear relationships between eDNA concentrations and both juvenile and adult pike abundance and biomass (range R^2: 0.82 – 0.91, **Paper I**). Furthermore I demonstrate that the eDNA signal decreases rapidly after fish have been removed, with eDNA levels dropping significantly within 24 hours, yet remaining detectable for several days (**Paper I**).

This provides information on eDNA–biomass relationships for both adult and juvenile pike, in line with findings from similar studies on other species (Rourke et al. 2021) and constituted an important first step towards eDNA based quantitative monitoring.

4.2 Optimization of eDNA sampling techniques

This thesis shows that choice of methodology can greatly influence outcomes of eDNA studies. I show that Chelex 100 achieved equal and even outperformed two of the most widely utilized DNA extraction approaches within the field (**Paper I**). Of the extraction methods tested in this thesis, Chelex yielded the highest eDNA concentrations in samples with high density of pike. In samples with low density of pike, Chelex yielded equal eDNA concentrations as the other methods. In addition, the Chelex protocol used here produced considerably larger sample volumes and required fewer steps of sample manipulation. Thereby reducing risks of handling errors or contamination during DNA extraction.

Prior to my test of the Chelex protocol, most published eDNA surveys have utilized commercial column-based extraction kits (Rourke et al. 2021). Such commercial kits offer relatively quick, easy, and high quality extraction of DNA, but they also require many stages of manipulation of samples and they are comparatively costly. I show that Chelex offer a robust alternative for isolation of eDNA, enabling greater numbers of samples to be processed by being less demanding in terms of labour and cost, which is important for implementation of eDNA in aquatic monitoring.

This thesis also show that highly repetitive nuclear DNA markers can provide significantly earlier amplification and detection of pike DNA compared to more widely used mitochondrial markers (**Papers III** and **IV**). The 5S rDNA loci tested here provided higher numbers of detectable eDNA copies compared to COI in environmental samples, which implies increased detection probabilities for pike. The repetitive nuclear DNA assay developed in this thesis showed substantially earlier amplification in pike tissue (~6.0 Cq, **Paper III**), in samples from semi-natural conditions (~3.9 Cq, **Paper III**) and in natural environments (~4.8 Cq, **Paper IV**), compared to mitochondrial DNA. Relationships between repetitive nuclear eDNA and abundance and biomass was also in general slightly weaker than with mitochondrial DNA (**Papers III** and **IV**). This indicates that highly repetitive nuclear markers can considerably increase eDNA signal strength and detection possibilities compared to mitochondrial markers. However, analysis of eDNA biomass relationships showed higher variability using the nuclear marker compared to the mitochondrial marker. Since the targeted region, 5S rDNA, is repetitive across fish species it is likely the region will be suitable for marker development of other species. Such development would be of particular interest when targeting rare or invasive species, where reducing false negatives is vital. I further demonstrate the importance of in vitro validation of marker specificity, especially for regions where reference material is lacking (**Paper III**).

Improved sensitivity of eDNA analysis will have significant implications for detection of rare or endangered species, invasive species management, conservation, and management of aquatic ecosystems.

4.3 eDNA relationships in natural environments

Through surveys in natural environments this thesis show positive linear relationships between eDNA concentration and standardized CPUE of pike (CPUE derived from angling). By including temperature and size of bay as explanatory variables, the log-linear relationship with eDNA concentration had a relatively high amount of variation explained (R^2 = 0.48, **Paper II**), comparable to similar studies (Yates et al. 2019). By ranking each coastal bay based on CPUE and eDNA concentrations respectively, a positive correlation was observed (Figure 3). This indicates that both methods, eDNA and angling, could separate the bays with the highest and lowest number of pike.

Although a positive relationship between eDNA concentration and CPUE was observed, it was relatively weak and a stronger effect from temperature was observed (**Paper II**). This is likely a result from temperature increasing fish activity and triggering spawning, thus increasing eDNA concentrations (Bylemans et al. 2017). The somewhat weak relationship between eDNA and CPUE could partly be due to both methods holding their own inherent biases. As angling (used to calculate CPUE) is size-selective and omits smaller sized individuals, the survey methods are perhaps not expected to correlate fully.

To improve the design of future eDNA-based monitoring programmes, variation among samples at various spatial scales was explored (**Paper II**). Small-scale spatial differences were lesser compared with more large-scale and temporal processes during spring when pike spawn. Yet, variation between technical replicates was higher than anticipated, potentially explained by the low levels of eDNA copies. This indicates that more replicates are necessary in cases where observed eDNA levels are low (**Paper IV**).

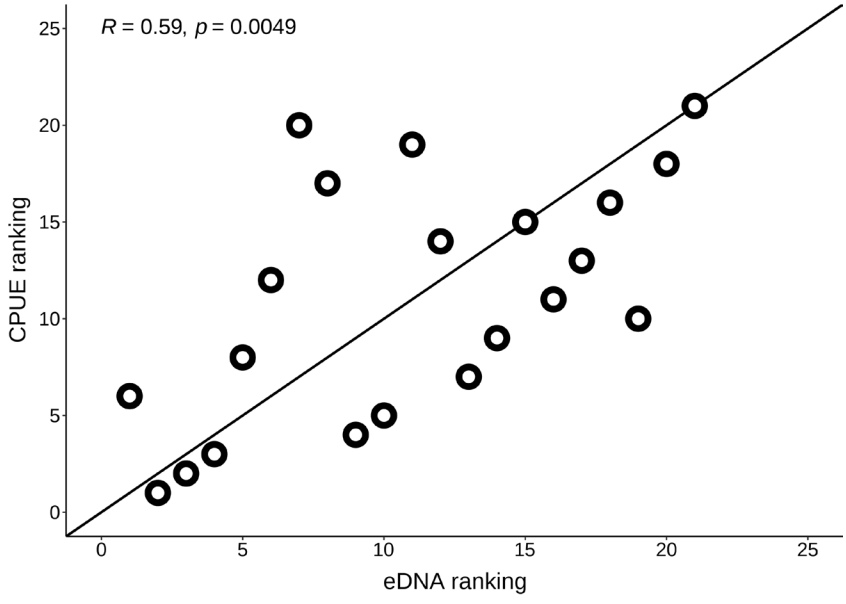

Figure 3. Relationship between standardized CPUE (angling) and eDNA based on ranking of population densities of 21 coastal bays (each bay indicated by points). CPUE is ranked by the highest value of two visits and eDNA is ranked by the lowest mean concentration of the two visits. Line depicts 1:1 relationship (**Paper II**).

In the study where the spring spawning migration of pike was monitored, I show that eDNA in samples taken at the entrance of the spawning ground (the wetland) had a strong positive relationship with the number of pike migrating in and out of the wetland (**Paper IV**). The best fitting models were between eDNA concentration and number and biomass of pike passing past the entrance of the wetland (passing a fish counter) within 60 and 72 hour prior to sampling. No relationship could however be observed between cumulative number of pike within the wetland and eDNA levels in the water emerging from the wetland.

Whilst the best fitting models included number and biomass of pike within 60 and 72 hours, number and biomass of pike within all time-points exceeding 1 hour, i.e., at 6, 12, 24, 36 and 48 hours all provided strong positive relationships as explanatory variable for observed eDNA levels (Figure 4).

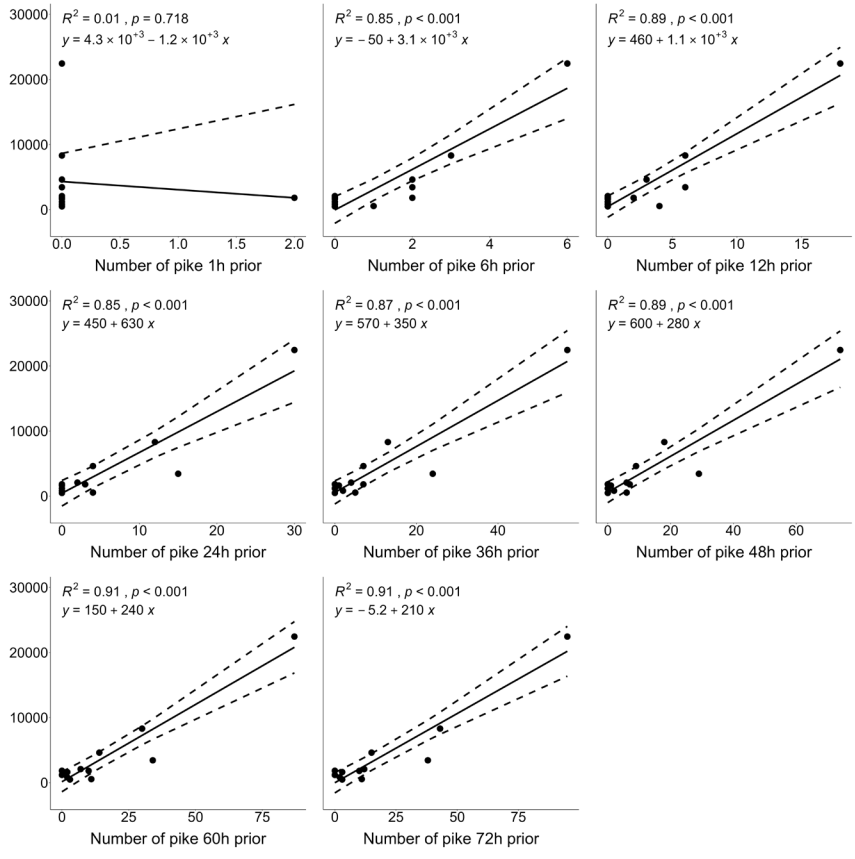

Figure 4. Linear regressions of eDNA concentration (mtDNA, COI) as a function of number of pike (*Esox lucius*) migrating into the spawning ground (the wetland) prior to eDNA sampling, for each time frame respectively (from top left panel to bottom right panel: 1, 6, 12, 24, 36, 46, 60 and 72 hours). Individual points denote mean eDNA for each of the 12 sampling occasions. Dashed lines indicate the 95% confidence interval (**Paper IV**).

Based on the results shown in **Paper I**, which demonstrated rapid degradation of eDNA, with eDNA levels drastically reduced within 24 hours, it is uncertain whether eDNA released 72 hours prior to sampling still meaningfully contribute to the eDNA signal. Yet, it is important to recognise that whilst the eDNA signal was best explained by number and biomass of pike passing the fish counter (entering the spawning ground in the wetland) within 60 and 72 hours, this signal is not necessarily directly due to eDNA being deposited during the passage of pike through the fish counter. The explanation could be that, after completing their spawning migration and

reaching the wetland, pike initially stay in proximity of the entrance, where the sampling site and fish counter was located. Additionally, this could be explained by pike engaging in spawning activity shortly after reaching the wetland, thereby elevating eDNA levels through release of milt and eggs (**Paper IV**).

In contrast to the findings from the coastal bays studied previously (**Paper II**), only a minor effect of temperature could be observed in the study of spawning migration into the wetland (**Paper IV**). This could be explained by the temperature ranges differing considerably between studies, with the wetland study having higher temperatures in general. Higher temperatures will induce higher activity and subsequent release of eDNA (Lacoursière-Roussel et al. 2016). Additionally, the temperatures in the wetland were consistently above the lower threshold for pike spawning (Frost & Kipling 1967), allowing for potential spawning throughout the wetland study.

Altogether, the wetland study of the spawning migration demonstrated strong positive linear relationship between eDNA levels and number and biomass of migrating pike, supporting the notion that eDNA constitutes a viable method for monitoring of annual spawning migration.

5. Conclusions and future outlooks

In this thesis I show that eDNA based methods can be used to infer relative abundance of pike both under controlled and natural environments. Additionally, I have developed and tested new methods within the field that can reduce cost and labour (**Paper I**), and methods that can increase detection levels and quantitative abilities (**Paper III**). This thesis further reveals the effect of temperature on eDNA dynamics, as well as highlighting the effect of number of biological replicates on precision and in extension reliability, of eDNA inferred quantitative estimates (**Paper IV**).

Through experiments under controlled conditions I show that eDNA levels in water show a strong positive relationship with both juvenile and adult pike (**Paper I**). This is a crucial first step in the process towards developing eDNA as method for quantitative monitoring of pike populations.

Additionally, I have developed and tested new methodologies that holds the potential to make eDNA analysis more efficient (**Paper I**) and sensitive (**Paper III**). I show that a comparatively simple Chelex based protocol can be an effective method for extracting eDNA from filters (**Paper I**). In addition to entailing fewer steps compared to more broadly used column based extractions kits, which will lower the risk of cross-contamination, it will greatly reduce processing time and costs and allow for a greater number of samples being processed. As revealed in **Papers II** and **IV**, number of biological replicates will affect the precision of eDNA derived abundance estimates, and increasing the possible number of biological replicates may be key for species and ecosystems where eDNA levels are low. Enabling sufficient replication is thus vital towards the future implementation of eDNA based aquatic monitoring.

Furthermore this thesis show that genetic markers targeting highly repetitive nuclear DNA can greatly enhance eDNA signal strength compared

to the currently more widely used markers targeting mitochondrial DNA (**Paper III**). Increasing the detectable and quantifiable amounts of eDNA allows for more accurate detection and precise quantification of aquatic species, reducing the risks of attaining false negative results, which is of particular importance when targeting rare or invasive species. As demonstrated in **Paper II**, eDNA levels encountered in natural environments can often be under the quantifiable limit, and increasing the quantity of targeted DNA will greatly increase the quantitative prospects of eDNA based monitoring. The development of the nuclear marker also revealed the necessity of *in vitro* testing of markers where genetic reference material is limited, as the sole use of *in silico* testing otherwise can inflate specificity of markers.

Use of eDNA in natural environments revealed positive relationships between both abundance and biomass of pike and eDNA concentrations over both spatial and temporal scales (**Paper II** and **IV**). Biomass together with abiotic data was able to explain nearly 50% of the observed variance in eDNA levels across a range of coastal bays (**Paper II**), in line with observations from similar studies (Yates et al. 2019). The observed influence of temperature, suggested to induce increased activity and spawning, means that thorough identification of ideal sampling periods are necessary for future studies. This will be vital to enable long-term monitoring of pike, and is proposed to occur at temperatures where the majority of the reproducing population has spawned, yet still stay aggregated.

By temporally observing eDNA levels over the spring spawning migration of Baltic Sea pike, eDNA showed a very strong and positive relationship with number and biomass of pike migrating into the freshwater wetland (**Paper IV**). Analysis of inter-replicate variance also show that to achieve acceptable precision in eDNA derived estimates, an excess of six biological replicates may be necessary in instances where eDNA levels are low. This will be crucial for future research and monitoring purposes, as it will have an effect on scale and cost of surveys.

Together the results of this thesis support the idea that eDNA can constitute an effective and non-invasive method to infer relative quantitative abundance estimates of fish populations, comparative to current monitoring methods. Whether the method can take monitoring beyond that, in providing estimates of absolute abundance and biomass remain more uncertain and poses a greater challenge. While this thesis has recorded strong linear

relationships between eDNA and abundance and biomass, the slope and intercept of these relationships has varied substantially and raises uncertainty regarding the generality of these relationships and potential for application to additional environments and situations (Figure 5). However, for species such as pike, where conventional monitoring methods are unsuitable, being able to provide relative abundance estimates will be of great value. Towards the aim of future implementation of eDNA based tools in monitoring in aquatic ecosystems, future research should now focus on the long-term eDNA dynamics over spatial and temporal scales to assess the method for inferring trends in fish populations.

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Popular science summary

Management of aquatic resources depends on reliable information about the status and development of fish stocks. Traditional monitoring methods, such as standardized test fishing using gill-nets, have generated valuable time series for most species of socio-economic and ecological value. Species with low catchability using these methods are often overlooked, leading to a lack of reliable data, which is necessary for management authorities to make well-informed decisions.

Pike (*Esox lucius*) is an important species in freshwater and coastal ecosystems and has become a popular species in recreational fishing in recent decades. Pike is one such species where traditional, passive (and lethal) monitoring methods have proven ineffective due to the pike's stationary behaviour. The limited information available on Swedish pike stocks indicates a drastic decline along Sweden's east coast, particularly for larger individuals. Several reasons have been proposed to explain this decline, such as reduced recruitment opportunities due to the loss of spawning habitats, increased predation from grey seals and cormorants, increased predation on juvenile pike by sticklebacks, and high fishing pressure from recreational fisheries during the 1990s.

Since pike form genetically stable and distinct populations over relatively small geographical areas, there is a need for monitoring methods that can provide information at the local level. Pike congregate for spawning in shallow, vegetation-covered areas in early spring and are strongly attached to their birthplace, meaning that monitoring requires good geographical coverage.

Environmental DNA (eDNA) has emerged as a method for monitoring biodiversity and has shown potential for estimating the quantity (number and biomass) of aquatic species. Several studies have shown that eDNA can

provide relative estimates of fish abundance, but with varying results depending on species and system. Therefore, it is necessary to evaluate the method at the species and system levels. The variability in previous studies can be explained by differences in methodology, hydrological conditions, the presence of substances that inhibit polymerase chain reaction (PCR), the size and age structure of the fish population, and water temperature.

To evaluate how the quantity of pike relates to the concentration of eDNA, a series of experiments were conducted in this thesis to establish the relationship between eDNA and pike abundance, first under controlled environments and then under natural conditions. The results of this thesis show a strong positive linear relationship between eDNA concentration and pike biomass under controlled conditions for both adult and juvenile pike. Field studies in coastal bays showed that the variation in measured eDNA concentrations could be explained by just under 50% of standardized rod fishing catches together with water temperature and the size of the surveyed bay. Water temperature was found to be crucial for a positive relationship between eDNA and catches, and temperature therefore needs to be considered when choosing eDNA sampling to enable long-term studies and monitoring.

Experiments to evaluate the possibility of tracking spawning migration over time were conducted by comparing measured eDNA concentrations with number of pike that migrated into a spawning ground (a wetland), measured with a camera fish counter. eDNA concentrations, through both nuclear (nuDNA) and mitochondrial (mtDNA) analyses, showed consistent patterns with measurements on number of pike from the fish counter. However, eDNA concentrations in the water flowing out of the wetland did not show any correlation with the cumulative number of pike that had gathered in the wetland to spawn. Instead, eDNA concentrations reflected the intensity of the migration of pike that swam upstream to spawn.

The results of the thesis also compare different DNA extraction methods, and show that on methods, called Chelex 100, can be a fast and cost-effective alternative for DNA extraction from samples, compared to other currently widely used methods. In addition, a genetic marker based on extremely repetitive nuclear DNA was developed for eDNA analyses of pike. Analysis of eDNA samples from experiments in controlled and natural environments showed that the newly developed marker could result in samples amplifying

earlier than with a previously established mitochondrial marker, which would imply higher sensitivity of the newly developed marker.

Overall, this thesis supports the growing body of research demonstrating positive correlations between fish abundance (number and biomass) and eDNA concentrations, and that eDNA can offer an efficient and non-invasive method for relative measures of fish abundance. For species like pike, where traditional monitoring methods are unsuitable, the ability to generate estimates of relative occurrence can be of great value. To enable future eDNA-based fish monitoring, future studies should focus on the long-term dynamics of eDNA on spatial and temporal scales to determine whether the method can detect trends in fish populations.

Populärvetenskaplig sammanfattning

Förvaltning av akvatiska resurser, så som fisk, är beroende av tillförlitlig information om beståndens status och utveckling. Traditionella övervakningsmetoder, såsom standardiserade provfiskenät, har gett värdefulla tidserier för de flesta arter av socio-ekonomiskt och ekologiskt värde. Arter som är svåra att fånga med dessa metoder förbises dock ofta, vilket leder till brist på tillförlitlig data som krävs för att förvaltande myndigheter ska kunna fatta välgrundade förvaltningsbeslut.

Gädda (*Esox lucius*) utgör en viktigt art i sötvattens- och kustnära ekosystem och har på senare decennier blivit en populär art inom fritidsfisket. Gädda är en sådan art där traditionella, passiva (och dödliga) övervakningsmetoder har visat sig vara ineffektiva på grund av gäddans stillastående beteende. Den begränsade information som finns om svenska gäddbestånd indikerar en drastisk nedgång längst Sveriges ostkust, framförallt för större individer. Flera orsaker har presenterats för att förklara nedgången, såsom försämrade möjligheter till reproduktion till följd av förlust av lekhabitat, ökad predation från gråsäl och skarv, ökad predation på yngel-stadier av gädda från storspigg, samt ett högt fisketryck från fritidsfisket under 1990-talet.

Eftersom gädda bildar genetiskt stabila och skilda populationer över relativt små geografiska områden finns det behov av övervakningsmetoder som kan ge information på lokal nivå. Gäddan samlas för lek i grunda vegetationsbeklädda områden under tidig vår och är starkt bundna till sin födelseort vilket innebär att övervakning behöver ha en god geografisk täckning.

Miljö-DNA (eDNA) har framträtt som en metod för övervakning av biologisk mångfald, och har visat potential för uppskattning av mängd (antal och biomassa) av akvatiska arter. Flera studier har visat att eDNA kan ge

relativa uppskattningar av mängd fisk, dock med varierande resultat beroende på art och typ av ekosystem. Det är därför nödvändigt att utvärdera metoden på art- och ekosystemnivå. Skillnaderna mellan dessa studier kan förklaras med skillnader i val av metodik, hydrologiska förhållanden, förekomst av ämnen som hämmar polymeraskedjereaktion (PCR), storlek- och åldersstruktur av fiskbeståndet och vattentemperatur.

För att utvärdera hur mängden gädda relaterar till koncentration av eDNA utfördes i denna avhandling en serie experiment för att etablera förhållanden mellan eDNA och mängd gädda, först under kontrollerade miljöer och sedan under naturliga förhållanden. Resultaten visar ett starkt positivt linjärt samband mellan eDNA-koncentration och gäddbiomassa under kontrollerade former för både vuxna och unga gäddor. Försök i kustvikar i Östersjön visade att variationen i uppmätta eDNA koncentrationer kunde förklaras till strax under 50 % av standardiserade spöfiskefångster tillsammans med vattentemperatur och storlek av undersökt vik. Vattentemperatur visade sig vara avgörande för ett positivt förhållande mellan eDNA och fångster, och kommer därför behöva tas i beaktning vid val av provtagning med eDNA för att möjliggöra långsiktiga studier och övervakning.

Försök för att utvärdera möjligheter att följa lekvandringen över tid utfördes genom att jämföra uppmätta eDNA-koncentrationer med mängden gädda som vandrat upp i våtmarken, mätt med en kamera-fiskräknare. Koncentration av eDNA, genom både nukleära (nuDNA) och mitokondriella (mtDNA) analyser, visade konsekventa mönster med kvantitativa mått från fiskräknaren. Dock uppvisade eDNA-koncentrationer i vattnet som strömmade ut från våtmarken inte något samband med den kumulativa mängden gädda som samlats i våtmarken för lek (reproduktion). Istället återspeglade eDNA-koncentrationerna intensitet i migrationen av gädda som vandrade upp för att leka.

Avhandlingen jämförde också olika metoder för att utvinna DNA, och resultaten visar att en metod, kallad Chelex 100, kan utgöra ett snabbt och kostnadseffektivt alternativ jämfört med befintliga, vanligen använda, metoder. I avhandlingen utvecklades också en genetisk markör baserad på extremt repeterat nukleärt DNA för eDNA-analyser av gädda. Analys av eDNA-prover från försök i kontrollerade och naturliga miljöer visade att den nyutvecklade markören kunde resultera i att prover amplifierade tidigare än vid analys med en etablerad mitokondriell markör. Detta innebär en högre

känslighet hos den nyutvecklade markören, och öppnar upp för användningsområden vid övervakning av arter/förhållanden där eDNA nivåerna är låga.

Sammantaget stödjer den här avhandlingen den växande mängden forskning som påvisar positiva samband mellan mängd fisk (antal och biomassa) och eDNA-koncentrationer, och att eDNA kan erbjuda en effektiv och skonsam metod för relativa mått på mängd av fisk.

För arter som gädda, där traditionella övervakningsmetoder är olämpliga, kan möjligheten att generera uppskattningar av relativ förekomst vara av stort värde. För att möjliggöra framtida eDNA-baserad övervakning av fisk bör framtida studier fokusera på den långsiktiga dynamiken av eDNA på rumslig och tidsmässig skala för att undersöka om metoden kan upptäcka trender i fiskpopulationer.

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ORIGINAL ARTICLE

Environmental DNA
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Strong positive relationships between eDNA concentrations and biomass in juvenile and adult pike (*Esox lucius*) under controlled conditions: Implications for monitoring

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Abstract

Reliable abundance information is the foundation for managing aquatic resources. Species with low catchability are, however, often overlooked in monitoring programmes. Thus, governing bodies lack the data necessary to make well-informed management decisions. Environmental DNA (eDNA) can produce quantitative estimates of fish abundances, but the precision varies greatly depending on the species and system. It is, therefore, necessary to evaluate its performance and investigate how fish biomass and density affects eDNA dynamics on a case-by-case basis before eDNA-based monitoring can be a viable option. Here, we evaluate how biomass and density of an ecologically and socioeconomically important top predator, the Northern pike (*Esox lucius*), relate to eDNA concentrations in controlled aquarium and mesocosm experiments. We carried out experiments using both juvenile and adult individuals and evaluated eDNA, biomass and density relationships at three different time points using a previously developed TaqMan assay, targeting the cytochrome oxidase I gene. We also evaluated the performance of multiple extraction methods (DNeasy Blood & Tissue kit, DNeasy PowerWater kit, and Chelex 100), and filtering systems (single- vs. double-membrane filters). The results from both pike experiments showed a strong positive linear relationship between eDNA concentration and pike biomass ($R^2 = 0.74 - 0.87$). Levels of eDNA dropped drastically within the initial 24 h of juvenile pike being removed from the aquaria, and low levels were detectable for up to 308 h. Of the extraction methods, Chelex 100 yielded the highest DNA concentration, offering a quick and cost-effective alternative compared with existing widely used extraction methods. Using double membrane filters of different material showed no increase in DNA yield regardless of the extraction method but it allowed more water to be processed. Although several challenges remain, our results show that eDNA holds promise to become a useful tool for monitoring fish biomass in natural environments.

KEYWORDS
chelex, eDNA extraction, fish, membrane filter, mesocosm, qPCR

1 | INTRODUCTION

Governing bodies rely on having robust and reliable data attained through monitoring programmes to make informed decisions on conservation efforts and regulations (Magnusson & Hilborn, 2007; McAllister & Kirkwood, 1998). Monitoring methods for freshwater fish are traditionally passive, such as gillnets or fyke nets, and rely on fish being active swimmers (Stoner, 2004). As a consequence, sedentary species may not be caught in a representative manner (Ruetz et al., 2007). The low catchability of such species implies that they may be over-looked in monitoring programmes (Bagenal, 1972; Olsson, 2019; Pierce, 1997). More targeted efforts using active gear, such as a rod-and-reel, can result in catches large enough to gain quantitative estimates of abundance, size, and occurrence (Karlsson & Kari, 2020; Kuparinen et al., 2010). Still, there are issues with standardization as catchability is influenced by the size and type of bait, as well as the fishing effort (Arlinghaus et al., 2008, 2017; Kuparinen et al., 2010).

The northern pike (*Esox lucius* L.) is one of many sedentary species where conventional, passive monitoring methods are not adequate. Pike are a keystone top predator, native to brackish and freshwater systems throughout the northern hemisphere (Craig, 2008). Pike are expressly cannibalistic with intraspecific predation often being an important regulatory factor for local population abundance (Craig, 2008). Furthermore, recreational fishing is a popular and economically important activity, with pike being a prized target among many anglers (Paukert et al., 2001). As a result, pike are vulnerable to overexploitation (Arlinghaus et al., 2010; Pierce & Tomcko, 1995).

Within the Nordic countries, monitoring programmes for coastal and freshwater species are harmonized and standardized to enable international comparisons (Appelberg et al., 1995; Thoresson, 1993). Monitoring with gillnets and other passive gear has resulted in a long and valuable time series for most species of economic and/or socio-economic interest, but abundance and occurrence data on sedentary species, including pike, is missing (Olsson, 2019). Currently, there is no monitoring programme that can provide sufficient data to reliably determine the status of Swedish pike stocks (Sandström et al., 2019).

The emergence of environmental DNA (eDNA) has rapidly proven to be a cost-effective tool for biodiversity monitoring relying on presence/absence data (Dejean et al., 2011; Dunker et al., 2016; Evans et al., 2017; Takahara et al., 2013; Thomsen et al., 2012). Being both cost-effective and non-lethal, eDNA has gained particular interest for monitoring rare and/or endangered species (Boothroyd et al., 2016; Nevers et al., 2018). Furthermore, eDNA analysis is particularly suitable in areas where conventional techniques are prohibited or restricted, and for species with low catchability using conventional methods (Hinlo et al., 2018; Jerde et al., 2011; Thomsen et al., 2012). More recently, eDNA has also been shown to be useful in making abundance estimates of aquatic species (Doi et al., 2015; Itakura et al., 2019; Lacoursière-Roussel et al., 2016; Salter et al., 2019; Spear et al., 2021; Tillotson et al., 2018). Whilst several studies have established a positive relationship between fish abundance and eDNA concentrations, both in controlled (Eichmiller et al., 2016; Klymus et al., 2015) and natural conditions (Itakura et al., 2019; Salter et al., 2019; Spear et al., 2021), the strength and shape of these correlations and how they are affected by environmental factors vary between species and by habitat (Coulter et al., 2019; Rourke et al., 2021). Thus, it is important that eDNA–biomass relationships are established and validated at the species level. Additionally, eDNA–biomass relationships for large, sedentary species are very scarce, and only a few studies to date have evaluated whether eDNA–biomass relationships differ between juvenile and adult fish (Maruyama et al., 2014). Fish metabolism typically scales with body mass allometrically (Jobling, 1994), and it has recently been demonstrated that eDNA shedding rates scale with mass in brook trout, *Salvelinus fontinalis* (Yates et al., 2021a). Populations of the same species in the same habitat type can however have different biomass–eDNA relationships if size structures of those populations are substantially different, meaning that the potential universality of this relationship across species is yet to be established (Yates et al., 2021). Therefore, it is important to experimentally establish eDNA-biomass relationships for different life stages and sizes before applying the developed methodology to natural conditions and for monitoring purposes (Rourke et al., 2021).

In this study we investigated how eDNA estimates, based on real-time quantitative polymerase reaction assay (qPCR), correlate with juvenile and adult pike biomass. More specifically, we evaluated (i) how juvenile pike density and biomass correlate with eDNA concentrations in controlled aquarium settings and (ii) how eDNA concentrations correlate with the individual biomass of adult pike in large outdoor mesocosms. In addition, (iii) we tested the performance of three DNA extraction methods and two filter combinations to identify the most sensitive and cost-effective approach for future eDNA monitoring.

2 | MATERIALS AND METHODS

2.1 | Aquarium experiment using juvenile pike

2.1.1 | Fish collection and holding

To determine if eDNA concentrations correlate with fish biomass and abundance, we first performed a controlled laboratory experiment on juvenile pike. Young-of-the year (YOY) pike (1.2 – 6.9 g wet weight) were collected using electrofishing on 26 June 2019 in the Långsjön wetland (58°38'8" N, 16°58'40" E), Sweden. The fish were transported to the laboratory at the Institute of Freshwater Research, Drottningholm, where they were kept in flowing water in holding tanks (200 × 82 × 29 cm) with natural, sand-filtered water from Lake Mälaren (59°20'02" N, 17°52'32" E), Sweden. The water temperature followed local conditions, and the light:dark cycle was set to 17 h:7 h (mimicking natural conditions). The pike (total $n = 125$) were kept in groups of no more than 18 individuals and sorted by size to prevent cannibalism, which was achieved by dividing the tanks into sections. To further standardize the conditions, the pike were

acclimatized for six days without being fed to reduce any potential effect caused by their capture, transportation and altered environment (Lacoursière-Roussel et al., 2016). In addition, the starvation period allowed the fish to clear their guts, thereby reducing the risk of fish excrement influencing the eDNA-signal and potentially distorting the eDNA–biomass relationship (Klymus et al., 2015).

2.1.2 | Experimental design

After the acclimatization period, the juvenile pike were introduced into aerated aquaria (40 × 20 × 25 cm) filled with 14 L of sand-filtered water from Lake Mälaren. To investigate the DNA–biomass relationship, we tested four pike density treatments by placing 0, 1, 3 or 9 pike in each aquarium (Figure 1a). Each treatment was run in triplicate resulting in 39 YOY pike divided over 12 aquaria (Figure 1a). The pike were assigned to the aquaria at random. Within each aquarium the pike were individually housed in plastic cages (Withlock-Vibert boxes, 14 × 9 × 6 cm) to prevent predation from their peers. The aquaria were kept in a temperature controlled room at ~19°C with an L12 h:D12 h light/dark cycle. Individual aquaria were placed on three stacked benches at different elevations, with one replicate per treatment on each level to control for potential bench effects (Figure 1a). The sides of the aquaria were covered with opaque sheets to prevent visual cues and potential stress from adjacent aquaria. We collected water samples (500 ml) for DNA quantification at 22, 48 and 70 h after the pike were introduced to ascertain that the eDNA levels had reached steady state (Figure 1b; Nevers et al., 2018). Three days after introduction, the Withlock-Vibert boxes containing pike were removed by hand, euthanized using an overdose of benzocaine, and weighed. To investigate the rate of decline in the eDNA concentration without the pike and the eDNA retention time in the aquaria, additional samples were taken 27, 74, 121, and 238 h after the pike were removed (Figure 1b).

2.1.3 | DNA extraction

The water samples were vacuum filtered immediately upon collection onto a 47 mm diameter cellulose nitrate membrane filter (MFS, Membrane Filtration Systems, Dublin, California) with a pore size of 1.2 μm and stored at −20°C (1 filter per aquarium and occasion).

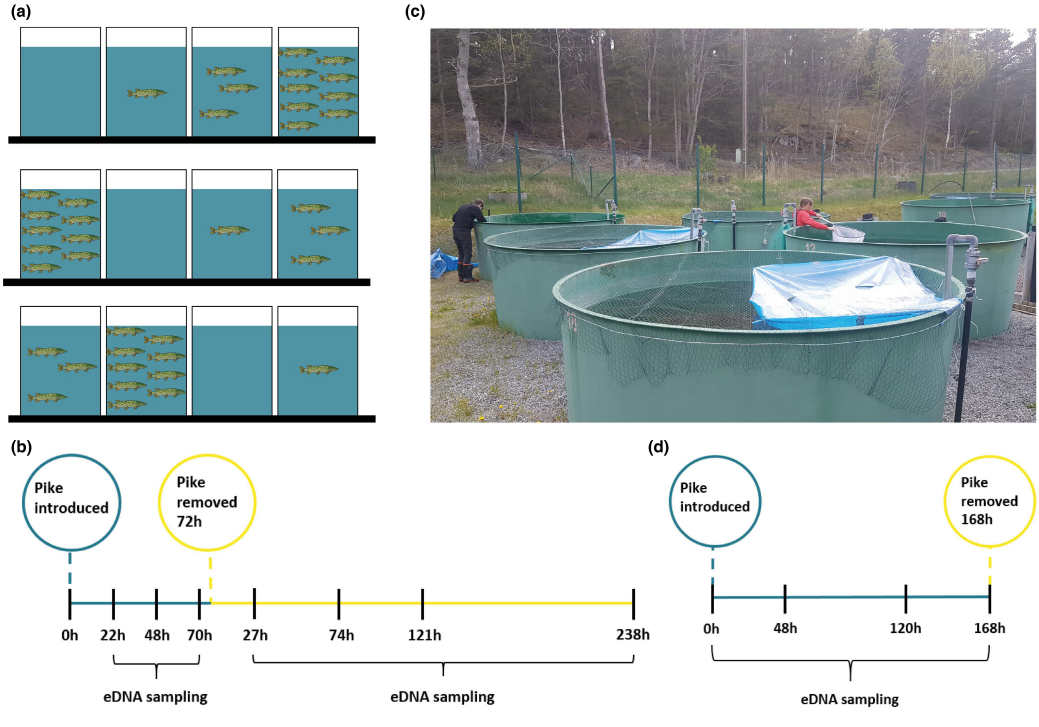

FIGURE 1 (a) Schematic illustration of the experimental setup of juvenile pike in aquaria with 0, 1, 3 and 9 individuals in 12 aquaria (the pike were held individually isolated within aquaria in Whitlock-Vibert boxes). (b) Sampling timeline for the juvenile pike experiment. (c) Experimental mesocosms used in the method evaluation and in the adult pike experiments. (d) Sampling timeline for the adult pike experiment

The equipment was cleaned and sterilized by soaking it in 50% commercial grade bleach for 5 min and then rinsing it thoroughly with tap water in between filtrations. DNA extraction was performed at the Institute of Technology, University of Tartu (Estonia) using the DNeasy PowerWater kit (Qiagen), with minor alterations to the standard protocol (the vortex time of the bead tubes was increased to 10 min and the final elution volume was reduced to 70 μl). In total, we extracted DNA from 86 filters with an additional five negative controls to test for contamination during extraction (the negative controls were kept at −20°C until DNA quantification). Negative controls were subjugated to the same manipulation and pipetting steps as the regular samples without a filter containing DNA being added at the start.

2.1.4 | DNA quantification using qPCR

To quantify the DNA released by the juvenile pike in the experimental aquaria we used a real-time quantitative polymerase reaction assay (qPCR). The primer and probe combination (F-primer: 5′-CCTTCCCCCGCATAAATAATATAA-3′, R-primer: 5′-GTACCAGCACCAGCTTCAACAC-3′ and probe: 5′-FAM-CTTCTG ACTTCTCCCC-BHQ-1-3′ (Microsynth AG)) was originally developed by Olsen et al. (2015, 2016) and has later been successfully used for pike detection in water samples (Dunker et al., 2016). The assay targets a 94-base-pair-long fragment of the Cytochrome oxidase I gene (COI). qPCR was performed on an Applied Biosystems 7500 Real-time PCR system with 20 μl reactions volumes. Reaction concentrations of the forward primer, reverse primer and probe were 200 nM each with 1× HOT FIREPol Probe Universal qPCR Mix (Solis Biodyne) in each well loaded with 4 μl of the sample template. The following qPCR program was used for all the reactions: 2 min at 60°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C.

eDNA quantification was achieved using a standard curve consisting of an 8-step, 10-fold dilution series of pike DNA (0.01 – 100 000 pg μl^{-1}). Pike DNA was extracted from pike liver tissue using a DNeasy Blood & Tissue kit (Qiagen). Samples and standard curves were run in quadruplicates with four no template control (NTC) reactions on each plate. Plate efficiency varied between 95.8% and 101.3%, with R^2 values between 0.995 and 0.999.

2.2 | DNA extraction methods and filter evaluation

2.2.1 | Experimental setup

To maximize eDNA yields, we conducted a separate experiment to evaluate three different extraction methods and two filter combinations. We placed adult pike at two different densities in two mesocosms (Figure 1c), with a single pike in one mesocosm (weight = 1.3 kg) and eight in the other (mean weight = 1.2 ± 0.27 kg) with flow-through water from Lake Mälaren (see Section 2.3.1 for additional details on adult pike collection and holding). To prevent escapement, the mesocosms were covered with PVC-coated chicken net. Approximately one third of the net's surface was covered with a blue plastic sheet to provide shade and cover (Figure 1c). Artificial plants made from 1 m long strips of green polyethylene tarp tied to a brick were submerged in each tank for shelter and enrichment. The pike were left for 6 days to acclimatize in the mesocosms prior to sampling for eDNA.

2.2.2 | eDNA sampling and extraction

Sampling was performed by first taking a large water sample from each mesocosm (~30 L) and then filtering 1 L through either a single cellulose nitrate filter (pore size of 0.8 μm) or a combination of a cellulose nitrate filter (pore size of 0.8 μm) with a glass microfiber filter (GFFA, pore size of approximately 1.6 μm). There were four replicates for each filter combination, extraction method and mesocosm (SI Section 2.1). A simple filtration technique was used where water was pushed through a Swinnex filter holder loaded with one or two filters using a plastic syringe (Supplementary Information (SI) Section 2.1). The filters were immediately frozen at −20°C using a portable freezer and then stored at −80°C until extraction was performed.

eDNA was extracted using three different methods: (1) DNeasy Blood & Tissue kit (Qiagen), (2) DNeasy PowerWater kit (Qiagen) and (3) Chelex 100 resin (Bio-Rad Laboratories). Each method was used to extract a total of 16 samples (four replicates per each density and filter combination). Extraction using DNeasy Blood & Tissue followed the manufacturer's protocol with minor modifications; for the initial lysis stage a 5-ml Eppendorf tube was used instead of the standard 1.5 ml size, and the volumes of the ATL buffer and Proteinase K were increased to 370 and 30 μl, respectively. These modifications were made to facilitate complete filter submersion during lysis. eDNA extraction using DNeasy PowerWater followed the same protocol as described for the juvenile pike aquarium experiment. eDNA extraction using Chelex 100 was conducted based on a modified Chelex 100 protocol (Walsh et al., 1991; SI Section 2.2). All extractions were performed at the Institute of Freshwater Research, Drottningholm. Samples that were extracted with DNeasy Blood & Tissue and Chelex were both diluted in a 1:8 ratio prior to qPCR to reduce variation between technical replicates likely originating from inhibition (McKee et al., 2015). Levels of potential inhibition of the qPCR reactions were not explicitly tested in this experiment.

DNA was quantified using qPCR on a CFX384 real-time PCR system (Bio-Rad Laboratories) based on the same primers and probe as used in the juvenile pike aquarium experiment. However, in contrast to the aquarium experiment, the concentration of both the primers and the probe were increased to 900 nM, and we used 1× of TaqMan Environmental Master Mix 2.0 to counteract potential inhibitory substances in the samples. The total reaction volume was 15 μl with 4 μl of DNA template. The standard protocol for the master mix was used for all the reactions: 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C.

Measurements of DNA concentrations were obtained from a standard curve consisting of a 6-step, 10-fold dilution series of pike DNA (0.01 – 1000 ng μl^{-1}). Reference DNA was extracted from pike muscle tissue using DNeasy Blood & Tissue kit (Qiagen). Four NTC negative controls were run on each plate. The estimated plate efficiency was 109.6%, with an R^2 value of 0.990.

2.3 | Mesocosm experiment using adult pike

2.3.1 | Experimental setup

To assess the fish eDNA–biomass relationship in a semi-natural environment, we performed a large mesocosm experiment using adult pike. Adult pike (n = 48, 758 – 8150 g) were collected by a local commercial fisherman using fyke nets, from Lake Mälaren in May 2020 and transported in a large fish-transporter tank to the Institute of Freshwater Research, Drottningholm. The pike were kept for 21 days in outdoor cylindrical mesocosms (the mesocosms are described in Section 2.2.1) containing 7000 L of unfiltered water from Lake Mälaren to acclimatize, standardize stress levels among individuals, and ensure that their digestive systems were empty (Seaburg & Moyle, 1964). After the acclimatization period, the pike were placed individually in new cylindrical mesocosms of the same type (n = 13, with an additional negative control mesocosm without fish) containing ~7000 L of unfiltered lake water (Figure 1c). The temperature was monitored continuously in individual tanks (SI Section 3.1) using a HOBO TidbiT v2 temperature logger (Adelaide, Australia). On the final sampling date, oxygen levels were measured using a Rinko ASTD-102 profiler (JFE Advantech Co., Ltd.). These measurements showed that the dissolved oxygen levels varied between 11.8 and 12.8 mg L^{-1}, which is well above critical levels for northern pike (Inskip, 1982).

The pike were not fed during the experiment. After 7 days of incubation, the pike were removed by means of a landing net, euthanized by a blow to the head and destruction of the brain. The fish were kept in a cooler until the following day when length (to the nearest mm) and weight (g) were measured.

2.3.2 | eDNA sampling and extraction

Based on the experience gained from the aquarium experiment using juvenile pike we adjusted and improved our sampling and analysis methodology. Water samples (1 L) were taken at four intervals during the experiment. Water was first collected prior to the introduction of the pike and then at 48, 120, and 168 h after the introduction, to assure that a steady state had been reached and to investigate how the eDNA–pike abundance relationship developed over time (Figure 1d). The water samples were collected from just below the water's surface, without any prior stirring to simulate a sampling event under natural conditions. We filtered the water samples immediately upon collection. We used a plastic syringe to push water through a Swinnex filter holder loaded with a cellulose nitrate filter (0.8 µm) and a glass microfiber filter (GFFA, approximately 1.6 µm, SI Section 2.3). The glass microfiber filter functioned as a pre-filter that allowed a larger volume of water to pass through (Capo et al., 2020). The filters were frozen immediately at −20°C after filtration using a portable freezer and then stored at −80°C until extraction. The equipment was sterilized by soaking them in 10%–20% commercial grade bleach for a minimum of 10 min and then they were rinsed thoroughly with tap water between sampling occasions.

Based on the results from the DNA extraction and filter evaluation, DNA from both filters was extracted using a modified Chelex 100 protocol (SI Section 3.2) at the Institute of Freshwater Research, Drottningholm. Pike DNA was quantified by qPCR on a CFX384 real-time PCR system (Bio-Rad Laboratories) using the same primers, probe and protocol used in the DNA extraction and filter evaluation (see Section 2.2.2).

Measurements of pike DNA concentrations were obtained from a standard curve consisting of a six-step, 10-fold dilution series of pike DNA (0.0028 – 280 pg μl^{-1}). Pike DNA was extracted from pike muscle tissue using DNeasy Blood & Tissue kit (Qiagen). Four NTC negative controls were run on each plate.

Limit of detection (LOD) and limit of quantification (LOQ) were determined by running a 10-fold dilution series used for the standard curve with DNA levels ranging 0.00275 – 275 pg μl^{-1}each in 16 technical replicates. LOD is defined as the lowest concentration of DNA where 95% of the technical replicates amplify and LOQ is defined as the lowest concentration of DNA with a coefficient of variation (CV) below 35% (Klymus et al., 2020). Effective LOD is defined as the lowest concentration with a detection probability of 95% given n replicates. The estimated qPCR efficiency varied between 98.5 and 100.8% with R^2 values between 0.998 and 0.996. LOD and LOQ were both determined to be 0.275 pg μl^{-1}. Analysis in quadruplicates gave an effective LOD of 0.00325 pg μl^{-1}. All samples from the mesocosms containing the pike were above this limit.

2.4 | Statistical analysis

2.4.1 | Experiment with juvenile pike in aquaria

Multiple linear regression was used to analyse the relationship between eDNA concentrations and juvenile pike biomass using data from samples taken at three occasions before the pike were removed from the aquaria. The model included pike *biomass* (g, continuous predictor), *sampling occasion* (categorical predictor), and their interaction, as explanatory variables.

2.4.2 | DNA extraction and filter evaluation

Factorial analysis of variance (ANOVA) was used to analyse how the extraction method and filter combinations affected eDNA

concentrations in the samples collected from the mesocosms containing two different densities of pike (1 vs. 8 individuals per tank). Due to an unfortunate handling error, half of the samples that were extracted using DNeasy PowerWater could not be included in the analysis, leaving only the samples extracted using DNeasy Blood & Tissue and Chelex 100 amenable for statistical analysis. Results from the remaining samples extracted using DNeasy PowerWater are presented visually (Figure 4).

2.4.3 | Mesocosm experiment using adult pike

Analysis of the relationship between individual pike biomass (g) and eDNA concentrations was performed using multiple linear regression on the data from eDNA samples taken at three occasions before pike removal. In the initial model we used *DNA concentration* as the dependent variable and *biomass* (continuous predictor), *sampling occasion* (categorical predictor), their interaction, and *temperature* (daily median °C) as explanatory variables. However, *temperature* was excluded based on the Akaike information criterion; the most parsimonious model included *biomass*, *sampling occasion*, and their interaction as explanatory variables. Two mesocosms were excluded before analyses; one due to the fish dying before the end of the experiment and another due to not having an empty stomach at the end of the experiment. For all analyses, mean values of the technical qPCR replicates were used to estimate eDNA concentrations. All analyses were performed using R version 4.1.1 (R Core Team, 2017).

2.5 | Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The fish sampled and handled in this study complied with the standards and procedures stipulated by the Swedish Ministry of Agriculture and the ethical permit was approved by the Stockholm ethical committee (DNR 99-19).

3 | RESULTS

3.1 | Aquarium experiment using juvenile pike

Following the introduction of the pike, eDNA levels were relatively stable over 72 h, except for the highest density treatment which showed a strong increase in pike eDNA (Figure 2). After the pike were removed, eDNA levels dropped drastically within the next 27 h, with average eDNA levels decreasing from 441.1 pg $\mu l^{-1}$to 35.8 pg μl^{-1}. At 96 h, there was still a weak but detectable positive relationship between eDNA concentration and pike density. 236 h after the pike were removed, a very weak eDNA signal was still detectable in 8 out of the 12 aquaria (average Cq = 37.4, equating to 0.024 pg μl^{-1}), Figure 2).

Juvenile pike biomass showed a strong, positive correlation with eDNA concentrations (R^2 = 0.87) (Figure 3, Table 1a). The interaction between biomass and sampling occasion was statistically significant (Table 1a). Visual inspection of the regression slopes for each of the three sampling occasions suggested that the first sampling occasion (22 h) was different from the other two (46 and 70 h). The first sampling occasion was, therefore, excluded in a consecutive model which showed no significant difference between the slopes of the last two sampling dates ($F_{1,20}$ = 2.45, p = 0.13, SI Section 1.1). Compared with biomass, juvenile pike density (continuous predictor) showed a similar relationship with eDNA concentrations but had slightly higher explanatory power (R^2 = 0.87 – 0.97, SI Section 1.1). Samples taken from aquaria without pike displayed a weak amplification signal (Figure 2). However, compared with tanks containing the experimental fish, the amplification signal occurred more than ten cycles later suggesting that this weak signal likely originated

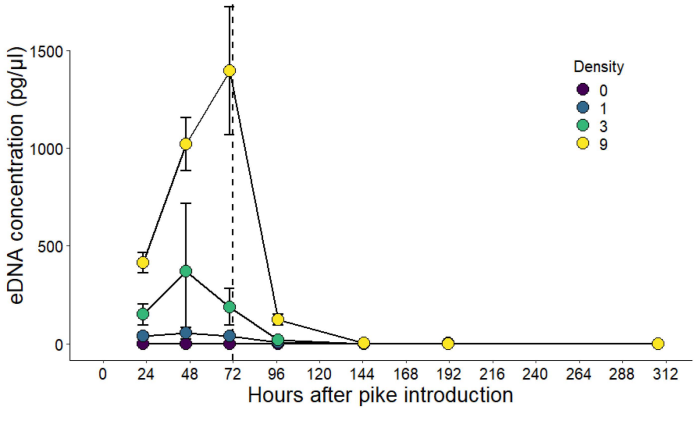

FIGURE 2 Temporal dynamics of eDNA concentration (pg µl-1) at different juvenile pike density levels. The data points denote the mean eDNA concentration of three replicates (the mean value of four technical replicates) and the error bars ±1 SD for each pike density (dark purple: 0 pike (control), blue: one pike, green: three pike, yellow: nine pike). The dotted vertical line represents the time-point when the pike were removed from the aquaria (at 72 h).

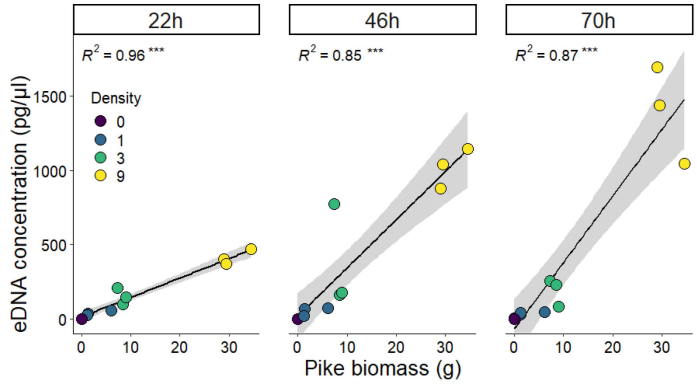

FIGURE 3 eDNA concentrations (pg µl-1) as a function of juvenile pike biomass (g) from aquaria with different densities (dark purple: 0 pike (control), blue: one pike, green: three pike, yellow: nine pike) at three sampling occasions (22, 46 and 70 h after the start of the experiment). Each point denotes the mean value of four technical replicates taken from a sample. The grey shaded areas around the regression lines shows the 95% confidence interval. R2 values were calculated for each time-point separately (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The results of the integrated analysis comparing the regression slopes between the consecutive sampling occasions are presented in Table 1a

TABLE 1 An ANOVA table (type III errors) for linear models with eDNA concentrations (pg ul^{-1}) as a function of pike biomass (g) and sampling occasion, after the pike were introduced in (a) the juvenile pike aquarium experiment and (b) the adult pike mesocosm experiment

	Model parameters	Sum Sq	F (df)	*p*
(a) Aquarium experiment	Biomass (g)	70247501	11.56 (1, 30)	**0.002**
	Sampling occasion	6318454	0.52 (2, 30)	0.600
	Biomass (g): Sampling occasion	173059537	14.24 (2, 30)	**<0.001**
(b) Mesocosm experiment	Biomass (g)	125151	7.80 (1, 30)	**0.009**
	Sampling occasion	35073	1.09 (2, 30)	0.348
	Biomass (g): Sampling occasion	256886	8.01 (2, 30)	**0.002**

Note: Significant *p*-values (<0.05) are in bold.

from the water itself (from Lake Mälaren). No extraction controls or NTCs showed any amplification during qPCR.

3.2 | DNA extraction and filter evaluation

In the high pike density treatment, Chelex 100 yielded a higher eDNA concentration than DNeasy Blood & Tissue ($F_{1,12} = 22.8$, $p < 0.001$, Figure 4). Neither filter combination ($F_{1,12} = 0.01$, $p = 0.92$) nor the interaction between the extraction method and filter combination ($F_{1,12} = 0.80$, $p = 0.39$) had any effect on the eDNA concentration. In the low pike density treatment, there was no difference among the extraction methods ($F_{1,12} = 3.1$, $p = 0.10$), filter combinations ($F_{1,12} = 0.41$, $p = 0.53$) or their interaction ($F_{1,12} = 2.28$, $p = 0.16$, Figure 4). Due to loss of replicates during DNA isolation, no statistical comparison between PowerWater and the other extraction methods could be performed. However, visual inspection (Figure 4) indicates that PowerWater yielded lower eDNA concentrations, except for a single replicate in the high density treatment using double filters (Figure 4).

3.3 | Mesocosm experiment using adult pike

Individual adult pike biomass had a positive effect on eDNA concentrations ($R^2 = 0.74$, Table 1b, Figure 5). However, the interaction between biomass and sampling occasion indicated that the relationship changed over time ($F_{2,30} = 8.00$, $p = 0.002$). The slope of the last sampling occasion (168 h) differed visually from the other two sampling occasions (48 and 120 h) and a subsequent model, excluding data from the last sampling occasion, showed that the slopes (48 vs. 120 h) did not differ statistically from each other ($F_{1,20} = 0.80$, $p = 0.38$, SI Section 3.3). Similar results were obtained using adult pike length (mm) instead of biomass (SI Section 3.3).

The negative control mesocosm contained very low levels of pike DNA with Cq between 38.8 and 40.0 (<0.07 pg μl^{-1} similar to

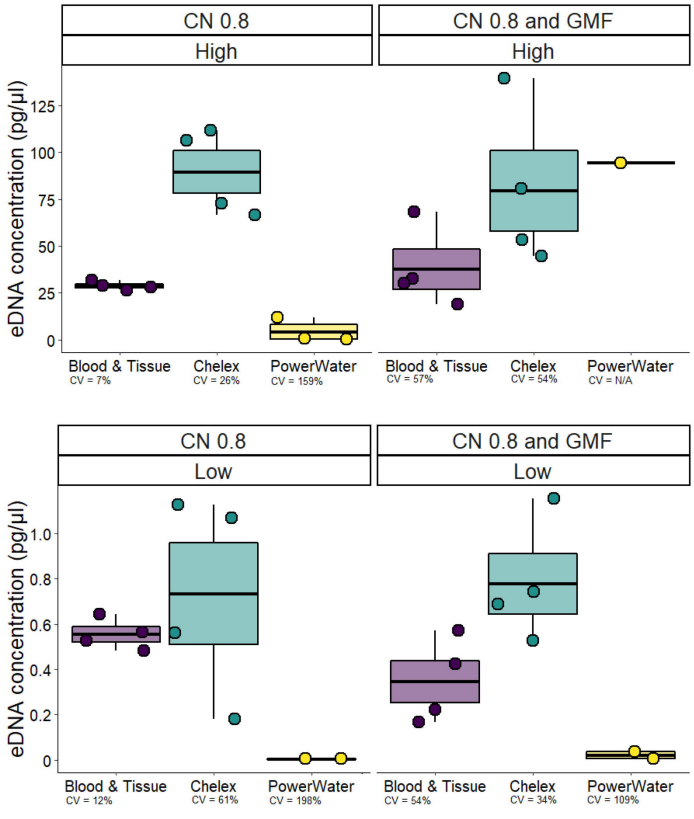

FIGURE 4 eDNA concentrations (pg µl-1) for samples extracted with DNeasy Blood & Tissue (purple), Chelex 100 (blue) and DNeasy PowerWater (yellow) in high (top panels) or low (bottom panels) pike densities, as well as the use of either single (CN 0.8) or double (CN 0.8 and GMF) filters. Each point denotes the mean value of four technical replicates taken from a sample. Black horizontal lines mark the mean, whereas the boundaries of the box indicate ±1 SE with whiskers above and below indicating minimum and maximum values. CV =the coefficient of variation, which is the mean normalized standard deviation

the aquarium measurements with juvenile pike) compared with the samples collected from mesocosms containing pike (mean Cq of 29.4 corresponding to 54.13 pg μl^{-1} of eDNA). One sampling control (taken at 120 h) showed amplification at 39.0 Cq (equating to eDNA concentration of 0.06 pg μl^{-1}) and no extraction controls amplified. Two NTC amplified on one plate (Cq = 38.8 and 38.3 equating to 0.07 and 0.10 pg μl^{-1}); the plate was not excluded as the observed signal was much stronger than contamination.

4 | DISCUSSION

We show that eDNA concentrations correlate positively with biomass and density of both juvenile and adult pike. Experiments in both aquaria and mesocosms showed strong linear correlations between eDNA concentrations and pike biomass, whilst also demonstrating that the eDNA signal decreased rapidly when the fish were removed. Additionally, we found that Chelex 100 outperformed the two most widely used eDNA extraction approaches in terms of yield, while the eDNA yield was similar regardless of whether a single or double filter was used.

4.1 | eDNA–biomass relationship

Our study shows a strong and positive linear relationship between eDNA and biomass for a large, sedentary species of fish (Rourke et al., 2021). The explanatory power of the relationships from our study are in line with prior research in controlled environments (average $R^2 = 0.82$; Yates et al., 2019). Previous experiments on common carp, *Cyprinus carpio*, found similar strong correlations between eDNA and biomass, whilst showing even stronger correlations with abundance (Doi et al., 2015; Eichmiller et al., 2016; Takahara et al., 2012), results that are analogous with our findings from the juvenile pike experiment. The shedding rate of eDNA per fish body weight (copies h^{-1} g^{-1}) has been shown to decrease with increasing size (allometric scaling) in bluegill sunfish, *Lepomis macrochirus* (Maruyama et al., 2014) and brook trout (Yates et al., 2021; Yates et al., 2021). This highlights a potential problem when using eDNA to infer biomass estimates on fish populations without information on the size or age structure of the population. Research on how individual size and biomass correlates with eDNA concentrations has thus far been largely overlooked with prior laboratory studies generally manipulating biomass by increasing the density (number of individuals per unit volume or area)

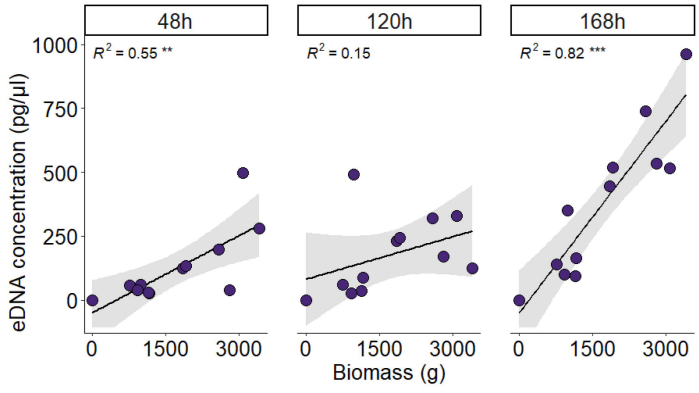

FIGURE 5 eDNA concentrations (pg µl-1) as a function of individual adult pike biomass (g) for three sampling occasions (48, 120, and 168 h after the pike were introduced to the mesocosms). Each data point represents the mean value from two replicates (the mean value of four technical replicates) taken at the same occasion and the color indicates individual mesocosms/replicates. The gray-shaded areas around the regression lines shows the 95% confidence interval. R2 values were calculated for each time point separately (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Results of the integrated analysis comparing the regression slopes between the consecutive sampling occasions are presented in Table 1b

(Doi et al., 2015; Klymus et al., 2015; Lacoursière-Roussel et al., 2016; Mizumoto et al., 2018; Takahara et al., 2012). Shedding rates of eDNA having been shown to increase disproportionately in some species when the fish are kept in groups (Thalinger et al., 2021). Pike are solitary ambush predators that commonly remain stationary while waiting for suitable prey, and they distribute spatially to avoid larger conspecific individuals (Nilsson, 2006). Applying eDNA–biomass relationships garnered through experiments where biomass is manipulated by varying amounts of individuals (often from small individuals with higher weight-specific shedding rates), therefore, run the risk of underestimating biomass/abundance of large pike, as the eDNA shed by a single large pike may be less than expected. Confirming and describing the positive relationship for large fish in controlled environments constitutes an important first step towards using eDNA for fish monitoring. Still, field validation of the methodology is necessary before the method can be deployed as a tool for ecologists and governing bodies. Promising findings in controlled environments are not necessarily repeated in natural systems (Yates et al., 2019), with numerous biotic and abiotic factors whose effects on eDNA dynamics are poorly understood. How the distribution and behavior of solitary, sedentary species affects the spatiotemporal dynamic of eDNA requires further understanding before abundance estimates can be inferred from environmental samples.

4.2 | DNA isolation—sometimes new is the well-forgotten old

The field of eDNA is very diverse when it comes to methods used to capture and isolate DNA for detection or quantification (Loeza-Quintana et al., 2020; Taberlet et al., 2018). Extracting eDNA from filters can be performed in many ways (Deiner et al., 2015). Currently the majority of eDNA studies on aquatic species use column-based extraction kits to extract eDNA from filters (Rourke et al., 2021). These kits are relatively quick, easy to use, and yield high eDNA concentrations (Eichmiller et al., 2016), but they also require several sample manipulations and are relatively costly. Chelex resin is a chelating polymer which historically has been used in forensic science and population genetics as a fast, extremely cost-effective and efficient technique to extract DNA for PCR (Walsh et al., 1991). However, during recent decades, more expensive column-based extraction methods have, to a large extent, replaced the use of Chelex in genetic research. To the best of our knowledge, Chelex has only been used in a single study on the spatial and temporal eDNA patterns of sea lamprey, *Petromyzon marinus* (Bracken et al., 2019). Here, we demonstrate that Chelex yields higher DNA concentrations than widely used commercial extraction kits. Reducing cost and labor is imperative for high-throughput processing in a monitoring context. As a consequence of reduced costs, the number of samples can increase, improving the signal-to-noise ratio and resulting in more reliable abundance estimates. The results of this study suggest that eDNA extraction from filters using Chelex is a cheap, quick and efficient alternative to current filter-based extraction methods. The Chelex protocol used in this study also returns considerably larger sample volumes (~400 µl compared with 100 µl (DNeasy PowerWater) and 200 µl (DNeasy Blood & Tissue)), giving the added possibility of further concentrating eDNA or using it for multiple analyses through qPCR, ddPCR, and/or metabarcoding. In addition, the Chelex protocol contains fewer sample manipulation steps which reduces the risk for contamination and handling errors during extraction (Walsh et al., 1991).

4.3 | Single or double filter?

The choice of filter material will affect the effectiveness of eDNA capture (Majaneva et al., 2018), with different materials and pore sizes capturing eDNA of different sizes and sources (Turner et al., 2014). Cellulose nitrate (CN, Dunker et al., 2016; Tillotson et al., 2018) as well as glass microfiber (GMF, Doi et al., 2017; Nevers et al., 2018) filters have both been used with good results in eDNA surveys. In addition, serial filtrations through multiple filters have shown to increase eDNA retention (Capo et al., 2020; Guivas & Brammell, 2020; Hunter et al., 2019). Some commercial companies use similar solutions (Hellström et al., 2019). Contrary to our hypothesis, filtering through double filters did not increase the DNA yield compared with using a single filter in our experiment. However, we have in a subsequent study observed that double filters enable more water to be pushed through the filters before clogging, which is likely due to the larger pore size of the GMF filter, which functions as a "pre-filter." Since larger water volumes are expected to increase eDNA yields (Schabacker et al., 2020; Wilcox et al., 2018), we expect that using double filters may be beneficial when sampling natural environments where clogging may be an issue (Hunter et al., 2019).

4.4 | Temporal eDNA dynamics

When organisms are introduced to new environments, such as aquaria or mesocosms, an equilibrium between the release and degradation of eDNA will be reached after some time, potentially having either higher (Maruyama et al., 2014; Takahara et al., 2012) or lower (Nevers et al., 2018) levels prior to the equilibrium. The time to reach equilibrium has been shown to be highly variable depending on the species and experimental setup, ranging from a few hours (Nevers et al., 2018; Sansom & Sassoubre, 2017; Sassoubre et al., 2016) to several days (Takahara et al., 2012). Our initial aquarium experiment seemingly reached equilibrium within the first 48 h, but our mesocosm experiment showed that eDNA levels were still increasing even by the end of the experiment, suggesting that equilibrium had not been reached. Temperature has been shown to increase eDNA shedding rates of brook charr, *Salvelinus namaycush*, attributed to increased metabolic rates (Lacoursière-Roussel et al., 2016), whilst studies on several carp species have failed to find a relationship between temperature and eDNA shedding rates (Klymus et al., 2015; Takahara et al., 2012). In addition, increased shedding rates may be counteracted by increased eDNA degradation through microbial activity with increased temperature (Dejean et al., 2011; Strickler et al., 2015). The combined indirect effects of temperature on eDNA concentration dynamics are, as a result, complex and uncertain. The effect of temperature could at least partially explain why eDNA concentrations did not reach equilibrium by the end of the mesocosm experiment, as temperature increased slowly throughout the experiment (the temperature increased by an average of 4.3°C by the end of the experiment compared with the start). Conversely, eDNA reached equilibrium by 48 h in the aquarium experiment, where temperature was kept constant. Shedding rates of eDNA has been shown to be highly variable even under constant conditions and the heterogenous distribution of eDNA in the water column may be a potential reason for the variation observed in our mesocosm experiment (Klymus et al., 2015). Furthermore, we observed the largest temporal increase in eDNA concentration for the highest biomass treatments in both experiments. It is likely that the pike experienced handling stress at the time of introduction to the mesocosms as well as during the confinement. The steeper increase in eDNA concentration in mesocosms with larger individuals could potentially be explained by size-specific tolerances to stress, where smaller/younger individuals generally are more tolerant than larger older ones (Barcellos et al., 2012) and, therefore, shed less DNA per unit mass than larger individuals (Thalinger et al., 2021). Similarly, the steeper increase in eDNA concentration in aquariums with higher density could be due to increased stress caused by confinement in close proximity to other similarly sized conspecifics, which are prone to cannibalization (Craig, 2008).

4.5 | Implications for monitoring

Northern pike inhabit diverse ecosystems, including rivers, lakes, and coastal habitats with very different environmental characteristics (Craig, 1996). The results presented here suggest that it is possible to quantify pike biomass using eDNA, but before it can be routinely applied for monitoring, several challenges need to be resolved. For example, spatiotemporal dynamics-related questions on how to allocate samples in a surveyed area and how different seasons affect eDNA concentrations (e.g., spawning time) need to be explored further. Future research should also focus on how environmental factors influence eDNA dynamics in pike. PCR is inhibited by naturally occurring substances such as humic, phytic, and tannic acids (Lance & Guan, 2020). If inhibiton was present in our study, it would have similar effect across replicates and treatments, and therefore, not affect the results. This will, however, not necessarily be the case when field samples from different locations with varying degrees of inhibition are analysed. Finding a streamlined and cost-effective way to quantify inhibition and adjust eDNA measurements accordingly, would constitute an additional important step towards using eDNA to infer meaningful temporal and spatial changes in fish population densities.

5 | CONCLUSIONS

We found strong linear eDNA–biomass and density relationships in controlled environments for both juvenile and adult pike. As such, our study adds much needed information on individual eDNA–biomass relationships for a large, sedentary fish species. Additionally, we highlight that Chelex is an effective method for eDNA isolation from filters, enabling a greater number of samples to be processed quicker and at a lower cost, potentially enabling implementation of eDNA

in large-scale aquatic monitoring. With the addition of research on the effects of biotic and abiotic factors on degradation, persistence, and inhibition on eDNA the methodological setup outlined here represents an important first step towards eDNA based monitoring to improve our knowledge on the population dynamics of sedentary fish like the northern pike.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception and design of experiments: EK, MO, GS, JS, OS, and AV. eDNA sampling and laboratory work was performed by EK, MO, GS, JS, OS. IN. EK performed the data analysis and wrote the paper with significant contributions from MO, GS, JS, and AV. All authors reviewed and approved the final draft.

DATA AVAILABILITY STATEMENT

Data for this study are included in the supplementary material.

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Supplementary Information

1. Aquarium experiment

1.1 Alternative regression models for the aquarium experiment

Table S1. Linear regression model using density (continuous predictor) and sampling occasion as explanatory variable (instead of biomass) (R^2 = 0.90)

	Estimate	Std. Error	t value	p
(Intercept)	-2.392	849.703	-0.003	0.997
Density	693.942	178.146	3.895	<0.001 ***
Sampling occasion (46h)	-237.556	1201.661	-0.198	0.845
Sampling occasion (70h)	-1693.320	1201.661	-1.409	0.169
Density : Sampling occasion (46h)	988.144	251.937	3.922	<0.001 ***
Density : Sampling occasion (70h)	1569.723	251.937	6.231	<0.001 ***

Table S2. Linear regression model exploring differences between second (46h) and last (70h) sampling occasion.

	Estimate	Std. Error	t value	p
(Intercept)	255.80	1137.96	0.225	0.824
Biomass (g)	471.93	70.25	6.717	<0.001 ***
Sampling occasion	-1205.10	1609.32	-0.749	0.463
Biomass(g) : Sampling occasion	155.65	99.35	1.567	0.133

Results of ANOVA (III) on model

	Sum	Df	F	p
(Intercept)	453165	1	0.05	0.8244

Biomass (g)	404676399	1	45.12	<0.001 ***
Sampling occasion	5028729	1	0.56	0.463
Biomass(g) : Sampling occasion	22008521	1	2.45	0.133
Residuals	179359026	20		

2. Method evaluation

2.1 Replicate allocation

Table S3. Allocation of replicates for a) high (8 pikes) or low (1 pike) pike densities, b) the use of either a single cellulose nitrate filter (CN) or combination of a cellulose nitrate (CN) and a glass microfiber (GMF) filter and c) extraction of DNA through DNeasy Blood & Tissue, Chelex 100 or DNeasy PowerWater.

Density	1 pike	1 pike	8 pikes	8 pikes
Filter	CN	CN + GMF	CN	CN + GMF
Chelex	4	4	4	4
Blood & Tissue	4	4	4	4
PowerWater	4	4	4	4

2.2 Extraction protocols

Chelex extraction of eDNA filters

General rules:

- *Always exchange pipette tip as soon as it has been in contact with a sample solution or has touched ANY surface or part of equipment.*
- *Always use filter tip pipette tips for extraction.*

- *Change gloves as soon as you suspect they may have come into contact with any type of solution (both sample and buffer) or filter piece from samples. Additionally change gloves as soon as they have been in contact with a surface that has not been disinfected, for example lab note book or pen.*

  Preferably gloves can be exchanged between each step

1. Dry filters overnight using silica gel.
2. Prepare Chelex solution (10% w/v).
3. Pre-heat a water bath to 100 ºC.
4. Using a sterilized pair of scissors and forceps cut sample filters into ~3x3 mm pieces and insert them in a 5 ml Eppendorf screw cap tube.
5. Put the chelex solution on a magnetic stirrer and pipette 1000 ul of chelex solution into the Eppendorf tube.
6. Sterilize scissors and forceps between samples by soaking them in 95% ethanol and burn under open flame.
7. Perform extraction in batches of 10 - 16 samples (including 1 extraction negative control, where no filter is inserted but all steps are performed as with samples).
8. Vortex the tubes briefly and centrifuge at 12 000 g for 1 min.
9. Vortex briefly again and incubate the tubes in the waterbath at 100 ºC for 10 min.
10. Vortex briefly and incubate in the waterbatch at 100 ºC for an additional 10 min.
11. Vortex samples and centrifuge samples at 12 000 g for 1.5 min.
12. Using a 1000ul pipette transfer supernatant to a new 1.5 ml Eppendorf tube (sticking the pipette tip into the “filter mush” is necessary here). Repeat step 9-10 until all liquid has been transferred.
13. Centrifuge the 1.5 ml tubes at 12 000 g for 1.5min.
14. Pipette out supernatant AVOIDING the pellet of chelex and transfer to a new 1.5 ml tube. Repeat step 11-12 until there is no visible chelex left in sample (atleast once).
15. Sample is now ready for down-stream analysis

2.3 Sampling equipment

Figure S1. Sterilized equipment ready to be used for mesocosm sampling 2020 (left to right); 300ml syringe, Swinnex filter holders pre-loaded with filters, extra filters, sample bags, plastic forceps.

3. Mesocosm experiment

3.1 Mesocosm temperature profiles

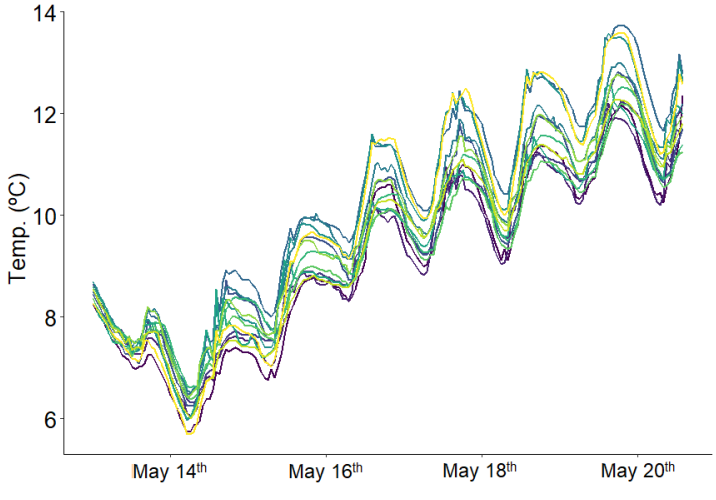

Figure S2 Hourly, mesocosm temperature throughout the length of the adult pike experiment (2020-05-13 to 2020-05-20). Each colour represents an individual mesosocosm/replicate.

Figure S3 Daily median temperature throughout the length of the adult pike experiment (2020-05-13 to 2020-05-20). Each colour represent a mesocosm/replicate used in the experiment.

3.2 Extraction protocol

Chelex extraction of eDNA filters

General rules:

- *Always exchange pipette tip as soon as it has been in contact with a sample solution or has touched ANY surface or part of equipment.*
- *Always use filter tip pipette tips for extraction.*
- *Change gloves as soon as you suspect they may have come into contact with any type of solution (both sample and buffer) or filter piece from samples. Additionally change gloves as soon as they have been in contact with a surface that has not been disinfected, for example lab note book or pen.*

  Preferably gloves can be exchanged between each step

16. Prepare Chelex solution (10% w/v).
17. Pre-heat a water bath to 100 ºC.

18. Using a sterilized pair of scissors and forceps cut sample filters into ~3x3 mm pieces and insert them in a 5 ml Eppendorf screw cap tube.
19. Put the chelex solution on a magnetic stirrer and pipette 1500 ul of chelex solution into the Eppendorf tube.
20. Sterilize scissors and forceps between samples by soaking them in 95% ethanol and burn under open flame.
21. Perform extraction in batches of 10 - 16 samples (including 1 extraction negative control, where no filter is inserted but all steps are performed as with samples).
22. Vortex the tubes briefly and centrifuge at 12 000 g for 1 min.
23. Vortex briefly again and incubate the tubes in the waterbath at 100 ºC for 10 min.
24. Vortex briefly and incubate in the waterbatch at 100 ºC for an additional 10 min.
25. Vortex samples and centrifuge samples at 12 000 g for 1.5 min.
26. Using a 1000ul pipette transfer supernatant to a new 1.5 ml Eppendorf tube (sticking the pipette tip into the "filter mush" is necessary here). Repeat step 9-10 until all liquid has been transferred.
27. Centrifuge the 1.5 ml tubes at 12 000 g for 1.5min.
28. Pipette out supernatant AVOIDING the pellet of chelex and transfer to a new 1.5 ml tube. Repeat step 11-12 until there is no visible chelex left in sample (atleast once).
29. Sample is now ready for down-stream analysis.

3.3 Alternative regression models for the mesocosm experiment

Table S4. Multiple linear regression model using length (mm) as explanatory variable (instead of biomass(g)) (R^2 = 0.62)

	Estimate	Std. Error	t value	p
(Intercept)	-128.84312	131.51963	-0.980	0.335
Length (mm)	0.41009	0.20083	2.042	0.050 .
Sampling occasion (120h)	94.39298	185.99685	0.507	0.616
Sampling occasion (168h)	-81.23540	185.99685	-0.437	0.665

Length (mm) : Sampling occasion (120h)	-0.06753	0.28402	-0.238	0.814
Length (mm) : Sampling occasion (168h)	0.54821	0.28402	1.930	0.063

Table S5. Multiple linear regression model exploring differences between first (48h) and second (120h)

	Estimate	Std. Error	t value	p
(Intercept)	-48.68976	70.55210	-0.690	0.498
Biomass (g)	0.10034	0.03530	2.843	0.010 *
Sampling occasion (120h)	129.94582	99.77573	1.302	0.208
Biomass (g) : Sampling occasion (120h)	-0.04487	0.04992	-0.899	0.380

ANOVA table (type III sums of squares)

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	7377	1	0.4763	0.498
Biomass (g)	125151	1	8.0800	0.010 *
Sampling occasion	26272	1	1.6962	0.208
Biomass (g) : Sampling occasion	12510	1	0.8077	0.380
Residuals	309778	20		

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Temperature moderates eDNA–biomass relationships in northern pike

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Abstract

Support for eDNA as a quantitative monitoring tool is growing worldwide. Despite advances, there are still uncertainties regarding the representability of the eDNA signal over varying spatiotemporal scales, the influence of abiotic forcing, and phenological changes affecting the behavior of the study organism, particularly in open environments. To assess the spatiotemporal variability and predictive power of quantitative eDNA analysis, we applied species-specific real-time quantitative PCR on water filtrates during two visits to 22 coastal bays in the Baltic Sea. Within bays, we collected water along four transects across each bay and compared the pooled eDNA concentration to temporally matched catches from standardized angling targeting the northern pike (*Esox lucius*), a species for which reliable monitoring data is lacking. We found the variability in eDNA concentrations between transects to be moderate (21%) but still considerably lower than across bays and visits (52%), suggesting small-scale spatial differences are of less importance during spring when pike spawn. Standardized angling catches, bay area, and water temperature together explained 48% of the variance in eDNA concentrations. DNA concentrations decreased with the increasing bay area, likely indicating a dilution effect. Notably, the relationship between eDNA and standardized catches was positive but varied with temperature and the eDNA-abundance relationship was only significant at higher temperatures, which also coincided with a higher proportion of spawning/spent fish. We conclude that temperature is a key moderating factor driving changes in pike behavior and spring DNA-dynamics. We recommend that future surveys focus on larger spatiotemporal scales during times when the influence of changing temperatures is minimized.

KEYWORDS
abundance, angling, Baltic Sea, biomass, coast, CPUE, eDNA, environmental DNA, *Esox lucius*, spawning, temperature

1 | INTRODUCTION

The monitoring of fish stocks requires quantitative data on their abundance. This can be difficult to obtain for species with sedentary lifestyles and whose catchability in passive gears is low, such as gillnets and traps (Villegas-Ríos et al., 2014). Environmental DNA (eDNA) has been suggested as a possible tool for fish stock monitoring in general, and monitoring of species with low catchability in particular (Kačergytė et al., 2021). While eDNA can be successfully used in biodiversity monitoring based on presence/absence data (Dejean et al., 2011; Dunker et al., 2016; Hernandez et al., 2020; Takahara et al., 2013; Thomsen et al., 2012), its potential use for biomass estimation is still under development. Quantitative relationships between eDNA concentrations and fish biomass have been demonstrated under controlled conditions with known biomass and to a lesser extent also in natural environments with unknown biomass (Rourke, Fowler, et al., 2022 and references therein). Moreover, the positive relationship between eDNA and fish biomass in the wild has most often been found in freshwater lakes (Spear et al., 2021), streams (Yates, Cristescu, & Derry, 2021), and to some extent pelagic marine environments (Li et al., 2022). Comparative eDNA surveys for semi-open coastal fish communities are still scarce in the literature (Rourke, Fowler, et al., 2022).

Although linear relationships have been obtained in controlled experiments, the precision of these estimates varies greatly in natural systems where eDNA on average explains 57% of the variance compared with 81% in controlled mesocosm experiments (Yates et al., 2019). The variability in these estimates can be attributed to differences in ground truthing methods (Rourke, Fowler, et al., 2022), hydrologic conditions (Song et al., 2017), DNA extraction methods (Bockrath et al., 2022; Karlsson et al., 2022), presence of polymerase chain reaction (PCR) inhibiting humic and tannic acids (Lance & Guan, 2020), sediment particles in the water (Stoeckle et al., 2017), size distribution of the local population (Yates, Cristescu, & Derry, 2021; Yates, Wilcox, et al., 2021) and ambient temperature (Lacoursière-Roussel et al., 2016). Here, two of these factors are focused on, namely, temperature (DNA shedding and degradation) and hydrology (distribution and dilution). Temperature is intimately linked to metabolic processes (Thalinger et al., 2021), activity (de Souza et al., 2016; Thalinger et al., 2021), and behavior (Tillotson et al., 2018) that all affect DNA shedding rates, particularly in poikilotherms like fishes. In extension, this likely also affects the detection probability and spatial distribution of organisms (Takahara et al., 2012). However, the extent to which temperature affects DNA shedding and degradation rates in aquatic environments is still not well understood and studies have reported contrasting results. For example, temperature did not seem to affect DNA concentrations in aquaria experiments with common carp (*Cyprinus carpio*, Linnaeus 1758) (Takahara et al., 2012), bighead carp (*Hypophthalmichthys nobilis*, Richardson 1845) and silver carp (*Hypophthalmichthys molitrix*, Valenciennes 1844) (Klymus et al., 2015) and round goby (*Neogobius melanostomus*, Pallas 1814) (Nevers et al., 2018) while higher temperature did increase DNA shedding rates in experiments with brook charr (*Salvelinus fontinalis*, Mitchill 1814) (Lacoursière-Roussel et al., 2016) and Japanese jack mackerels (*Trachurus japonicus*, Temminck & Schlegel 1844) (Jo et al., 2019). Increased shedding rates caused by higher temperatures may in turn be counteracted by increased microbial activity and degradation of DNA (Andruszkiewicz Allan et al., 2021; Jo et al., 2019; Tsuji et al., 2017). How and when temperature influences eDNA concentrations in the water thus seems to depend on species as well as experimental/environmental conditions.

Hydrological conditions govern the distribution of DNA. Therefore, it is important to consider that eDNA-abundance relationships deduced from field surveys likely are influenced by the area or volume of the sampled water body. Although the literature is scarce on this topic, a few studies have reported improved DNA-abundance relationships for river-dwelling salmonids when water flow has been accounted for, indicating that high water flows dilute the DNA signal (Curtis et al., 2021; Jane et al., 2015). Even less is known for lentic or marine environments but generally, biomasses scaled to the area of the water body seem to correlate well with quantified DNA copy numbers, suggesting that the sizes of water bodies should be considered in order to correctly reflect population sizes (Gaudet-Boulay et al., 2022; Seymour & Smith, 2023). Hence, it is important to include the influence of multiple abiotic factors in quantitative eDNA studies (Jo, 2023).

The northern pike (*Esox lucius*, Linnaeus 1758) is a species of growing research interest (Forsman et al., 2015). It is a keystone predator in freshwater and coastal ecosystems and it is important for ecosystem functioning as well as a focal species for the recreational fishery (Arlinghaus et al., 2018; Crane et al., 2015). Pike also provides an example of a species for which accurate abundance indices are difficult to obtain due to its low catchability in passive gears (Craig, 2008). In the Baltic Sea, large-scale patterns indicate that the pike populations on the east coast of Sweden have drastically declined (Olsson et al., 2023). The reasons are multifaceted but likely a consequence of increased predation on adults from gray seals and cormorants (Hansson et al., 2017; Svensson, 2021), predation on juvenile stages by three-spined stickleback (Donadi et al., 2020; Eklöf et al., 2020), loss of recruitment habitats (Sundblad & Bergström, 2014), and also a period of high recreational fishing mortality during the early 1990s (Bergström et al., 2022). For stationary species which form genetically stable distinct populations over rather small geographical areas (Diaz-Suarez et al., 2022; Laikre et al., 2005; Möller et al., 2021; Wennerström et al., 2016) management needs to be regional and there is a need for monitoring methods which can accurately assess the status of pike populations on a local scale. Northern pike aggregates in shallow areas to spawn during spring and have a strong homing behavior (Craig, 2008; Skov & Nilsson, 2018). This requires monitoring with a high level of spatial coverage, which poses challenges to the management of this species. Since traditional, passive, and lethal monitoring methods have proven ineffective, recent attempts to quantify pike abundance have employed active methods, such as standardized

rod-and-reel fishing during the spawning period to obtain measures of relative abundance (Catch-Per-Unit-Effort data, CPUE) and size structure of distinct populations that form local spawning aggregations. Standardization of such methods is however complicated as the size and type of bait used, catch-and-release (C&R) practices and angling effort can affect the catchability (Arlinghaus et al., 2008, 2017; Kuparinen et al., 2010), meaning that CPUE can underestimate population size in areas where fishing is intense and C&R is common. In fisheries research, this phenomenon is called *hyperdepletion*, which can seriously bias stock assessments (Alós et al., 2015). Environmental DNA analysis on the other hand offers several advantages over active rod-fishing, in the sense that it is not size selective, unaffected by fishing effort and gear use, can provide an adequate level of replication (Shelton et al., 2022), is noninvasive, cost-efficient, and potentially has a higher probability of better reflecting the local density of fish (Wilcox et al., 2016).

Strong positive relationships between eDNA and the biomass of pike have been shown in large outdoor mesocosms during the reproductive period (Karlsson et al., 2022). However, it is unknown if eDNA analysis can provide quantitative data on pike abundance also under natural conditions. In this paper, we test the hypothesis that relative pike population sizes can be estimated using eDNA analysis during the reproductive season when pike aggregates. We do this using data collected from a large number of coastal bays in the Baltic Sea where we compare eDNA concentrations to standardized angling while accounting for the potential effects of environmental factors.

2 | MATERIALS AND METHODS

2.1 | General design

To assess the potential of eDNA analysis to estimate pike population biomass/abundance under natural conditions, we collaborated with a project conducting standardized angling to support management actions. The multi-year project was initiated by the Stockholm County Administrative Board and aimed to assess pike population sizes in relation to current and future fishing closures during the pike spawning season.

The study area covered >200 km of the Stockholm archipelago in the Baltic Sea. The angling was performed during two visits in 24 coastal bays during April–May 2020 (coordinates for each bay can be found in the supplementary data file "DATA.xlsx"). The selection of bays to include in this study was therefore reliant on the evaluation of fishing closures. The design for that evaluation was based on paired bays, of which some used a Before-After-Control-Impact (BACI)-design (Eberhardt, 1976; Green, 1979) which enables future evaluations of fishing closures as a form of fisheries management by accounting for site-specific temporal changes in the environment. The paired bays were chosen to be in close proximity to each other and to be as similar as possible in terms of size, mean depth, and habitat conditions, but with one bay being either closed or soon-to be closed for fishing and the other one open to angling; thus likely providing a range of fish densities spanning from low to high, which was a prerequisite for the evaluation of the eDNA-biomass relationships in this study.

The eDNA sampling was performed in 22 out of the 24 fished bays a few days prior to each angling visit in a bay, to not risk the eDNA signal to be influenced by the fishing activity nor to disturb the fishing by simultaneously sampling eDNA (Figure 1).

2.2 | DNA analyses

2.2.1 | eDNA collection and filtration

Within each bay and visit, we collected water along four transects: three shallow water transects (A, B, and C), each trailing roughly a third of the coastal length of the bay, and one deep water transect (D) across the center of the bay (Figure 2, supplementary list of figures "Bay.info.pdf"). For each transect, 1 L of surface water was collected every 50 m. The distance between individual subsamples was chosen based on the reported detection distance for caged northern pike carcasses in a freshwater system (Dunker et al., 2016) and live Japanese striped jack (*Pseudocaranx dentex*, Bloch & Schneider, 1801) in a marine setting (Murakami et al., 2019). The total amount of water collected per bay and transect was approximately proportional to the bay area. The water from each transect was pooled in a large plastic container and the total volume of pooled water varied from four to 26 L, median = 10, interquartile range (IQR) = 7 (Supporting information file, "DATA.xlsx"), depending on the length of the transect. From this pool of water, duplicate samples of 1 L each were filtered on-site using an established filtration technique (Karlsson et al., 2022) with some modifications. Each water sample was pushed through a Swinnex filter holder (Merck KGaA) loaded with two stacked filters (cellulose nitrate filter, pore size of 0.8 μm and a glass microfiber filter on top (GF/A, pore size of approximately 1.6 μm; GE Healthcare)) using a plastic syringe. The glass microfiber filter allowed a larger volume of water to pass through (Capo et al., 2020). We re-used the filter holder and exchanged the filters in the field for the second technical replicate. Although some cross-contamination could be expected at this stage, we assumed the contamination would be diluted to the point that it would fall below the detection limit. This was later confirmed by finding no consistent increase in eDNA concentrations in consecutive eDNA samples (Figure S1). One field negative control using 1 L of distilled water was run per bay visit directly after the filtration of the eDNA samples. After filtration, the filters were enclosed in zip-loc bags and snap-frozen on dry ice until arrival to the laboratory where they were directly transferred to a −80°C freezer pending DNA extraction. Nitrile gloves and sterile pincers were used at all times during filter handling. All equipment was sterilized between field visits by immersion in 10%–20% commercial grade sodium hypochlorite bleach for a minimum of 10 min and then rinsed thoroughly in tap water.

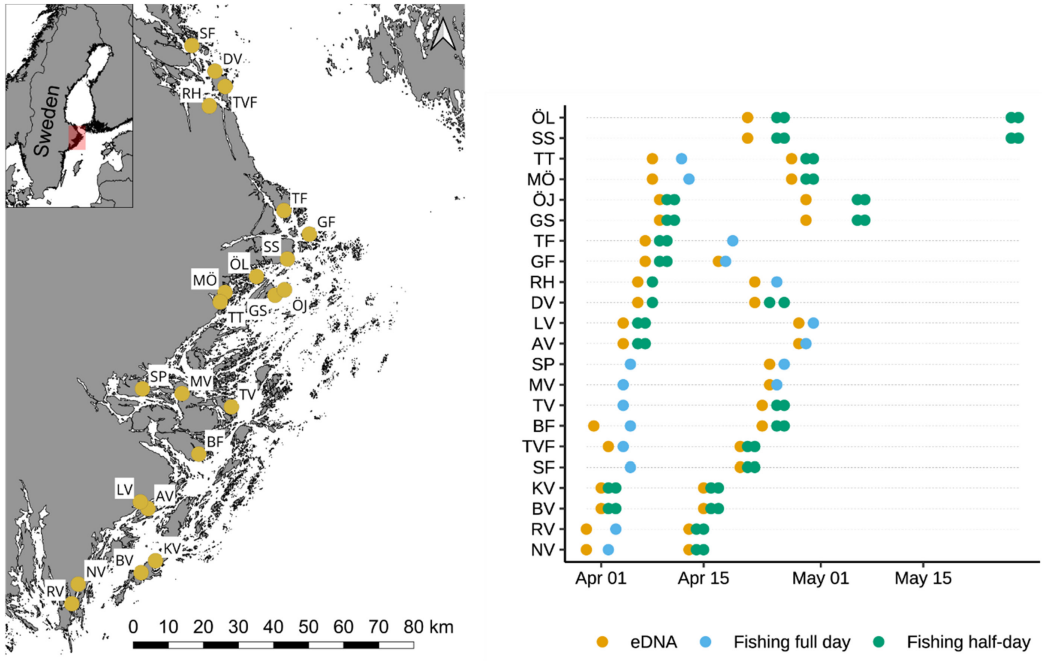

FIGURE 1 Overview map of bays sampled for eDNA and pike by angling (left panel) and sampling scheme (right panel) showing sampling dates for eDNA and angling ordered by bay-pair during the survey in 2020. Angling was either divided into two half days within a bay-pair and fished for two consecutive days, alternating morning and afternoon in each bay, or as a full day's fishing in a specific bay.

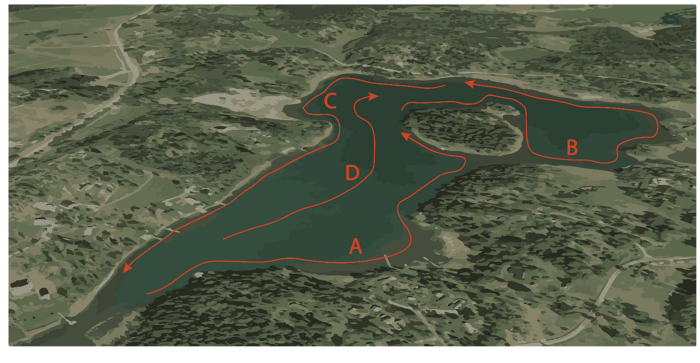

FIGURE 2 Schematic image showing the sampling design for eDNA. 1 L water samples were collected 50 m apart in four transects (A–D) and pooled within each transect. Transects A–C normally covered the shallowest vegetated areas while transect D was the deepest in the central part of the bay. Transect length was approximately proportional to the bay area.

2.2.2 | DNA extraction

DNA was extracted from both types of filters using a modified Chelex extraction protocol described in Karlsson et al. (2022). In brief, the filters were cut into smaller pieces using sterile equipment and then mixed with 750 µL of a 10% (w/v) Chelex suspension in 5 mL Eppendorf® screw cap tubes. The tubes containing the filter cuttings were heated at 100°C for 10 min to lyse cell material and denaturate the DNA, and then vortexed thoroughly. This step was repeated twice after which the supernatant was transferred to a smaller 1.5 mL tube and centrifuged at 16×10^3 g for 1.5 min to remove remaining filter debris and Chelex from the solution. After centrifugation, the supernatant was once again transferred to a clean 1.5 mL tube. If necessary, any remaining Chelex was removed by repeating the last centrifugation step and transferring the supernatant to a clean tube. Extraction–negative controls were added for each batch of samples that were extracted ($n = 21$).

2.2.3 | DNA quantification using qPCR

We used a real-time quantitative polymerase chain reaction assay (qPCR) for quantification of pike DNA in collected samples. The primer and probe combination (F-primer: 5′-CCT TCCCCCGCATA AATAATATAA-3′, R-primer: 5′-GTACCAGCACCAGCTTCAACAC-3′ and probe: 5′-FAM-CTTCTG ACTTCTCCCC-BHQ-1-3′ (Microsynth AG)) was originally developed and tested for specificity against co-occurring freshwater species, including closely related muskellunge, *Esox masquinongy* by Olsen et al. (2015, 2016). The assay has subsequently been successfully used for northern pike detection in water samples (Dunker et al., 2016; Karlsson et al., 2022). The assay targets a 94-base-pair-long fragment of the Cytochrome oxidase I gene (COI). qPCR was performed on a BioRad CFX384 Real-time PCR system with 15 μL reaction volumes. Reaction concentrations of the forward primer, reverse primer, and probe were 900 nM each with 7.5 μL 2× TaqMan™ Environmental Master Mix 2.0 (Thermo Fisher) in each well loaded with 4 μL of the sample template. An internal positive control (IPC) (Cy®5-QXL®670 Probe; EuroGentec) kit was run in duplex reactions to control for potential inhibition. 0.3 μL of 10×IPC mix and 0.2 μL of IPC template DNA was added to each reaction.

Inhibition in eDNA samples was determined based on aberrant IPC Cq-values. The expected Cq of the IPC over the range of the standard curve was on average 27.3 Cq with average minimum and maximum values ranging 26.5–28.5 Cq. Therefore, we classified samples >28.5 Cq as unacceptable. Such samples were purified using a Zymo OneStep PCR Inhibitor Removal Kit (Zymo Research Corp.) and reanalyzed in the qPCR. If purification of the sample did not improve, the IPC-value to within acceptable Cq-limits was excluded (Figure S2).

The following qPCR program was used for all the reactions: 10 min activation at 95°C followed by 45 cycles of 15 s at 95°C and 60 s at 60°C. Quantification of eDNA was achieved using a standard curve consisting of an 8-step, 10-fold dilution series of pike DNA ($1–1\times10^7$ copies/μL) with the addition of a lowest concentration at 0.25 copies/μL. As a standard, we used a synthetic 94 nucleotide oligo template targeting the mitochondrial COI-gene: 5′-CCT TCC CCC GCA TAA ATA ATA TAAGCT TCT GAC TTCTCCCCC CCT CCT TTT TAC TTC TCT TAG CCT CCT CAG TTC TCT GTG TTG AAG CTG GTG CTG GTA C-3′ and complementary strand: 5′-GTA CCA GCA CCA GCT TCA ACA CCT GAG GAG GCT AAG AGA AGT AAA AAG GAG GGG GGG AGA AGT CAG AAG CTT ATA TTA TTT ATG CGG GGG AAG G-3′ (Microsynth AG).

Samples and standard curves were run in quadruplicates with four no–template control (NTC) reactions on each plate. Plate efficiency varied between 101.3% and 110.7%, with R^2 values between 0.983 and 0.995.

2.2.4 | Determination of limits of detection (LOD) and quantification (LOQ)

The limits of detection (LOD) and quantification (LOQ) were determined by running a standard curve with DNA concentrations in the same range as all other standards ranging $0.25–1\times10^7$ copies/μL each in 16 technical replicates. The estimated qPCR efficiency was 118.2% with $R^2=0.981$. LOD is defined as the lowest concentration of DNA that can be detected with 95% probability in one single replicate and LOQ is defined as the lowest concentration of DNA with a coefficient of variation (CV) below 35% (Klymus et al., 2020). Effective LOD is defined as the lowest concentration with a 95% probability of detection given *n* technical replicates. LOD and LOQ were both determined to 1.97 copies/μL. Analysis in quadruplicates ($n=4$) gave an effective LOD of 0.58 copies/μL which is the LOD relevant to our assay. Concentrations are given per microliter of target sample (4 μL).

2.2.5 | qPCR data handling and curation

DNA concentrations below the LOQ cannot be adequately determined and are considered to be censored in statistical terms (Cohen & Ryan, 1989). This means that the true value is unknown but the threshold below or above which the true value can occur is well defined. In order to calculate an average DNA concentration per sample when some values are partially unknown is problematic. To solve this problem, the simplest approach is to remove the data. However, the consequence is that (i) valuable data are discarded and the sample characteristics are lost, lowering the overall statistical power of tests (Turkson et al., 2021) and (ii) arithmetic means calculated using excluded data become overestimated and standard deviations biased (Hornung & Reed, 1990). Censored data can be estimated using several statistical methods like Maximum likelihood estimation, Kaplan–Meier estimators, Cox-regression, or simply, by substitution with fixed values (Canales et al., 2018; Dinse et al., 2014; Hornung & Reed, 1990). Here, we chose the simpler approach of substitution which has been proven adequate for most applications (Glass & Gray, 2001). Furthermore, samples with very low average DNA concentrations usually have an unproportionally high frequency of non-detects across technical replicates (Lesperance et al., 2021; McCall et al., 2014). To accurately estimate the average DNA concentration per eDNA sample, it is important to assign a value of zero to true negatives, that is, non-detects. We visually determined the average DNA concentration per *Bay* and *Visit* where the proportion of non-detects clearly deviated from the mean. This threshold was approximately at 8 copies/μL (Figure S4). Hence, non-detects below this threshold value were assigned a DNA concentration value of zero while values above the threshold, but below the LOD, were assigned a value of one-half of the LOD (Cohen & Ryan, 1989; Glass & Gray, 2001). Detectable values but below the LOD were set to the LOD while values between the LOD and the LOQ were assigned the mean value of LOD + LOQ (Figure S3).

2.3 | Collection of angling data

In total, 24 coastal bays were fished at two occasions (visits), 8–20 days apart. Two of these bays were not sampled for eDNA due

to logistic reasons (Villinge N 59°5.7789′, E 18°36.7948′, Jungfruskär, N 59°8.4618′, E 18°40.9969′). During each visit, the fishing was divided into two consecutive half days à 4 h of active fishing each, alternating morning (08:00–12:00) and afternoon (13:00–17:00). In some cases, however, the fishing was instead performed during one full day (8 h, Figure 1) due to logistics and weather conditions. The fishing was performed by six teams, each team consisted of two highly experienced pike anglers. The aim was to fish efficiently and catch as much fish as possible by choosing what the anglers considered to be the most suitable angling gear and bait. Sampling effort was quantified as rod hours, that is, time fished per person. For each visit, the fishing teams recorded surface water temperature, number of seals at the site (estimated by eye), number of cormorants at the site (estimated by eye), numbers of other anglers present at the site (i.e. a boat with three anglers should be counted as three), and stationary fishing gear at the site (as indicated by buoys). Each pike caught was measured for total length using a tape measure, weight with a digital balance and sexed based on external characteristics (Casselman, 1974). Spawning status was visually assessed according to expert judgment and classified as either pre-spawning (large girth indicating developed gonads but no running roe or milt), spawning (running roe or milt), post-spawning (spent fish, no running roe or milt and flaccid abdomen) or undefined (usually small fish without external characteristics indicating sexual maturation).

2.4 | Abiotic data collection

Abiotic data were collected on each eDNA sampling visit to the bays using a Rinko ASTD-102 profiler (JFE Advantech Co., Ltd.). Depth, temperature, salinity, dissolved oxygen, turbidity and Chl-A-levels were measured from the surface to the bottom at the beginning and the end of each transect. The median value was calculated per depth profile and across transects to provide a grand median per bay visit.

2.5 | Statistical analyses

Statistical analyses were performed using R, version 4.2.0 (R Core Team, 2022) and the *tidyverse* suite of packages (Wickham et al., 2019). Linear mixed models were run using the *lme4* (Bates et al., 2015) and generalized linear models using the *MASS* package (Venables & Ripley, 2002). Associations between candidate variables in the models were first assessed graphically using pair plots and by Pearson's product–moment correlation coefficient. Highly correlated ($r > 0.7$) and biologically insignificant variables were excluded. Multicollinearity was also checked using the Variance Inflation Factor (VIF) and no variable included in the modeling had VIF values >1.6, indicating lack of significant multicollinearity. Model evaluations were performed using the *DHARMa* (Hartig, 2022) and *visreg* packages (Breheny & Burchett, 2017) in combination with visual inspection of the residuals, outliers and leverage. Model fit was assessed using AICc (AIC corrected for sample size) and R square values were calculated with the *rsq* package (Zhang, 2022). The level for statistical significance was set to $\alpha = 0.05$. R-scripts and data for the analyses are provided as Supporting Information.

2.5.1 | Standardization of angling data

To account for the potential influence of variables that might have affected catchability (i.e., rod-fishing efficiency), we ran a series of generalized linear mixed effects models to standardize the catch of pike in each bay and visit (hereafter called the CPUE-model). We modeled the number of pike caught per bay and visit using a Poisson distribution and the log of fishing effort as an offset ($n = 48$). We used *Bay* and *Visit* nested within *Bay* as random factors on the intercept. The latter also functioned as an observation level random effect (OLRE) to handle overdispersion in the count data (Harrison, 2014). For models that did not converge, the random effects were simplified to only include *Visit* nested within *Bay*. The number of other anglers (mean 3.0, range 0–25), number of cormorants (mean 9, range 0–100), and water temperature (mean 7.9°C, range 4–16°C) observed during angling were treated as continuous fixed effect variables. The other variables (number of seals and numbers of stationary fishing gear) were not included in the models since the data were too sporadic to be useful. Model selection consisted of fitting (i) a base model with only random effects, (ii) models with each fixed effect separately, (iii) models with pairwise combinations of the fixed effects and (iv) a full model with all variables, resulting in a total of 8 models. If two models were identified as equally parsimonious based on AICc, we chose the model with the strongest statistical significance for the fixed effects.

2.5.2 | Estimating spatiotemporal variation in eDNA concentrations

Because the eDNA and angling datasets were collected at different spatial scales, we modeled the average DNA concentration in each combination of bay and visit to make the two datasets compatible (hereafter called the eDNA-model). We used a generalized linear mixed model with a Poisson distribution (Chambert et al., 2018). Although qPCR data derived using standard curves can be treated as a continuous variable, it is appropriate to use Poisson or negative binomial models since qPCR quantifies discrete counts of DNA and the underlying distribution can be assumed to be driven by a Poisson process (Majumdar et al., 2017). Because the data were continuous but the Poisson model requires integer values, we also rounded the data up to the nearest integer (Chambert et al., 2018). As response variable, we used DNA copy number per μL, the interaction between *Bay* and *Visit* was used as the fixed effect. Random effects on the intercept were transects nested within visits nested within bays and sample filter ID, which acted as an OLRE to account for the overdispersion in the data. We chose a mixed model over a conventional generalized linear model due to the hierarchical nesting of our data.

To assess the relative variance associated with either spatial or temporal variation we calculated the intra-class correlation coefficients (ICCs, or variance components) and their uncertainty (Nakagawa et al., 2017; Nakagawa & Schielzeth, 2010). The model contained only random intercepts on *Bay*, *Visit* nested within *Bay* (*Bay:Visit*), *Bay:Visit:Transect*, *Bay:Visit:Transect:Filter* and the OLRE which consisted of each individual technical replicate in the qPCR dataset. Moreover, we fitted a second model, excluding the random effects for *Bay* and *Bay:Visit* and replaced them with the pooled effect of *Bay* and *Visit*, that is, the unique combinations of bays and visits. This was done in an attempt to account for the large uncertainty stemming from low within-level replication, especially at the finer scale such as within transects (two filters per transect).

2.5.3 | Modeling eDNA concentrations

To explain the variation in eDNA concentrations across bays and visits we tested and evaluated a range of generalized linear models. The response variable in these models was the average eDNA concentration (DNA copies per μL) estimated from the eDNA-model. Due to overdispersion, we chose a model assuming a negative binomial distribution over Poisson (Lindén & Mäntyniemi, 2011).

As predictor variables we chose a range of variables known to affect the eDNA signal. We chose *temperature* because it is a proximal variable that is intimately linked to physiological rate process in poikilotherms (Woods et al., 2003), and hence also DNA shedding and degradation (Jo et al., 2019), *bay size* because the eDNA concentration should be approximately proportional to the area or volume of a particular bay, assuming complete mixing of the water, that is, a dilution effect (Yates, Glaser, et al., 2021) and fish density estimated as CPUE from angling (Capo et al., 2019; Lacoursière-Roussel et al., 2016; Stoeckle et al., 2021; Yates, Glaser, et al., 2021). Furthermore, because eDNA concentrations have been shown to scale allometrically with fish size (Yates, Cristescu, & Derry, 2021; Yates, Wilcox, et al., 2021; Zhang et al., 2022), we also calculated the allometrically scaled average fish weight per bay and visit in the population (ASM) as:

$$\text{ASM} = \sum (M)\beta / N$$

where M equals the individual weight (g), β equals a scaling coefficient (0.7) (Yates et al., 2022), and N the total number of fish caught per bay and visit. Effectively, this approach was a slight modification of the allometrically scaled mass (ASM) proposed by Yates, Glaser, et al. (2021) and Stoeckle et al. (2021) since ASM in our case did not extend the calculation to the population level. Instead, we used it as a covariate together with CPUE (sensu Spear et al., 2021). Another potential variable that could be expected to affect eDNA concentrations is the spawning status of the population, since spawning events momentarily increase eDNA-levels due to increased activity of the fish (movements) but also release of gametes (Collins et al., 2022; Wu et al., 2023). This variable was strongly correlated with temperature and therefore omitted from the models by necessity. However, we calculated the variable *proportion spawned* to visualize the relationship between temperature and spawning status (Figure S6),

$$\text{Proportion spawned} = \frac{(\text{S} + \text{PS})}{(\text{S} + \text{PS} + \text{PrS} + \text{U})}$$

where S=spawning, PS=post-spawned, PrS=pre-spawning and U=undefined.

Based on the selected variables temperature, bay size, CPUE, and ASM, we used a forward selection process starting with the main effects of each variable, as well as the interaction between CPUE and ASM (Spear et al., 2021). After having selected the best fitting main effects, we included also potential interactions.

3 | RESULTS

3.1 | qPCR data and quality control

Inhibition, operationally defined as a sample displaying Cq-values >28.5 for the Cy5-labeled IPC was found in all or some of the technical replicates from five out of the 22 bays ("SP –Släpan/Ekefjärd", "TT –Tomtviken/Urö", "SS –Södersundet", "MÖ –Möcklingeviken" and ÖL –Östra Lemaren", Figure S2). Consequently, these samples did not pass the quality control and were excluded from further analyses. In total, 285 filter samples (1135 samples including technical replicates) passed the quality control and were amenable for downstream analysis. Out of these samples (n=1135), 61.9% were above the LOQ, 12.2% between LOD and LOQ, 2.2% below the LOD, and 23.6% were non-detects (Figure S3). In order to not overestimate sample averages, 86.6% of the non-detects were imputed with zeros based on their overall high sample Cq-values (Figure S4).

3.2 | Descriptive abiotic data

The surveyed parts of the bays were shallow, with a median depth of 1 m (IQR=0.7–1.4 m) across transect measurements. At the bay level, the temperature increased over the survey period from 3.2 to 11.7°C (min–max). At the visit level the temperature increased from 4.9 to 8.3°C on average. Salinity was relatively stable around 5 psu (median=5.7, IQR=5.1–5.9) but two bays situated in the innermost parts of the archipelago had lower salinity (SP – Släpan/Ekefjärd and MV –Myttingeviken, median=2.4 and 2.6 psu respectively). These two bays were also characterized by markedly higher fluorescence intensities in the 640–980 nm range which is a proxy for Chlorophyll A concentrations (median=13.8 and 5.9 ppb respectively relative the global median of 1.9 pbb).

3.3 | Spatiotemporal eDNA dynamics

The amount of variance associated with the different levels of the eDNA survey could not be partitioned into clear spatial and temporal dynamics with the original model (Figure 3a). However, using the simplified model with the combined effect of *Bay* and *Visit*, differences emerged (Figure 3b). Surprisingly, the variance within transects, that is, between the two filters from the same collection of water, seemed to have a rather high variance (22%, CI 14–33). The amount of variance explained at the local scale (within bays/visits, that is, across transects) was lower (21%, CI 11–34) than at the larger spatiotemporal scale between bays and visits (52%, CI 33–64) (Figure 3b). This indicates that while significant, small-scale spatial differences are of less importance compared with more large-scale and temporal processes during spring when pike spawn.

3.4 | Effects of fishing pressure on CPUE

The number of pike caught per rod-hour in the standardized angling was best explained by the negative effect of the number of other *anglers* present at the time of the survey (Table 1, model 4). It is worth noting that the negative effect of *cormorants* also appears important (Table 1). However, model 4 explained more of the fixed variance, had the lowest AICc and had a statistically significant predictor term ($p = 0.023$), which is why we chose this model to standardize the catches. The standardization model was used for the comparison with eDNA by predicting the catch (standardized pike abundance) in the absence of other anglers at an effort of 16 rod hours for each bay and visit.

3.5 | eDNA-abundance/biomass relationship

The forward selection process revealed that the eDNA concentration in the bays was primarily explained by temperature, followed by bay size (Table 2). Adding an interaction between temperature and bay size did not improve model fit, nor did adding either CPUE or ASM as main effects. However, the best fitting model included bay size and the interaction between temperature and CPUE (Model 18, Tables 2 and 3). Temperature and CPUE showed a significant and positive log-linear relationship with DNA concentration among bays and visits, which together with the negative effect of bay size explained 48% of the variance (Figure 4, Table 3).

4 | DISCUSSION

As eDNA-abundance relationships are being established for many different species in a wide range of habitats, evidence is accumulating in favor of eDNA analysis as a quantitative tool for monitoring fish populations. This suggests that the methodology bears potential for resource management and conservation purposes. However, the strength of these relationships has been variable, ranging from basically no relationship (Knudsen et al., 2019; Rourke, Walburn, et al., 2022) to rather high levels of correlation >80% (Salter et al., 2019; Spear et al., 2021; Yates, Glaser, et al., 2021). Reasons for this high level of heterogeneity are still not well understood but could be attributed to species-specific behavioral differences (Rourke, Walburn, et al., 2022) and responses to environmental factors (e.g., Curtis et al., 2021; Jo et al., 2019; Lacoursière-Roussel et al., 2016) as shown here. Therefore, it is important to evaluate eDNA-abundance relationships at the species level (Jane et al., 2015; Lance & Guan, 2020).

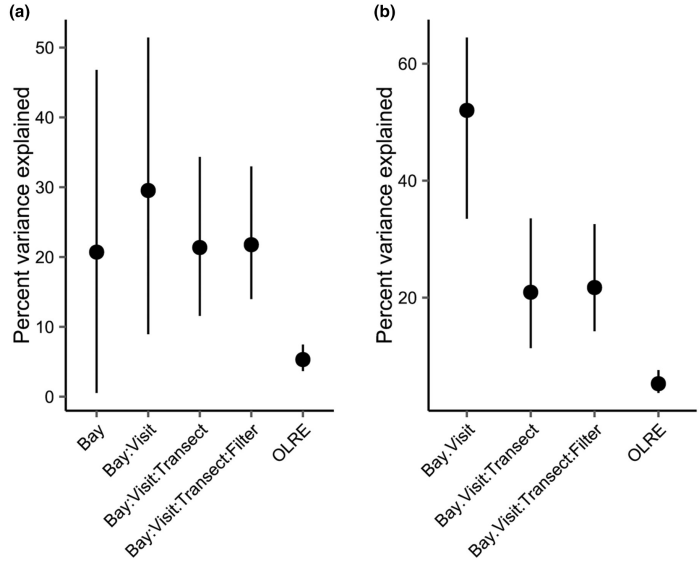

FIGURE 3 Intra-class correlation coefficients for the random effects in the eDNA-model showing the percentage of variance explained in the model at different hierarchical levels from the full model (a) or a simplified version where the effects of Bay and Visit are pooled to one random effect (b).

TABLE 1 Model selection for the CPUE-model.

Model	Fixed effect	Random effect	AICc	dAICc	Marginal R^2	Conditional R^2	Estimate	SE	*p*
1	Intercept	(1|Bay/Visit)	340.2	2.7	0.00	1.00	−0.99	0.24	**<0.001**
2	Temperature	(1|Bay/Visit)	342.3	4.8	0.00	1.00	0.04	0.06	0.587
3	Cormorants	(1|Bay:Visit)	341.0	3.5	0.08	0.91	−0.02	0.01	0.058
4	Anglers	(1|Bay/Visit)	337.5	0.0	0.09	0.91	−0.09	0.01	**0.023**
5	Cormorants	(1|Bay/Visit)	338.3	0.8	0.14	0.85	−0.01	0.01	0.189
	Anglers						−0.07	0.04	0.071
6	Temperature	(1|Bay/Visit)	339.7	2.2	0.10	0.90	0.03	0.06	0.586
	Anglers						−0.09	0.04	**0.023**
7	Temperature	(1|Bay:Visit)	343.3	5.8	0.08	0.91	0.02	0.07	0.751
	Cormorants						−0.02	0.01	0.060
8	Temperature	(1|Bay/Visit)	340.6	3.1	0.15	0.85	0.03	0.06	0.6000
	Cormorants						−0.01	0.01	0.192
	Anglers						−0.07	0.04	0.070

Note: AICc is Akaike's Information Criterion corrected for sample size and dAICc is the difference in AICc between a model and the best model. Marginal and conditional R^2 show the proportion of variance explained by fixed factors only and total including random effects, respectively. Note that the conditional R^2 is inflated by the use of observation level random effects. The estimate with associated standard error and *p*-value are given for each fixed effect. Significant *p*-values are highlighted in bold. Catch-Per-Unit-Effort (number of pikes caught per rod-hour) was used as the response variable in the models. The models assume a Poisson distribution and use a log-link function. The natural log of fishing effort was used as an offset term.

By comparing eDNA concentrations to standardized abundance metrics complemented by abiotic data, we add to the growing line of evidence that eDNA can reflect the densities of wild fish populations and be a useful tool for monitoring. Specifically, our results show that eDNA analysis can be applied to species that are generally undersampled by standard monitoring gear like gill nets. Our results also show that this method can be used in semi-open brackish water habitats. However, the positive relationship between eDNA concentrations and the standardized pike abundance was not straightforward and we identified a number of confounding factors that will need to be taken into consideration as the eDNA methodology develops.

4.1 | Spatiotemporal variation in eDNA concentrations

Using a high level of spatial replication within and across 22 bays, we were able to assess the spatial variability of eDNA in a semi-enclosed coastal system. We found considerable variability across bay visits but lower variation within bay visits. Within specific bays, the variability in eDNA concentrations across transects was without typical patterns (Figures S1 and S5). We initially predicted that the central transect (D), which was situated in the deeper part of the bay, would consistently show lower DNA concentrations because it normally would fall outside the preferred vegetated habitat of spawning pike (Frost & Kipling, 1967; Pursiainen et al., 2021). This was however not the case and we found no such discernible patterns.

The spatial distribution of eDNA has been shown to be patchy and vary seasonally in both marine and freshwater environments (Hervé et al., 2022; Littlefair et al., 2021). However, the degree to which concentrations vary mainly depends on the spatial distribution of the target species but also hydrographic and environmental conditions. For example, in larger lakes and marine systems, it is common to find eDNA to be vertically stratified by thermoclines that form during periods of limited vertical mixing, effectively concentrating eDNA released from cold water species below the thermocline (Hervé et al., 2022, Littlefair et al., 2021). During our survey, the bays were thoroughly mixed which likely smoothed out any spatial differences (Table S1). This lack of patchiness was also consistent over the two bay visits, albeit the average concentrations tended to be somewhat higher at the second visit as water temperatures rose (Figures S1, S5, and S6). Moreover, our integrative approach of pooling water samples along the transects likely also contributed to decrease spatial patterns.

At smaller spatial scales, caging experiments have shown a rather limited detection distance in the range of 30–50 m in lakes (Dunker et al., 2016) and coastal waters (Murakami et al., 2019). In our case, we subsampled the transects with 50 m intervals and pooled the water at the end of each transect. In doing so, we averaged out some level of variation making transects more similar to each other. Nevertheless, it should also be noted that the level of variation between filter replicates was of the same magnitude as across transects. This could partly be explained by low sample sizes and a statistical difficulty in partitioning the variance components but it could also be an effect of low DNA copy numbers and stochasticity which would warrant a higher level of in-field replication and filtration of larger volumes of water. Although the filtration of large water quantities may be cumbersome, it may be performed using larger filter pore sizes. Such filters have a higher probability of capturing longer multi-copy nuclear

TABLE 2 Selection of models explaining eDNA concentrations.

Model index	Independent variables	*K*	AICc	dAICc	R^2
1	ASM × CPUE	5	243.9	28.8	0.01
2	ASM	3	240.9	25.9	0.00
3	CPUE	3	239.6	24.6	0.00
4	Bay size	3	231.4	16.3	0.09
5	Temp.	3	**223.6**	8.5	0.10
6	Temp. + ASM × CPUE	6	226.6	11.5	0.16
7	Temp. + ASM	4	225.1	10.0	0.05
8	Temp. + CPUE	4	221.8	6.7	0.13
9	Temp. + Bay size	4	**219.8**	4.8	0.23
10	Temp. × Bay size	5	220.7	5.7	0.31
11	Temp. + Bay size + ASM × CPUE	7	225.1	10.0	0.28
12	Temp. + Bay size + ASM	5	222.2	7.1	0.19
13	Temp. + Bay size + CPUE	5	**219.7**	4.6	0.22
14	Temp. + Bay size + ASM + CPUE	6	222.6	7.6	0.22
15	Temp. + Bay size × CPUE	6	221.1	6.1	0.24
16	Temp. + Bay size × ASM	6	216.2	1.2	0.49
17	Temp. × ASM + Bay size	6	225.1	10.1	0.15
18	Temp. × CPUE + Bay size	6	**215.0**	**0.0**	0.48
19	Temp. × Bay size + CPUE	6	219.0	3.9	0.40
20	Temp. × Bay size + ASM	6	223.4	8.3	0.22

Note: The table is divided by a forward selection process. ASM is the allometrically scaled mean size in the population, CPUE is the standardized pike abundance, Temp. is water temperature (°C) and K is the number of parameters in the model. AICc is the Akaike Information Criterion corrected for sample size and dAICc is the difference in AICc between a model and the best model. R^2 shows the proportion of variance explained by the model. Bold AICc indicates the best candidate model at each forward selection step. The models assume a negative binomial distribution and use a log-link function.

TABLE 3 Model summary for the best-performing model predicting eDNA concentrations (Model 18, Table 2).

Coefficients	Estimate	SE	*z*	*p*-Value	R^2
Intercept	2.65	0.98	2.702	0.007	0.48
Temperature	0.02	0.11	0.189	0.850	
CPUE	−0.13	0.05	−2.446	0.014	
Bay size	−0.04	0.01	−2.983	0.003	
Temperature × CPUE	0.02	0.01	2.845	0.004	

Note: The model assumes a negative binomial distribution and uses a log-link function.

eDNA fragments (Jo et al., 2020), which due to their higher degradation rates compared with shorter mitochondrial DNA, better reflect instantaneous fish densities (Jo et al., 2022) and could possibly have improved the precision of our measurements.

4.2 | eDNA-abundance relationship

4.2.1 | Temperature drives eDNA dynamics

Although we found a positive relationship between CPUE and eDNA concentrations, we found an even stronger influence of temperature. Moreover, the effect of CPUE was only evident at higher temperatures suggesting either that (i) pike abundance in the bays increased over the survey period and that there was an interaction with catchability (increased fish density but unchanged CPUE), (ii) eDNA shedding rates increased with temperature (becoming detectable and fully quantifiable above a threshold temperature), and/or (iii) that spawning, which increased with temperature (Figure S6), had an additive effect.

We cannot rule out that pike abundance in the bays increased as the bays became warmer (i above) but temperature had no significant effect on CPUE (Table 1), suggesting that the abundance of pike was relatively stable over the survey period. Moreover, catches were sometimes substantial already at temperatures as low as 3–4°C (Figure 5) indicating that arrival to the spawning grounds

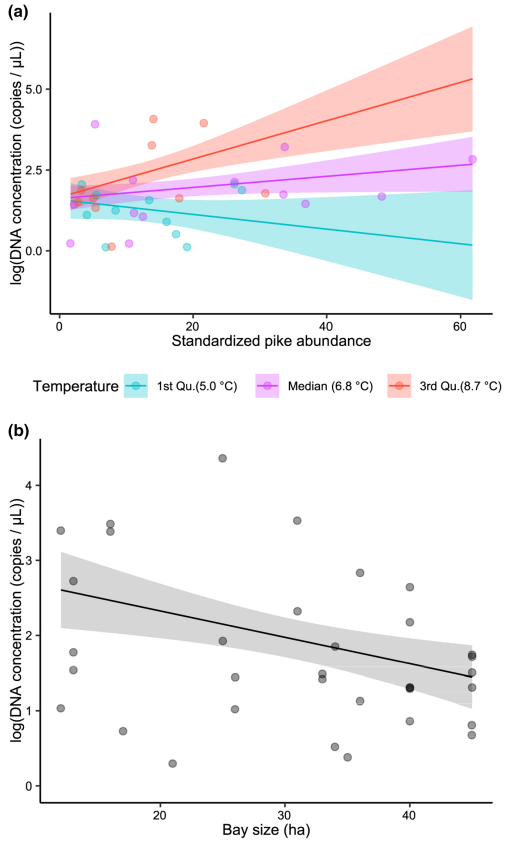

FIGURE 4 Model estimated DNA concentration (log-scale copies per µL) as a function of (a) standardized pike abundance, at different temperatures, and (b) bay size (hectares). Points are partial residuals. R^2 for the model was 0.48.

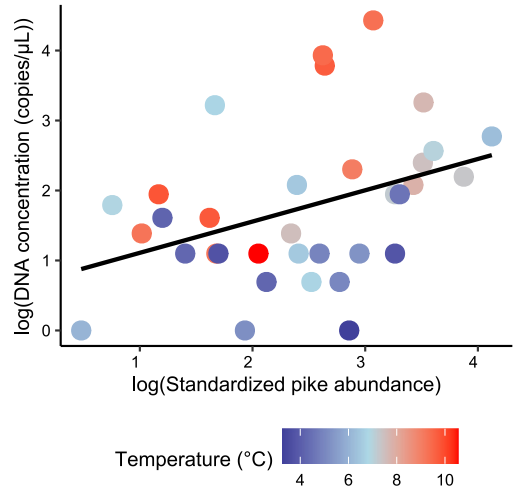

FIGURE 5 Raw data plot (log–log scale) of the relationship between DNA concentration and standardized pike abundance (CPUE) colored by water temperature.

happens well before temperatures have reached optimal spawning conditions which normally fall between 6 and 8°C (Clark, 1950; Frost & Kipling, 1967). This is also supported by observations from other fresh and brackish water systems where arrival to the spawning grounds can precede the actual spawning event by several weeks or even months (Flink et al., 2023; Raat, 1988). Therefore, direct effects of temperature on eDNA concentrations (ii and iii above) are more probable.

In line with our field observations, a laboratory study on brook trout has also shown a temperature-mediated effect, resulting in a stronger eDNA-abundance/biomass relationship at higher temperatures (Lacoursière-Roussel et al., 2016). The authors suggested that the temperature–biomass interaction was driven by increased activity levels and metabolism. This is indeed very likely since metabolism and in extension DNA shedding rates are dependent on temperature (Bean, 2010; Jo et al., 2019; Kitchell et al., 1977). Additional changes also take place as temperature rises, not the least an increase in the proportion of spawning fish (Figure S6). As the fish spawn, their activity and physical interactions increase (Lucas, 1992). Simultaneously, the spawning event itself leads to the release of sperm which becomes readily incorporated in the eDNA pool (Holmes et al., 2022; Tillotson et al., 2018; Tsuji & Shibata, 2021). The contribution of sperm could potentially be estimated by comparing ratios of nuclear and mitochondrial eDNA (Bylemans et al., 2017). Such comparisons rely on robust assays for both mitochondrial and nuclear DNA, but the latter is currently lacking for pike. In summary, we believe that temperature, especially during early spring in temperate regions, is a key driver affecting physiological processes, such as metabolism and shedding rates, as well as behavior and spawning activity–all of which have a strong influence on eDNA concentrations.

4.2.2 | CPUE based on angling likely underestimates true abundance

Even though we did our best to estimate the "true" pike abundance by modeling the effect of other anglers and deriving a standardized pike abundance, we found a relatively weak relationship between CPUE and eDNA concentrations. This could be a result of using angling data instead of census data from, for example, mark-recapture experiments (Spear et al., 2021). It is well known that angling success can vary due to local environmental conditions, and for species that are the target of catch-and-release practices like pike, also previous fishing intensity (Arlinghaus et al., 2017; Chen & Zeng, 2022; Kuparinen et al., 2010). Even though we corrected for the number of anglers present during the rod-fishing,

fishing pressure the days before remains unknown, which could also influence catchability. Similarly, poor predictive capability of angler-based abundance was obtained in a study investigating the eDNA–abundance relationships for brook charr (*Salvelinus fontinalis*, Mitchill 1814) in a series of Canadian lakes (Gaudet-Boulay et al., 2022). In that study, the CPUE of brook charr estimated from angling data predicted eDNA concentration in the lakes poorly, but the explanatory power of the model increased once the surface area of the lakes was accounted for, indicating that fish density measured per unit area is a better predictor (marginal R^2 in models with only fish density as a predictor varied from 0.1 to 0.44). That observation is in accordance with our study where bay size as a covariate had a strong negative effect on eDNA concentrations (Table 2). Accounting for the size of the study area makes sense assuming that fish are heterogeneously distributed and concentrated to certain habitats. In the case of pike, it is very likely that most fish were aggregated close to the vegetated shore where spawning usually takes place (Clark, 1950; Lucas, 1992). Since the proportion of preferred habitat scales disproportionately with the square of bay area, and given that the eDNA is thoroughly mixed within the bay, this results in a dilution effect. Similar patterns of DNA dilution have been observed in rivers with elevated water discharges (Pont et al., 2023).

4.2.3 | Abundance or biomass as eDNA predictors?

Apart from using standardized abundance as a predictor of eDNA concentration, we also tested to include allometrically scaled biomass (Yates et al., 2022; Yates, Wilcox, et al., 2021) by calculating the allometrically scaled mean population weight and using this as a covariate in our modeling. Several authors have recently shown improved relationships between fish biomass and eDNA concentrations when accounting for the size distribution of the fish community. Spear et al. (2021) saw an improvement in model R^2 from 0.62 to 0.81 when the mean size of walleye (*Sander vitreus*, Mitchill 1818) was used as a covariate together with the estimated population biomass, while Yates, Glaser, et al. (2021) saw an improvement in model R^2 from 0.59 (fish/ha) and 0.63 (kg/ha) to 0.78 when accounting for allometric scaling in a study on brook charr. Although the evidence for allometric effects on eDNA production seem to be generalizable across species (Yates et al., 2022), we did not find a significant effect of including ASM in our pike models. The reason for this lack of effect is not clear but could potentially be attributed to a relatively homogeneous size distribution across bays. Indeed, the average pike weight per bay and visit in our study only differed by approximately a factor of three, while in the study by Yates, Glaser, et al. (2021), the difference was substantially larger across lakes (factor ten difference). Furthermore, it is likely that the angling approach underestimated the abundance of smaller individuals which likely were not captured as efficiently by the anglers ($\bar{x} = 60$ cm, SD = 10 cm). Such a size selectivity would effectively inflate the average size of the population, decrease the variance, and hence also influence allometry. Nevertheless, it is probable that allometric effects may be of greater importance during other seasons when local size distributions are more variable (Neumann & Willis, 1995). Another potential cause for the lack of allometric effect in our study could also be attributed to a temperature-dependent effect on the actual scaling coefficient itself. Temperature-dependent effects on metabolic scaling coefficients have been shown to vary greatly among teleost species (Glazier, 2005) and seem, to a high extent be related to temperature (Killen et al., 2010; Lindmark et al., 2018; Ohlberger et al., 2012). Assuming that metabolism is closely linked to eDNA shedding rates (Thalinger et al., 2021), it is plausible that temperature also can affect the allometric relationship between body mass and eDNA shedding rates. However, to the best of our knowledge, there are no studies to date that have tested the influence of temperature on allometric relationships in an eDNA context, which is an avenue worth exploring.

4.3 | Conclusions

Our study supports the growing body of evidence showing a positive relationship between fish abundance/biomass and eDNA concentrations in the wild. Including abiotic data, we were able to explain nearly 50% of the variance in eDNA concentrations. This is in line with similar studies performed on other species and in different ecosystems (Yates et al., 2019). With the additional support from established eDNA-biomass relationships under more controlled conditions (Karlsson et al., 2022), it is likely that eDNA could be used to infer relative abundance data in wild pike populations. However, we also found temperature to be important, likely acting as a driver of fish activity and spawning that has a strong effect on eDNA concentrations. Temperatures that change rapidly, especially in temperate regions, will therefore induce unwanted variance, which may be difficult to account for. Hence, choosing appropriate sampling times will be crucial in order to make longitudinal data comparable. We therefore recommend that quantitative eDNA-surveys targeting species that converge for spawning should be performed at temperatures when spawning has peaked but fish maintain a high probability to stay aggregated in close proximity to their spawning areas.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the study, EK, MO, GS, JS, and PB collected the data, MO and GS conducted the statistical analyses and created the figures, MO and GS drafted the manuscript, with contributions from all co-authors. All authors approved the final submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data and script for this study are available at Figshare, https://doi.org/10.6084/m9.figshare.21781622 following best practices (Roche et al., 2015), and was made available to editors and reviewers upon initial submission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Figures

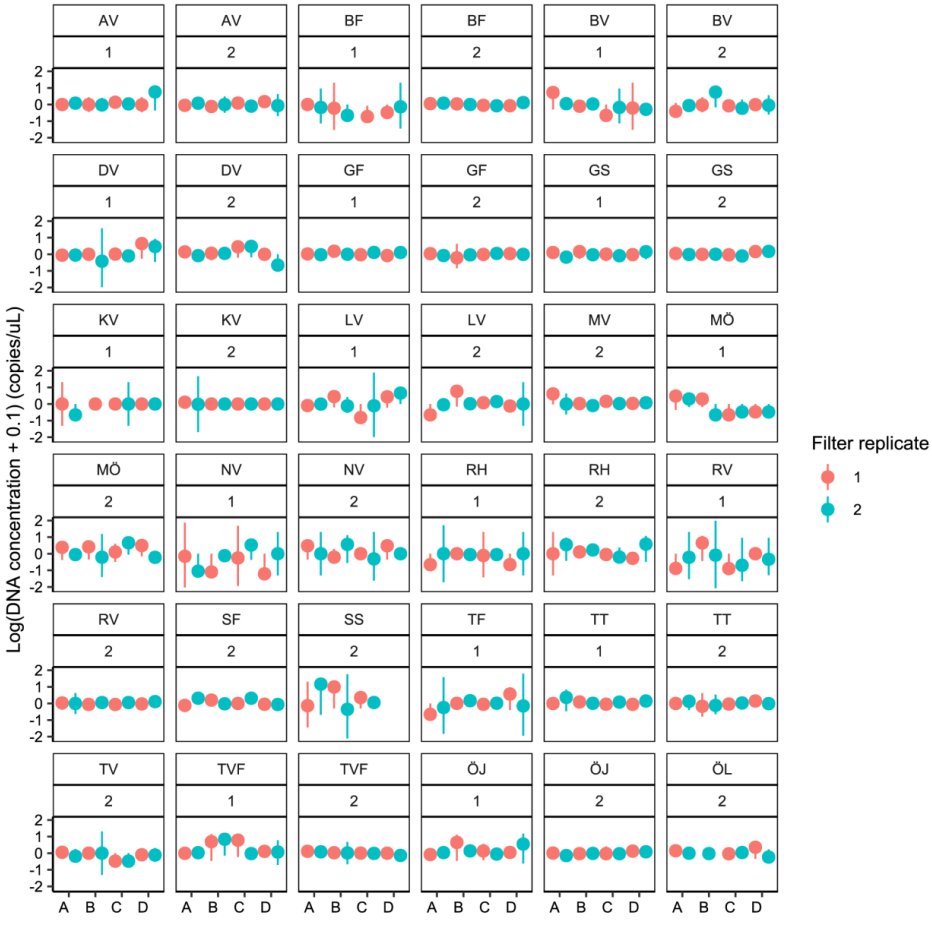


Figure S 1. Filter plot showing Log transformed eDNA concentrations (copies μL^{-1}) per bay, visit (1 and 2), transect (A-D) and filter replicate (1 and 2). Each filter consists of four technical qPCR replicates. Bay ID: AV = Askviken, BF = Björnöfjärden/Torpe Infjärd, BV = Byviken/Ryssundet, DV = Dalviken, GF = Gisslingöfladen, GS = Granösundet, KV = Kyrkviken/Utö, LV = Lännåkersviken, MV = Myttingeviken, MÖ = Mulö/Lögla, NV = Nynäsviken, RH = Rotholmaviken, RV = Rassa vikar, SF = Söderöfjärden/Sladdarön, SP = Släpan/Ekefjärd, SS = Södersundet, TF = Tofladen/Gropaviken, TT = Tomtviken/Urö, TV = Tranvik/Djuröviken, TVF = Tranviksfjärden, ÖJ = Öjaren/Söderöra/Norröra, ÖL = Östra Lemaren.

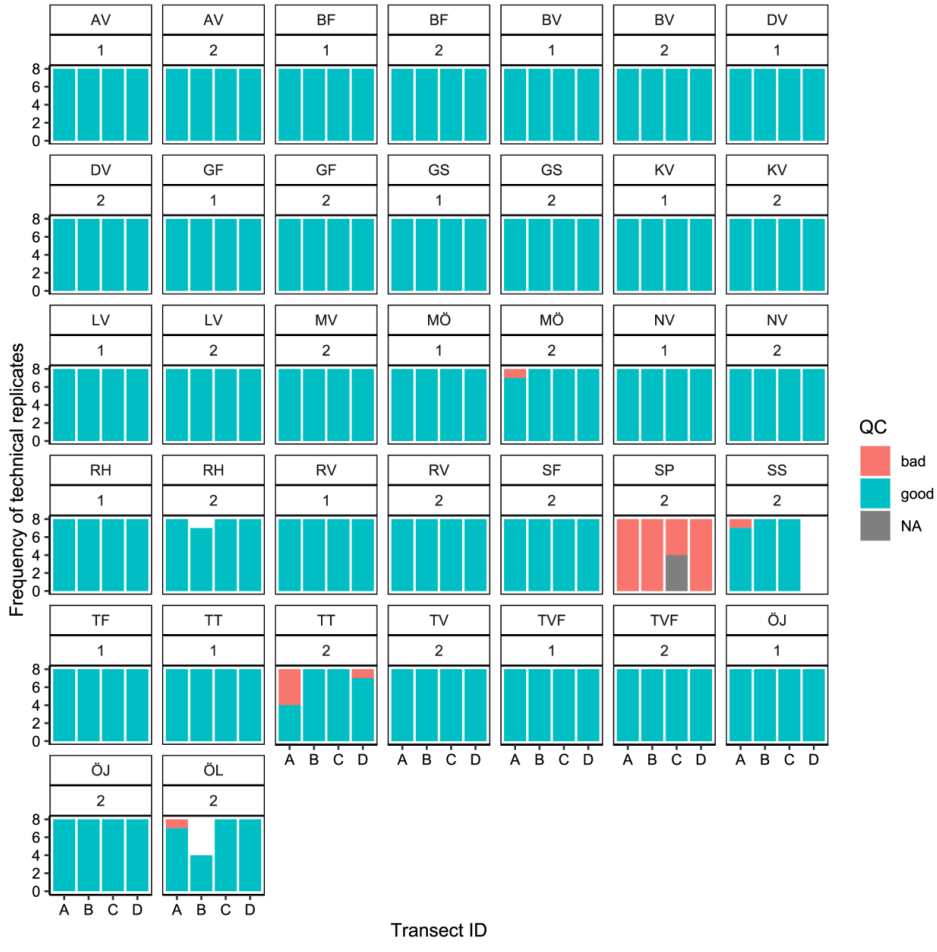
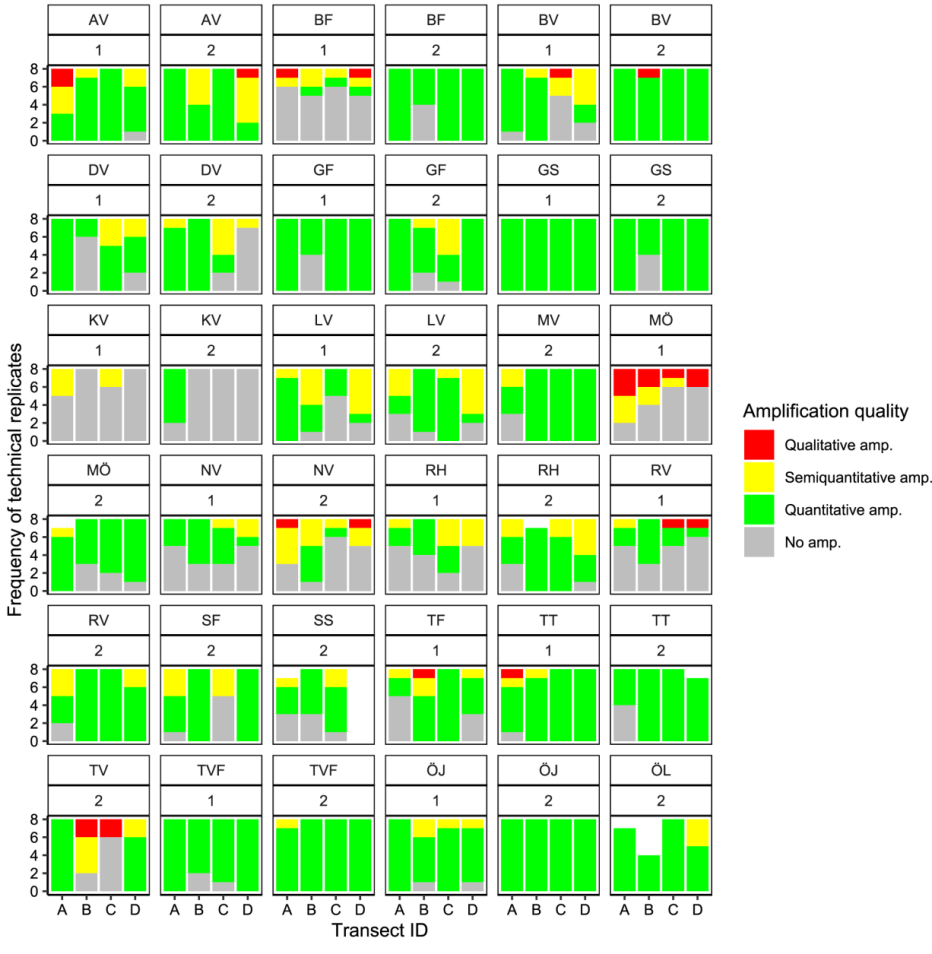


Figure S 2. Barplot showing the frequency of technical qPCR replicates flagged for inhibition by having Cq values > 28.5 for the internal positive control. Data is divided per bay, visit and transect. Bay ID: AV = Askviken, BF = Björnöfjärden/Torpe Infjärd, BV = Byviken/Ryssundet, DV = Dalviken, GF = Gisslingöfladen, GS = Granösundet, KV = Kyrkviken/Utö, LV = Lännåkersviken, MV = Myttingeviken, MÖ = Mulö/Lögla, NV = Nynäsviken, RH = Rotholmaviken, RV = Rassa vikar, SF = Söderöfjärden/Sladdarön, SP = Släpan/Ekefjärd, SS = Södersundet, TF = Tofladen/Gropaviken, TT = Tomtviken/Urö, TV = Tranvik/Djuröviken, TVF = Tranviksfjärden, ÖJ = Öjaren/Söderöra/Norröra, ÖL = Östra Lemaren



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19 *Figure S 3. Barplot showing the frequency of technical qPCR replicates classified as either quantitative (green*
20 *bars, values above the limit of quantification –LOQ), semi-quantitative (yellow bars, between LOQ and LOD –*
21 *the limit of detection), qualitative (red bars, below LOD), and non-detects (grey bars, No. amp). Data is divided*
22 *per bay, visit and transect. Bay ID: AV = Askviken, BF = Björnöfjärden/Torpe Infjärd, BV = Byviken/Ryssundet,*
23 *DV = Dalviken, GF = Gisslingöfladen, GS = Granösundet, KV = Kyrkviken/Utö, LV = Lännåkersviken, MV =*
24 *Myttingeviken, MÖ = Mulö/Lögla, NV = Nynäsviken, RH = Rotholmaviken, RV = Rassa vikar, SF =*
25 *Söderöfjärden/Sladdarön, SP = Släpan/Ekefjärd, SS = Södersundet, TF = Tofladen/Gropaviken, TT =*
26 *Tomtviken/Urö, TV = Tranvik/Djuröviken, TVF = Tranviksfjärden, ÖJ = Öjaren/Söderöra/Norröra, ÖL = Östra*
27 *Lemaren*

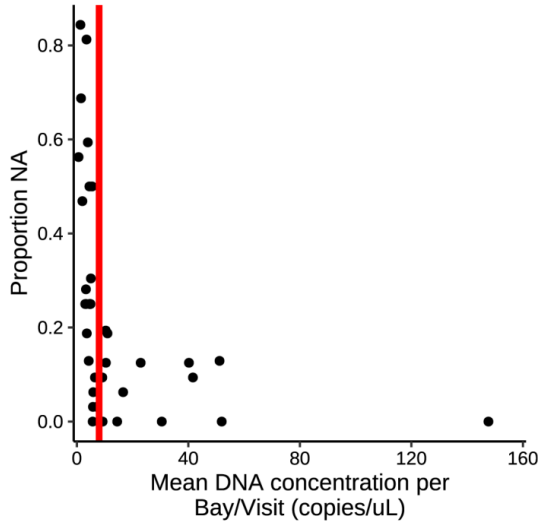

Figure S 4. Proportion of qPCR NA-values as a function of mean eDNA concentration per bay and visit. The vertical red line denotes a subjective threshold concentration for the delineation of true negatives. Non-detects below this line were assigned a values of zero while non-detects above the threshold were left unchanged.

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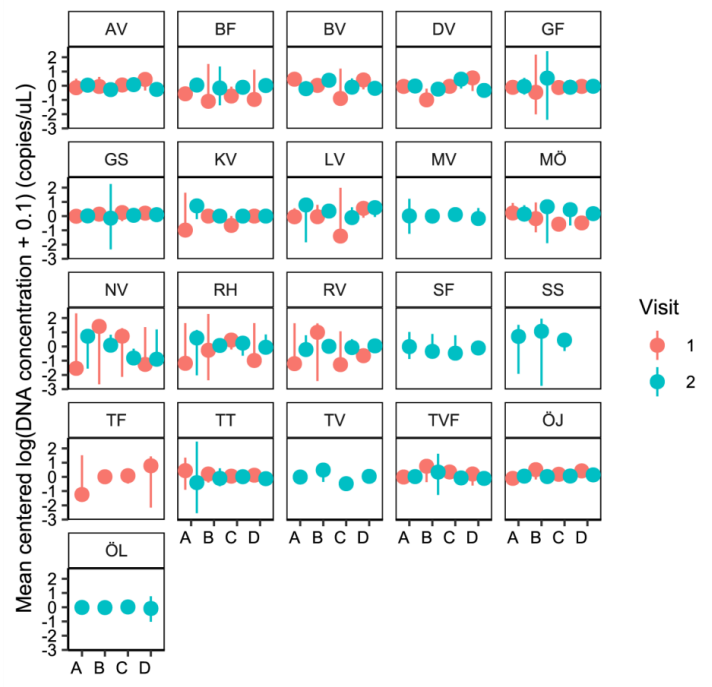

Figure S 5. Transect plot showing mean centered and log transformed eDNA concentrations (copies μL^{-1}) per bay, transect (A-D) and visit (1 and 2). Each visit consists of two filter replicates where each filter consists of four technical qPCR replicates. Bay ID: AV = Askviken, BF = Björnöfjärden/Torpe Infjärd, BV = Byviken/Ryssundet, DV = Dalviken, GF = Gisslingöfladen, GS = Granösundet, KV = Kyrkviken/Utö, LV = Lännåkersviken, MV = Myttingeviken, MÖ = Mulö/Lögla, NV = Nynäsviken, RH = Rotholmaviken, RV = Rassa vikar, SF = Söderöfjärden/Sladdarön, SP = Släpan/Ekefjärd, SS = Södersundet, TF = Tofladen/Gropaviken, TT = Tomtviken/Urö, TV = Tranvik/Djuröviken, TVF = Tranviksfjärden, ÖJ = Öjaren/Söderöra/Norröra, ÖL = Östra Lemaren.

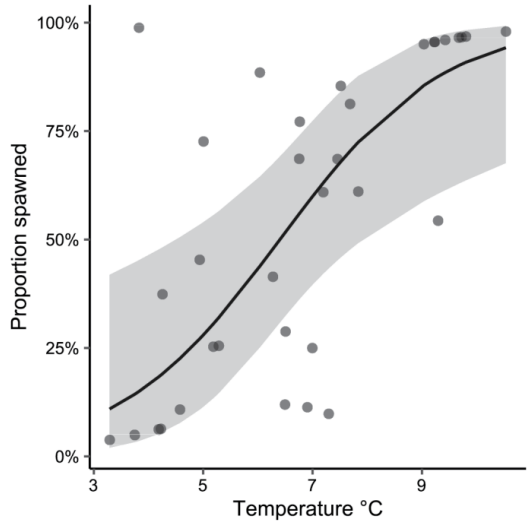

Figure S 6. Proportion of spawned pike per bay and visit as a function of temperature. The relationship was fitted using a GLM with a binomial distribution (estimate 0.67, se=0.24, z=2.76, p=0.0058) and plotted using ggpredict.

Tables

Table S 1. Summary statistics (mean ± (SD)) for measured abiotic variables per bay and visit. Bay ID: AV = Askviken, BF = Björnöfjärden/Torpe Infjärd, BV = Byviken/Ryssundet, DV = Dalviken, GF = Gisslingöfladen, GS = Granösundet, KV = Kyrkviken/Utö, LV = Lännåkersviken, MV = Myttingeviken, MÖ = Mulö/Lögla, NV = Nynäsviken, RH = Rotholmaviken, RV = Rassa vikar, SF = Söderöfjärden/Sladdarön, SP = Släpan/Ekefjärd, SS = Södersundet, TF = Tofladen/Gropaviken, TT = Tomtviken/Urö, TV = Tranvik/Djuröviken, TVF = Tranviksfjärden, ÖJ = Öjaren/Söderöra/Norröra, ÖL = Östra Lemaren.

Bay ID	Visit	Depth (m)	Salinity (PSU)	Oxygen (mg/L)	Turbidity (FTU)	Chlorophyll A (ppb)	Temperature (°C)
AV	1	1.5 (0.21)	6.3 (0.01)	13.9 (0.09)	2.9 (0.13)	1.9 (0.19)	4.2 (0.13)
AV	2	1.4 (0.06)	6.3 (0.08)	11.5 (0.26)	5.0 (1.48)	2.7 (0.21)	9.7 (0.51)
BF	1	1.0 (0.48)	5.0 (0.01)	14.9 (0.20)	1.1 (0.09)	4.0 (0.50)	3.3 (0.19)
BF	2	1.5 (0.78)	5.1 (0.02)	12.9 (0.14)	1.2 (0.24)	2.8 (0.32)	9.0 (0.51)
BV	1	0.8 (0.65)	5.9 (0.27)	14.0 (0.31)	2.1 (0.28)	2.8 (0.68)	4.3 (0.47)
BV	2	0.4 (0.41)	6.3 (0.19)	12.5 (0.44)	1.6 (0.28)	1.8 (0.48)	7.5 (0.92)
DV	1	1.8 (0.63)	5.2 (0.01)	15.0 (0.19)	0.9 (0.02)	1.9 (0.17)	3.8 (0.23)
DV	2	1.6 (0.46)	5.2 (0.00)	14.0 (0.18)	0.6 (0.02)	1.2 (0.08)	6.5 (0.39)
GF	1	0.7 (0.06)	5.5 (0.04)	13.1 (0.40)	1.5 (1.08)	4.1 (0.33)	6.3 (0.35)
GF	2	0.3 (0.21)	5.6 (0.06)	12.7 (0.61)	1.7 (0.44)	2.5 (0.47)	7.0 (0.86)
GS	1	0.5 (0.09)	5.9 (0.01)	13.8 (0.44)	1.2 (0.43)	1.0 (0.04)	7.3 (0.75)
GS	2	0.5 (0.40)	5.8 (0.01)	12.7 (0.15)	1.7 (1.35)	0.9 (0.03)	7.7 (0.43)
KV	1	1.0 (0.58)	6.7 (0.01)	15.1 (0.24)	0.8 (0.08)	1.0 (0.21)	3.9 (0.14)
KV	2	1.2 (0.48)	6.6 (0.03)	14.0 (0.45)	0.9 (0.02)	0.8 (0.07)	6.0 (0.25)
LV	1	1.2 (0.44)	6.1 (0.06)	13.8 (0.15)	6.0 (5.51)	2.8 (0.31)	4.9 (0.10)
LV	2	0.6 (0.29)	6.4 (0.01)	11.6 (0.08)	4.4 (2.02)	2.1 (0.09)	9.2 (0.07)
MV	2	1.7 (0.15)	2.6 (0.31)	15.6 (0.42)	1.5 (0.19)	5.9 (1.26)	8.6 (1.29)
MÖ	1	1.4 (0.41)	5.4 (0.00)	13.7 (0.11)	0.7 (0.15)	1.2 (0.04)	5.2 (0.12)
MÖ	2	1.2 (0.32)	5.5 (0.00)	11.3 (0.50)	3.8 (1.90)	1.6 (0.20)	9.8 (0.13)
NV	1	1.5 (0.36)	5.7 (0.07)	15.1 (0.21)	3.4 (0.24)	3.5 (0.57)	3.7 (0.04)
NV	2	1.5 (0.16)	5.8 (0.09)	13.6 (0.08)	2.1 (0.29)	2.3 (0.17)	6.8 (0.06)
RH	1	1.0 (0.19)	5.1 (0.00)	14.3 (0.14)	1.5 (0.46)	1.8 (0.07)	5.0 (0.37)
RH	2	1.1 (0.23)	5.1 (0.00)	12.7 (0.14)	2.1 (0.46)	1.4 (0.06)	9.2 (0.06)
RV	1	1.4 (0.04)	6.3 (0.02)	13.5 (0.11)	1.1 (0.10)	1.7 (0.25)	4.2 (0.04)
RV	2	1.7 (0.09)	6.5 (0.01)	12.8 (0.15)	0.8 (0.05)	1.2 (0.21)	7.2 (0.07)
SF	2	1.0 (0.21)	5.4 (0.03)	13.1 (0.29)	1.3 (0.19)	1.2 (0.11)	7.8 (0.60)
SP	2	0.9 (0.23)	2.4 (0.17)	14.8 (0.76)	5.2 (0.87)	13.9 (3.43)	10.4 (0.55)
SS	2	0.9 (0.13)	5.8 (0.00)	13.7 (0.15)	0.7 (0.04)	1.1 (0.03)	7.5 (0.59)
TF	1	1.0 (0.36)	5.7 (0.02)	14.1 (0.52)	0.9 (0.32)	1.8 (0.24)	5.3 (0.66)
TT	1	0.9 (0.17)	4.6 (0.08)	13.9 (0.32)	2.8 (0.39)	2.9 (0.13)	6.5 (0.30)

Table S 2 continued.

Bay ID	Visit	Depth (m)	Salinity (PSU)	Oxygen (mg/L)	Turbidity (FTU)	Chlorophyll A (ppb)	Temperature (°C)
TT	2	1.3 (0.26)	5.0 (0.03)	12.6 (0.14)	2.1 (0.11)	2.6 (0.23)	9.7 (0.21)
TV	2	1.0 (0.34)	4.3 (0.01)	12.6 (0.28)	2.0 (0.84)	2.8 (0.26)	10.5 (0.33)
TVF	1	0.4 (0.08)	5.2 (0.04)	14.3 (0.09)	1.1 (0.33)	1.9 (0.08)	4.6 (0.09)
TVF	2	0.6 (0.19)	5.4 (0.00)	13.2 (0.21)	3.7 (0.82)	2.3 (0.31)	9.3 (0.11)
ÖJ	1	0.6 (0.12)	5.9 (0.01)	13.6 (0.27)	0.6 (0.09)	0.9 (0.02)	6.9 (0.05)
ÖJ	2	0.5 (0.06)	5.8 (0.01)	13.0 (0.13)	0.7 (0.09)	0.9 (0.05)	6.8 (0.08)
ÖL	2	0.7 (0.10)	5.8 (0.02)	13.3 (0.56)	1.5 (0.51)	1.3 (0.17)	9.4 (0.15)

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2024:85

Environmental DNA (eDNA) is a promising tool for species detection and biodiversity assessment, offering potential for quantitative estimates. This thesis investigates the utility of eDNA in providing quantitative monitoring of northern pike (*Esox lucius*), a key predator in freshwater ecosystems currently lacking suitable monitoring methods. The thesis demonstrates strong positive relationships between eDNA concentrations and pike abundance and biomass under controlled and natural conditions. Furthermore, a new sensitive nuclear assay was developed for pike, potentially enhancing eDNA detection probability.

Erik Karlsson received his undergraduate and postgraduate education at Stockholm University.

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