

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

Erik Karlsson<sup>1</sup>  | Martin Ogonowski<sup>1</sup> | Göran Sundblad<sup>1</sup> | Josefin Sundin<sup>1</sup> | Ofir Svensson<sup>1</sup> | Ilkka Nousiainen<sup>2</sup> | Anti Vasemägi<sup>1,2</sup>

<sup>1</sup>Department of Aquatic Resources, Swedish University of Agricultural Sciences, Drottningholm, Sweden

<sup>2</sup>Department of Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

## Correspondence

Erik Karlsson, Department of Aquatic Resources, Swedish University of Agricultural Sciences, Drottningholm 17893, Sweden.  
Email: [erik.karlsson@slu.se](mailto:erik.karlsson@slu.se)

## Funding information

Naturvårdsverket, Grant/Award Number: NV-03728-17

## 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

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Environmental DNA* published by John Wiley & Sons Ltd.

## 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).

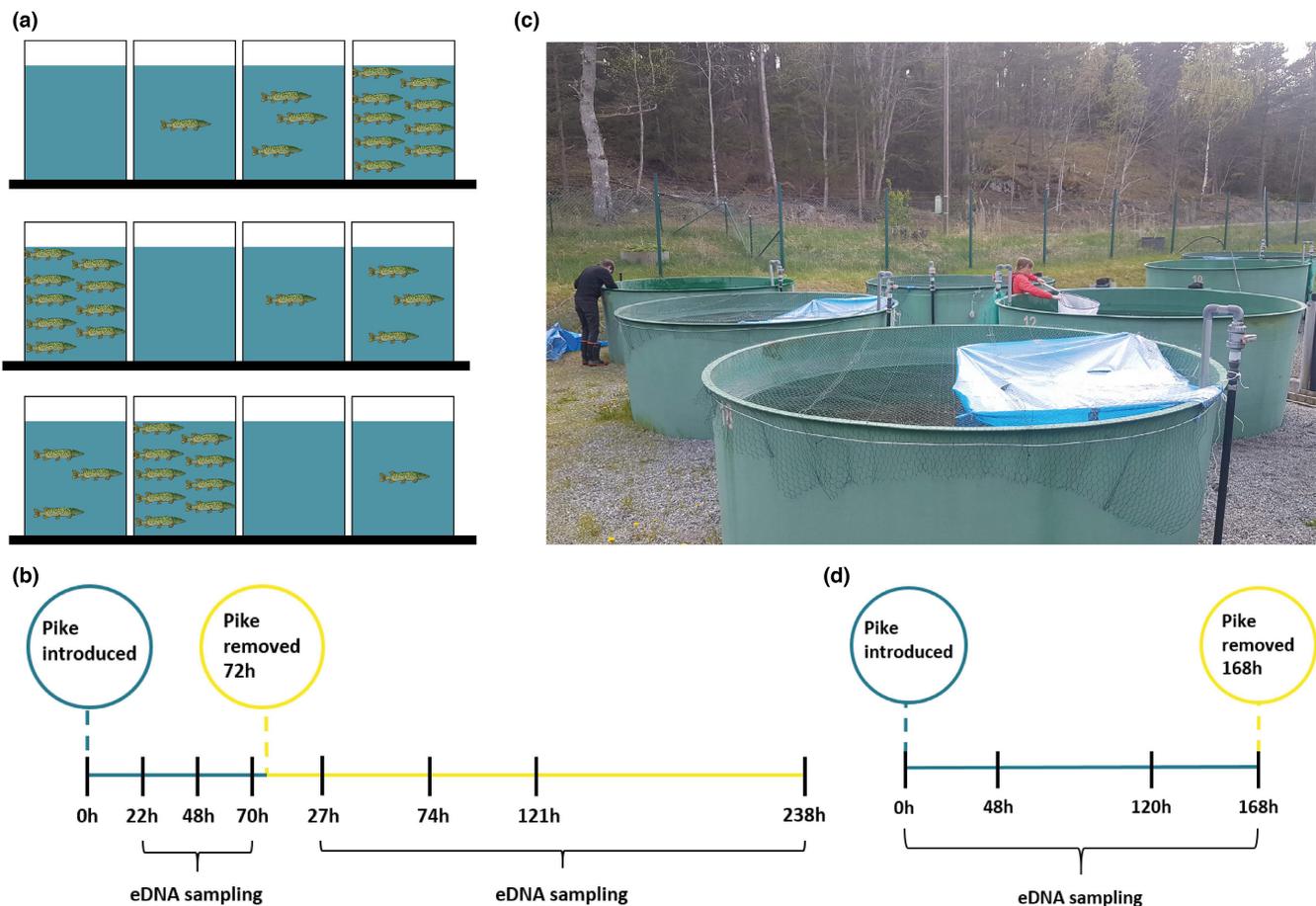
### 2.1.2 | Experimental design

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  $\mu$ l). 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  $-20^{\circ}\text{C}$  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'-GTACCAGCACAGCTTCAACAC-3' and probe: 5'-FAM-CTTCTGACTTCTCCCC-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  $\mu$ l reactions volumes. Reaction concentrations of the forward primer, reverse primer and probe were 200 nM each with 1 $\times$  HOT FIREPol Probe Universal qPCR Mix (Solis Biotyne) in each well loaded with 4  $\mu$ l of the sample template. The following qPCR program was used for all the reactions: 2 min at  $60^{\circ}\text{C}$  and 10 min at  $95^{\circ}\text{C}$  followed by 40 cycles of 15 s at  $95^{\circ}\text{C}$  and 60 s at  $60^{\circ}\text{C}$ .

eDNA quantification was achieved using a standard curve consisting of an 8-step, 10-fold dilution series of pike DNA (0.01 – 100 000  $\text{pg } \mu\text{l}^{-1}$ ). 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 =  $1.2 \pm 0.27$  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  $\mu$ m) or a combination of a cellulose nitrate filter (pore size of 0.8  $\mu$ m) with a glass microfiber filter (GFFA, pore size of approximately 1.6  $\mu$ m). 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  $-20^{\circ}\text{C}$  using a portable freezer and then stored at  $-80^{\circ}\text{C}$  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  $\mu$ l, 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 $\times$  of TaqMan Environmental Master Mix 2.0 to counteract potential inhibitory substances in the samples. The total reaction volume was 15  $\mu$ l with 4  $\mu$ l of DNA template. The standard protocol for the master mix was used for all the reactions: 10 min at  $95^{\circ}\text{C}$  followed by 40 cycles of 15 s at  $95^{\circ}\text{C}$  and 60 s at  $60^{\circ}\text{C}$ .

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  $\mu\text{l}^{-1}$ ). 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  $\text{L}^{-1}$ , 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  $\mu\text{m}$ ) and a glass microfiber filter (GFFA, approximately 1.6  $\mu\text{m}$ , 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  $-20^\circ\text{C}$  after filtration using a portable freezer and then stored at  $-80^\circ\text{C}$  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  $\mu\text{l}^{-1}$ ). 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  $\mu\text{l}^{-1}$  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  $\mu\text{l}^{-1}$ . Analysis in quadruplicates gave an effective LOD of 0.00325 pg  $\mu\text{l}^{-1}$ . 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

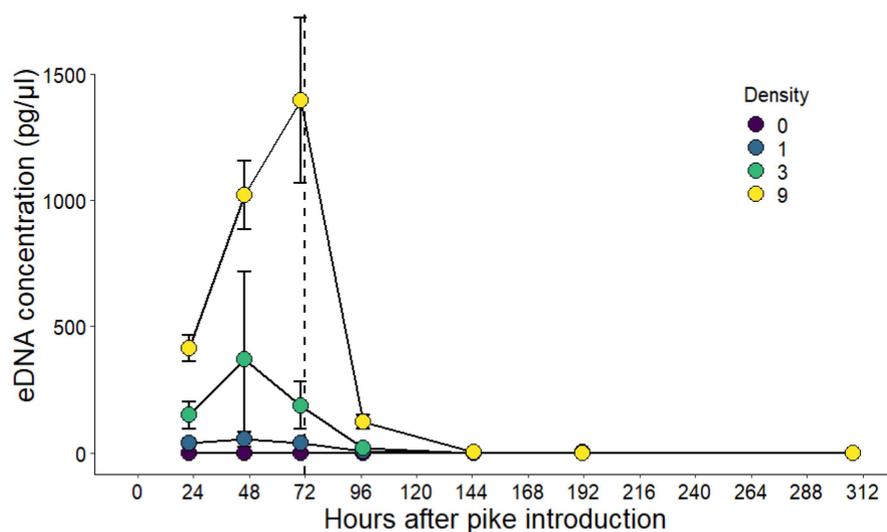
stipulated by the Swedish Ministry of Agriculture and the ethical permit was approved by the Stockholm ethical committee (DNR 99-19).

## 3 | RESULTS

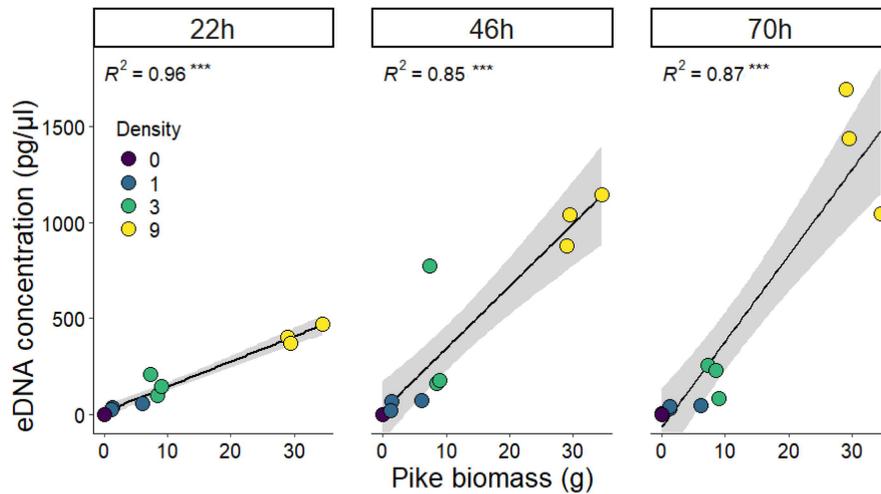
### 3.1 | Aquarium experiment using juvenile pike

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  $\mu\text{l}^{-1}$  to 35.8 pg  $\mu\text{l}^{-1}$ . 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  $\mu\text{l}^{-1}$ ), 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  $\mu\text{l}^{-1}$ ) 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  $\pm 1$  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 ( $\text{pg } \mu\text{l}^{-1}$ ) 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.  $R^2$  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 ( $\text{pg } \mu\text{l}^{-1}$ ) 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)	<i>p</i>
(a) Aquarium experiment	Biomass (g)	70247501	11.56 (1, 30)	<b>0.002</b>
	Sampling occasion	6318454	0.52 (2, 30)	0.600
	Biomass (g): Sampling occasion	173059537	14.24 (2, 30)	<b>&lt;0.001</b>
(b) Mesocosm experiment	Biomass (g)	125151	7.80 (1, 30)	<b>0.009</b>
	Sampling occasion	35073	1.09 (2, 30)	0.348
	Biomass (g): Sampling occasion	256886	8.01 (2, 30)	<b>0.002</b>

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

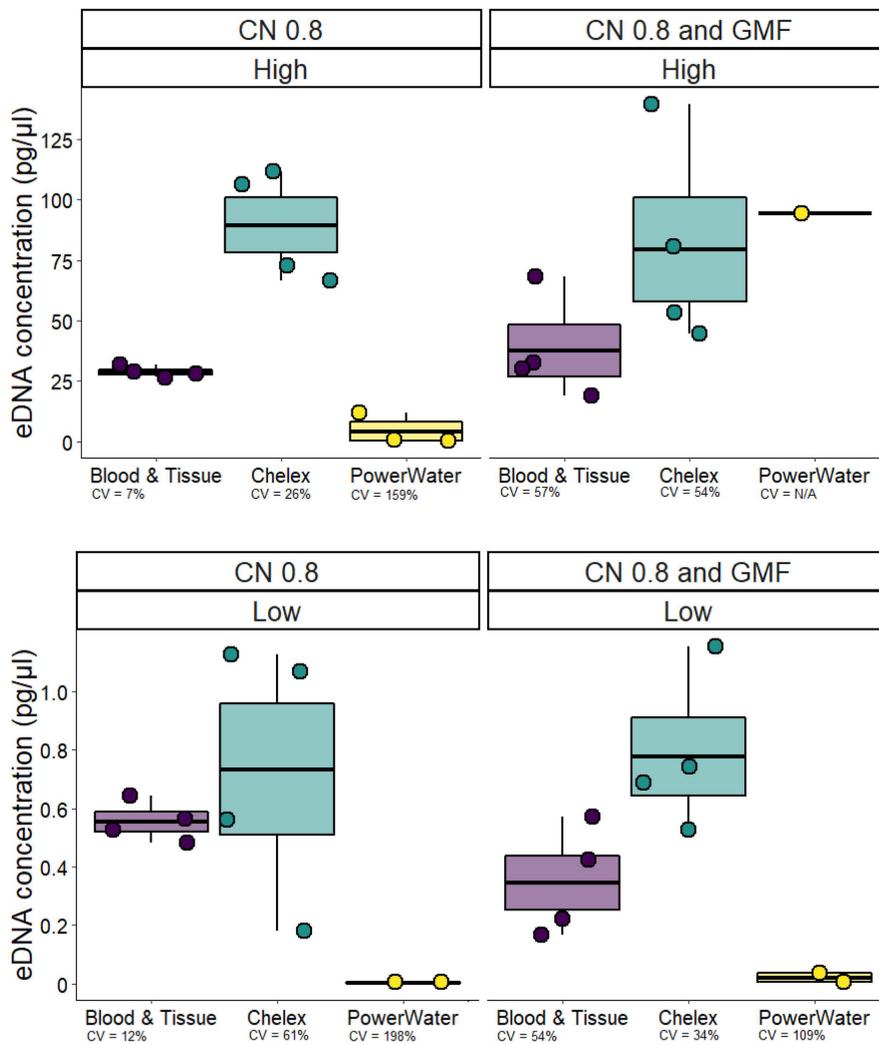
In the high pike density treatment, Chelex 100 yielded a higher eDNA concentration than DNeasy Blood & Tissue ( $F_{1,12} = 22.8$ ,  $p < 0.001$ , [Figure 4](#)). Neither filter combination ( $F_{1,12} = 0.01$ ,  $p = 0.92$ ) nor the interaction between the extraction method and filter combination ( $F_{1,12} = 0.80$ ,  $p = 0.39$ ) 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 ( $<0.07 \text{ pg } \mu\text{l}^{-1}$  similar to



**FIGURE 4** eDNA concentrations ( $\text{pg } \mu\text{l}^{-1}$ ) 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  $\pm 1$  SE with whiskers above and below indicating minimum and maximum values. CV = the coefficient of variation, which is the mean normalized standard deviation

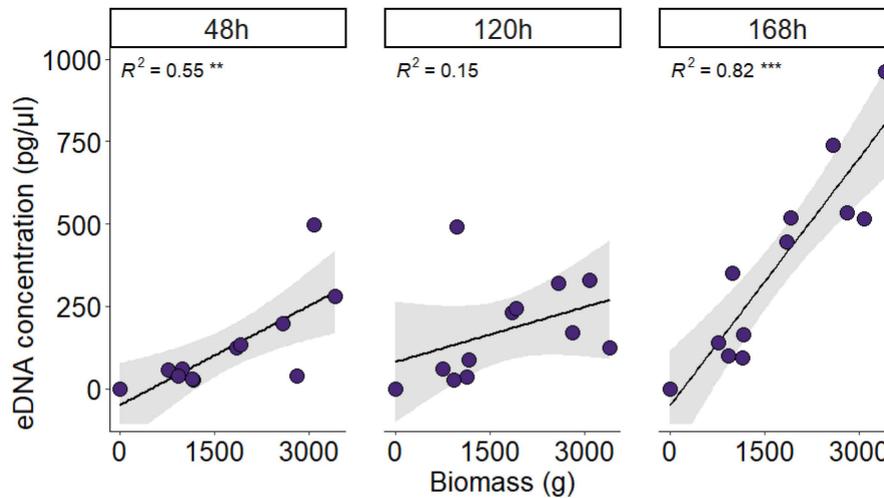
the aquarium measurements with juvenile pike) compared with the samples collected from mesocosms containing pike (mean Cq of 29.4 corresponding to  $54.13 \text{ pg } \mu\text{l}^{-1}$  of eDNA). One sampling control (taken at 120 h) showed amplification at 39.0 Cq (equating to eDNA concentration of  $0.06 \text{ pg } \mu\text{l}^{-1}$ ) and no extraction controls amplified. Two NTC amplified on one plate (Cq = 38.8 and 38.3 equating to 0.07 and  $0.10 \text{ pg } \mu\text{l}^{-1}$ ); 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 ( $\text{copies h}^{-1} \text{ g}^{-1}$ ) 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  $\mu\text{l}^{-1}$ ) 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.  $R^2$  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  $\mu\text{l}$  compared with 100  $\mu\text{l}$  (DNeasy PowerWater) and 200  $\mu\text{l}$  (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 inhibition 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.

## ACKNOWLEDGEMENTS

We thank Veljo Kisand and Riho Gross for their guidance and help with DNA extraction and quantification with qPCR in Tartu, Estonia. We also thank Stefan Nyberg and Ola Renman for their innovation and refinement of the filtration technique and Linda Söderberg for her guidance and help in the laboratory. Thanks to Nils Ljunggren and the Swedish Anglers Association for their assistance with collection of juvenile pike, and Per Vidlund for providing adult pike. This research was funded by the Swedish Environmental Protection Agency (SEPA) for the ePIKE project [grant number NV-03728-17] in collaboration with the Swedish Agency for Marine and Water Management (SwAM). Editorial assistance, in the form of language editing and correction, was obtained from XpertScientific Editing and Consulting Services.

## 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.

## ORCID

Erik Karlsson  <https://orcid.org/0000-0002-9464-4004>

## REFERENCES

- Appelberg, M., Berger, H.-M., Hesthagen, T., Kleiven, E., Kurkilahti, M., Raitaniemi, J., & Rask, M. (1995). Development and intercalibration of methods in nordic freshwater fish monitoring. *Water, Air, & Soil Pollution*, 85(2), 401–406. <https://doi.org/10.1007/BF00476862>
- Arlinghaus, R., Alós, J., Pieterek, T., & Klefoth, T. (2017). Determinants of angling catch of northern pike (*Esox lucius*) as revealed by a controlled whole-lake catch-and-release angling experiment—The role of abiotic and biotic factors, spatial encounters and lure type. *Fisheries Research*, 186, 648–657. <https://doi.org/10.1016/j.fishres.2016.09.009>
- Arlinghaus, R., Klefoth, T., Kobler, A., & Cooke, S. J. (2008). Size selectivity, injury, handling time, and determinants of initial hooking mortality in recreational angling for northern pike: The influence of type and size of bait. *North American Journal of Fisheries Management*, 28(1), 123–134. <https://doi.org/10.1577/M06-263.1>
- Arlinghaus, R., Matsumura, S., & Dieckmann, U. (2010). The conservation and fishery benefits of protecting large pike (*Esox lucius*) by harvest regulations in recreational fishing. *Biological Conservation*, 143(6), 1444–1459. <https://doi.org/10.1016/j.biocon.2010.03.020>
- Bagenal, T. B. (1972). The variability of the catch from gill nets set for pike *Esox lucius* L. *Freshwater Biology*, 2(1), 77–82. <https://doi.org/10.1111/j.1365-2427.1972.tb01582.x>
- Barcellos, L. J. G., Kreutz, L. C., Koakoski, G., Oliveira, T. A., da Rosa, J. G. S., & Fagundes, M. (2012). Fish age, instead of weight and size, as a determining factor for time course differences in cortisol response to stress. *Physiology & Behavior*, 107(3), 397–400. <https://doi.org/10.1016/j.physbeh.2012.09.008>
- Boothroyd, M., Mandrak, N. E., Fox, M., & Wilson, C. C. (2016). Environmental DNA (eDNA) detection and habitat occupancy of threatened spotted gar (*Lepisosteus oculatus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26(6), 1107–1119. <https://doi.org/10.1002/aqc.2617>
- Bracken, F. S. A., Rooney, S. M., Kelly-Quinn, M., King, J. J., & Carlsson, J. (2019). Identifying spawning sites and other critical habitat in lotic systems using eDNA “snapshots”: A case study using the sea lamprey *Petromyzon marinus* L. *Ecology and Evolution*, 9(1), 553–567. <https://doi.org/10.1002/ece3.4777>
- Capo, E., Spong, G., Königsson, H., & Byström, P. (2020). Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via droplet digital PCR. *Environmental DNA*, 2(2), 152–160. <https://doi.org/10.1002/edn3.52>
- Coulter, D. P., Wang, P., Coulter, A. A., Susteren, G. E. V., Eichmiller, J. J., Garvey, J. E., & Sorensen, P. W. (2019). Nonlinear relationship between silver carp density and their eDNA concentration in a large river. *PLoS One*, 14(6), e0218823. <https://doi.org/10.1371/journal.pone.0218823>
- Craig, J. F. (1996). *Pike: Biology and exploitation*. Chapman & Hall.
- Craig, J. F. (2008). A short review of pike ecology. *Hydrobiologia*, 601(1), 5–16. <https://doi.org/10.1007/s10750-007-9262-3>
- Deiner, K., Walser, J.-C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*, 183, 53–63. <https://doi.org/10.1016/j.biocon.2014.11.018>
- Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., & Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS One*, 6(8), e23398. <https://doi.org/10.1371/journal.pone.0023398>
- Doi, H., Inui, R., Akamatsu, Y., Kanno, K., Yamanaka, H., Takahara, T., & Minamoto, T. (2017). Environmental DNA analysis for estimating the abundance and biomass of stream fish. *Freshwater Biology*, 62(1), 30–39. <https://doi.org/10.1111/fwb.12846>
- Doi, H., Uchii, K., Takahara, T., Matsushashi, S., Yamanaka, H., & Minamoto, T. (2015). Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. *PLoS One*, 10(3), e0122763. <https://doi.org/10.1371/journal.pone.0122763>
- Dunker, K. J., Sepulveda, A. J., Massengill, R. L., Olsen, J. B., Russ, O. L., Wenburg, J. K., & Antonovich, A. (2016). Potential of environmental DNA to evaluate northern pike (*Esox lucius*) eradication efforts: An experimental test and case study. *PLoS One*, 11(9), e0162277. <https://doi.org/10.1371/journal.pone.0162277>
- Eichmiller, J. J., Miller, L. M., & Sorensen, P. W. (2016). Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. *Molecular Ecology Resources*, 16(1), 56–68. <https://doi.org/10.1111/1755-0998.12421>
- Evans, N. T., Shirey, P. D., Wieringa, J. G., Mahon, A. R., & Lamberti, G. A. (2017). Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries*, 42(2), 90–99. <https://doi.org/10.1080/03632415.2017.1276329>
- Guivas, R. A., & Brammell, B. F. (2020). Use of environmental DNA to determine fantail darter (*Etheostoma flabellare*) density in

- a laboratory setting: Effects of biomass and filtration method. *International Journal of Zoology*, 2020, e4731686. <https://doi.org/10.1155/2020/4731686>
- Hellström, M., Wijkmark, N., Edbom-Blomstrand, C., Hellström, P., & Näslund, J. (2019). *Footsteps in the snow—Pilot study for future monitoring of individual lynx (Lynx lynx) from eDNA in snow tracks*. *AquaBiota Report*. p. 10.
- Hinlo, R., Lintermans, M., Gleeson, D., Broadhurst, B., & Furlan, E. (2018). Performance of eDNA assays to detect and quantify an elusive benthic fish in upland streams. *Biological Invasions*, 20(11), 3079–3093. <https://doi.org/10.1007/s10530-018-1760-x>
- Hunter, M. E., Ferrante, J. A., Meigs-Friend, G., & Ulmer, A. (2019). Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. *Scientific Reports*, 9(1), 5259. <https://doi.org/10.1038/s41598-019-40977-w>
- Inskip, P. D. (1982). *Habitat suitability index models: Northern pike*. Western Energy and Land Use Team, Office of Biological Services, Fish and Wildlife Service, U.S. Department of the Interior.
- Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T., & Minamoto, T. (2019). Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(3), 361–373. <https://doi.org/10.1002/aqc.3058>
- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4(2), 150–157. <https://doi.org/10.1111/j.1755-263X.2010.00158.x>
- Karlsson, K., & Kari, E. (2020). Recreational anglers as citizen scientists can provide data to estimate population size of pike. *Esox Lucius. Fisheries Management and Ecology*, 27(4), 367–380. <https://doi.org/10.1111/fme.12419>
- Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, 2(3), 271–282. <https://doi.org/10.1002/edn3.29>
- Klymus, K. E., Richter, C. A., Chapman, D. C., & Paukert, C. (2015). Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biological Conservation*, 183, 77–84. <https://doi.org/10.1016/j.biocon.2014.11.020>
- Kuparinen, A., Klefoth, T., & Arlinghaus, R. (2010). Abiotic and fishing-related correlates of angling catch rates in pike (*Esox lucius*). *Fisheries Research*, 105(2), 111–117. <https://doi.org/10.1016/j.fishres.2010.03.011>
- Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, 16(6), 1401–1414. <https://doi.org/10.1111/1755-0998.12522>
- Lance, R. F., & Guan, X. (2020). Variation in inhibitor effects on qPCR assays and implications for eDNA surveys. *Canadian Journal of Fisheries and Aquatic Sciences*, 77(1), 23–33. <https://doi.org/10.1139/cjfas-2018-0263>
- Loeza-Quintana, T., Abbott, C. L., Heath, D. D., Bernatchez, L., & Hanner, R. H. (2020). Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)—Advancing collaboration and standardization efforts in the field of eDNA. *Environmental DNA*, 2(3), 255–260. <https://doi.org/10.1002/edn3.112>
- Magnusson, A., & Hilborn, R. (2007). What makes fisheries data informative? *Fish and Fisheries*, 8(4), 337–358. <https://doi.org/10.1111/j.1467-2979.2007.00258.x>
- Majaneva, M., Diserud, O. H., Eagle, S. H. C., Boström, E., Hajibabaei, M., & Ekrem, T. (2018). Environmental DNA filtration techniques affect recovered biodiversity. *Scientific Reports*, 8(1), 4682. <https://doi.org/10.1038/s41598-018-23052-8>
- Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from Juvenile and Adult Fish. *PLoS One*, 9(12), e114639. <https://doi.org/10.1371/journal.pone.0114639>
- McAllister, M. K., & Kirkwood, G. P. (1998). Using Bayesian decision analysis to help achieve a precautionary approach for managing developing fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, 55(12), 2642–2661. <https://doi.org/10.1139/f98-121>
- McKee, A. M., Spear, S. F., & Pierson, T. W. (2015). The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation*, 183, 70–76. <https://doi.org/10.1016/j.biocon.2014.11.031>
- Mizumoto, H., Urabe, H., Kanbe, T., Fukushima, M., & Araki, H. (2018). Establishing an environmental DNA method to detect and estimate the biomass of Sakhalin taimen, a critically endangered Asian salmonid. *Limnology*, 19(2), 219–227. <https://doi.org/10.1007/s10201-017-0535-x>
- Nevers, M. B., Byappanahalli, M. N., Morris, C. C., Shively, D., Przybyla-Kelly, K., Spoljaric, A. M., Dickey, J., & Roseman, E. F. (2018). Environmental DNA (eDNA): A tool for quantifying the abundant but elusive round goby (*Neogobius melanostomus*). *PLoS One*, 13(1), e0191720. <https://doi.org/10.1371/journal.pone.0191720>
- Nilsson, A. (2006). Avoid your neighbours: Size-determined spatial distribution patterns among northern pike individuals. *Oikos*, 113(2), 251–258. <https://doi.org/10.1111/j.2006.0030-1299.14555.x>
- Olsen, J. B., Lewis, C. J., Massengill, R. L., Dunker, K. J., & Wenburg, J. K. (2015). An evaluation of target specificity and sensitivity of three qPCR assays for detecting environmental DNA from Northern Pike (*Esox lucius*). *Conservation Genetics Resources*, 7(3), 615–617. <https://doi.org/10.1007/s12686-015-0459-x>
- Olsen, J. B., Lewis, C. J., Massengill, R. L., Dunker, K. J., & Wenburg, J. K. (2016). Erratum to: An evaluation of target specificity and sensitivity of three qPCR assay for detecting environmental DNA from Northern Pike (*Esox lucius*). *Conservation Genetics Resources*, 8(1), 89. <https://doi.org/10.1007/s12686-016-0526-y>
- Olsson, J. (2019). Past and current trends of coastal predatory fish in the Baltic sea with a focus on perch, pike, and pikeperch. *Fishes*, 4(1), 7. <https://doi.org/10.3390/fishes4010007>
- Paukert, C. P., Klammer, J. A., Pierce, R. B., & Simonson, T. D. (2001). An overview of northern pike regulations in north America. *Fisheries*, 26(6), 6–13. [https://doi.org/10.1577/1548-8446\(2001\)026<0006:AOONPR>2.0.CO2](https://doi.org/10.1577/1548-8446(2001)026<0006:AOONPR>2.0.CO2)
- Pierce, R. B. (1997). Variable catchability and bias in population estimates for northern pike. *Transactions of the American Fisheries Society*, 126, 658–664. [https://doi.org/10.1577/1548-8659\(1997\)126<0658:VCABIP>2.3.CO2](https://doi.org/10.1577/1548-8659(1997)126<0658:VCABIP>2.3.CO2)
- Pierce, R. B., & Tomcko, C. M. (1995). Exploitation of northern pike in seven small north-central Minnesota lakes. *North American Journal of Fisheries Management*, 15, 601–609. [https://doi.org/10.1577/1548-8675\(1995\)015<0601:EONPIS>2.3.CO2](https://doi.org/10.1577/1548-8675(1995)015<0601:EONPIS>2.3.CO2)
- Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, 4(1), 9–33. <https://doi.org/10.1002/edn3.185>
- Ruetz, C. R., Uzarski, D. G., Krueger, D. M., & Rutherford, E. S. (2007). Sampling a littoral fish assemblage: Comparison of small-mesh fyke netting and boat electrofishing. *North American Journal of Fisheries Management*, 27(3), 825–831. <https://doi.org/10.1577/M06-147.1>
- Salter, I., Joensen, M., Kristiansen, R., Steingrund, P., & Vestergaard, P. (2019). Environmental DNA concentrations are correlated with

- regional biomass of Atlantic cod in oceanic waters. *Communications Biology*, 2(1), 1–9. <https://doi.org/10.1038/s42003-019-0696-8>
- Sandström, A., Bryhn, A., Sundelöf, A., Lingman, A., Florin, A.-B., Petersson, E., Vitale, F., Sundblad, G., Wickström, H., Ahlbeck Bergendahl, I., Lövgren, J., Lundström, K., Edsman, L., Wennerström, L., Ogonowski, M., Ulmestrand, M., Cardinale, M., Lindmark, M., Bergenius, M., & Hekim, Z. (2019). *Fisk- och skaldjursbestånd i hav och sötvatten 2018*. Swedish Agency for Marine and Water Management.
- Sansom, B. J., & Sassoubre, L. M. (2017). Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. *Environmental Science & Technology*, 51(24), 14244–14253. <https://doi.org/10.1021/acs.est.7b05199>
- Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science & Technology*, 50(19), 10456–10464. <https://doi.org/10.1021/acs.est.6b03114>
- Schabacker, J. C., Amish, S. J., Ellis, B. K., Gardner, B., Miller, D. L., Rutledge, E. A., Sepulveda, A. J., & Luikart, G. (2020). Increased eDNA detection sensitivity using a novel high-volume water sampling method. *Environmental DNA*, 2(2), 244–251. <https://doi.org/10.1002/edn3.63>
- Seaburg, K. G., & Moyle, J. B. (1964). Feeding Habits, Digestive Rates, and Growth of Some Minnesota Warmwater Fishes. *Transactions of the American Fisheries Society*, 93(3), 269–285. [https://doi.org/10.1577/1548-8659\(1964\)93<269FHDRAG>2.0.CO;2](https://doi.org/10.1577/1548-8659(1964)93<269FHDRAG>2.0.CO;2)
- Spear, M. J., Embke, H. S., Krysan, P. J., & Vander Zanden, M. J. (2021). Application of eDNA as a tool for assessing fish population abundance. *Environmental DNA*, 3(1), 83–91. <https://doi.org/10.1002/edn3.94>
- Stoner, A. W. (2004). Effects of environmental variables on fish feeding ecology: Implications for the performance of baited fishing gear and stock assessment. *Journal of Fish Biology*, 65(6), 1445–1471. <https://doi.org/10.1111/j.0022-1112.2004.00593.x>
- Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85–92. <https://doi.org/10.1016/j.biocon.2014.11.038>
- Taberlet, P., Bonin, A., Coissac, E., & Zinger, L. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press.
- Takahara, T., Minamoto, T., & Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS One*, 8(2), e56584. <https://doi.org/10.1371/journal.pone.0056584>
- Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS One*, 7(4), e35868. <https://doi.org/10.1371/journal.pone.0035868>
- Thalinger, B., Rieder, A., Teuffenbach, A., Pütz, Y., Schwerte, T., Wanzenböck, J., & Traugott, M. (2021). The effect of activity, energy use, and species identity on environmental DNA shedding of freshwater fish. *Frontiers in Ecology and Evolution*, 9, 73. <https://doi.org/10.3389/fevo.2021.623718>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One*, 7(8), e41732. <https://doi.org/10.1371/journal.pone.0041732>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L., & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21(11), 2565–2573. <https://doi.org/10.1111/j.1365-294X.2011.05418.x>
- Thoresson, G. (1993). *Guidelines for Coastal Fish Monitoring*, (No. 1, Kustrapport).
- Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, 220, 1–11. <https://doi.org/10.1016/j.biocon.2018.01.030>
- Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods in Ecology and Evolution*, 5(7), 676–684. <https://doi.org/10.1111/2041-210X.12206>
- Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, 10(4), 506–513.
- Wilcox, T. M., Carim, K. J., Young, M. K., McKelvey, K. S., Franklin, T. W., & Schwartz, M. K. (2018). Comment: The Importance of Sound Methodology in Environmental DNA Sampling. *North American Journal of Fisheries Management*, 38(3), 592–596. <https://doi.org/10.1002/nafm.10055>
- Yates, M. C., Fraser, D. J., & Derry, A. M. (2019). Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. *Environmental DNA*, 1(1), 5–13. <https://doi.org/10.1002/edn3.7>
- Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Molecular Ecology*, 30(13), 3068–3082. <https://doi.org/10.1111/mec.15543>
- Yates, M. C., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., & Derry, A. M. (2021). Allometric scaling of eDNA production in stream-dwelling brook trout (*Salvelinus fontinalis*) inferred from population size structure. *Environmental DNA*, 3(3), 553–560. <https://doi.org/10.1002/edn3.150>

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** 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, 881–893. <https://doi.org/10.1002/edn3.298>