

Temperature moderates eDNA–biomass relationships in northern pike

M. Ogonowski¹  | E. Karlsson¹  | A. Vasemägi^{1,2} | J. Sundin¹ | P. Bohman¹ | G. Sundblad¹

¹Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, Drottningholm, Sweden

²Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Correspondence

M. Ogonowski, Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, Stångholmsvägen 2, SE-17893, Drottningholm, Sweden.
Email: martin.ogonowski@slu.se

Funding information

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

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

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.

© 2023 The Authors. *Environmental DNA* published by John Wiley & Sons Ltd.

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*, Mitchell 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 (Díaz-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 >200km 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.

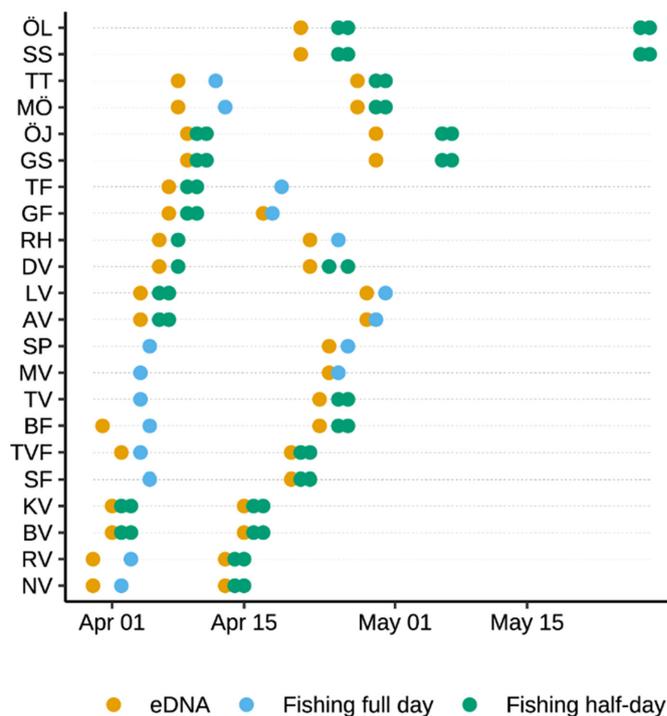
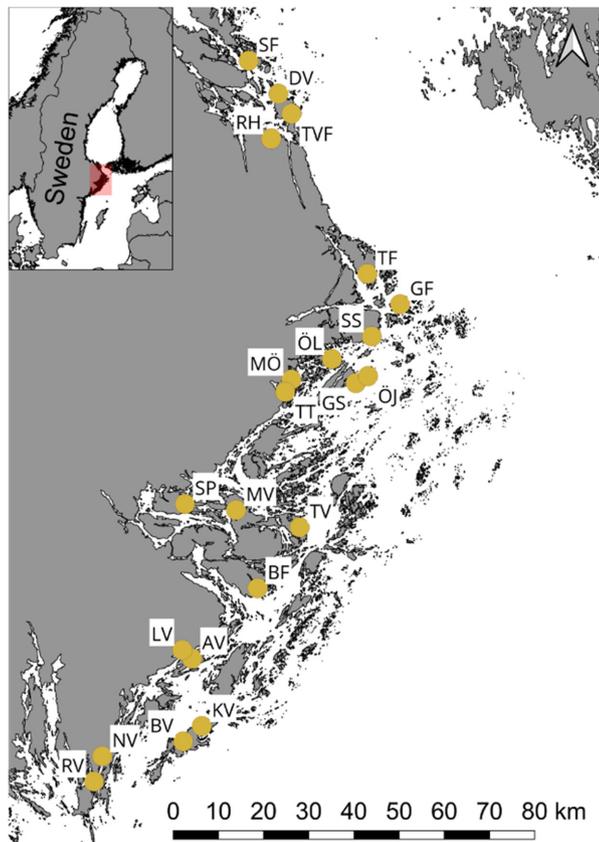
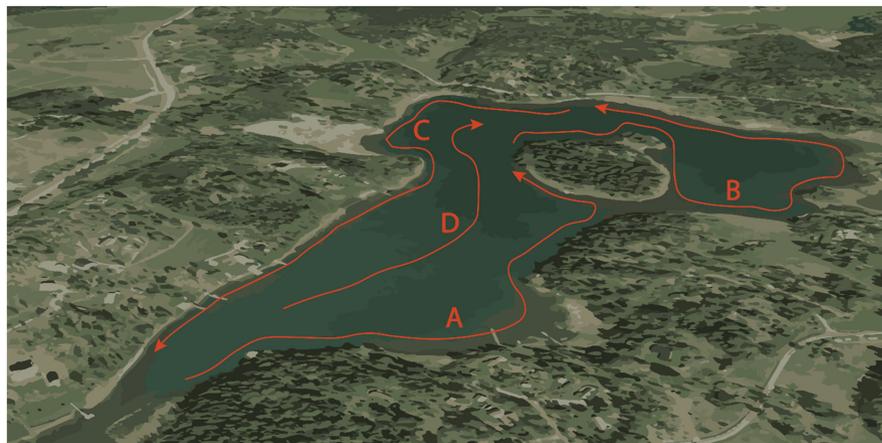


FIGURE 1 Overview map of bays sampled for eDNA and pike by angling (left panel) and sampling scheme (right panel) showing sampling dates for eDNA and angling ordered by bay-pair during the survey in 2020. Angling was either divided into two half days within a bay-pair and fished for two consecutive days, 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 μ L 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 denature 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 TCCCCGCATA 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 μ L reaction volumes. Reaction concentrations of the forward primer, reverse primer, and probe were 900 nM each with 7.5 μ L 2 \times TaqMan[™] Environmental Master Mix 2.0 (Thermo Fisher) in each well loaded with 4 μ L 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 μ L of 10 \times IPC mix and 0.2 μ L of IPC template DNA was added to each reaction.

Inhibition in eDNA samples was determined based on aberrant IPC C_q-values. The expected C_q of the IPC over the range of the standard curve was on average 27.3 C_q with average minimum and maximum values ranging 26.5–28.5 C_q. Therefore, we classified samples >28.5 C_q 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 C_q-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 \times 10⁷ copies/ μ L) with the addition of a lowest concentration at 0.25 copies/ μ L. 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 TTCTCCCC 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 \times 10⁷ copies/ μ L 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/ μ L. Analysis in quadruplicates ($n=4$) gave an effective LOD of 0.58 copies/ μ L which is the LOD relevant to our assay. Concentrations are given per microliter of target sample (4 μ L).

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/ μ L (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 (8h, [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 (Casselmann, 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),

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

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 *C_q*-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 -Tomtvikén/Urö", "SS -Södersundet", "MÖ -Möcklingeåker" 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 *C_q*-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 ppb).

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).

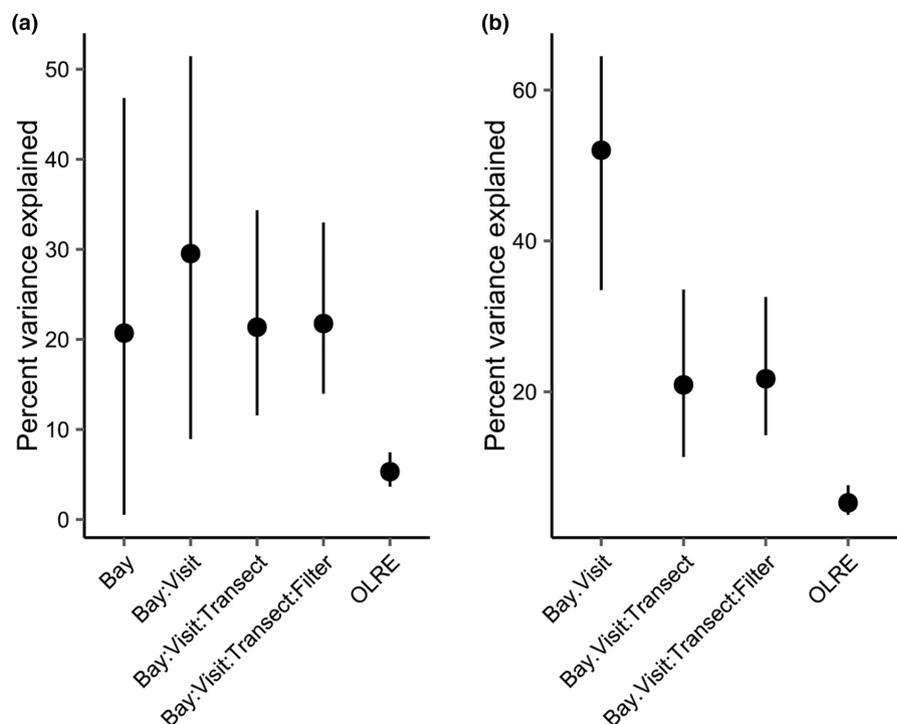


FIGURE 3 Intra-class correlation coefficients for the random effects in the eDNA-model showing the percentage of variance explained in the model at different hierarchical levels from the full model (a) or a simplified version where the effects of *Bay* and *Visit* are pooled to one random effect (b).

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. R² 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

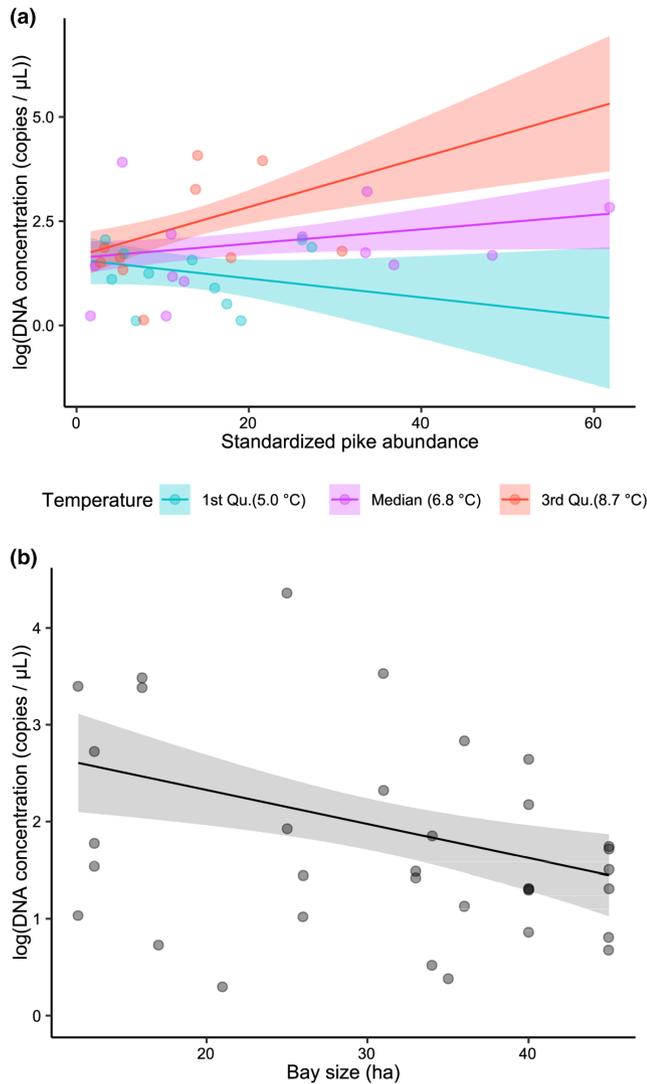


FIGURE 4 Model estimated DNA concentration (log-scale copies per μL) as a function of (a) standardized pike abundance, at different temperatures, and (b) bay size (hectares). Points are partial residuals. R^2 for the model was 0.48.

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

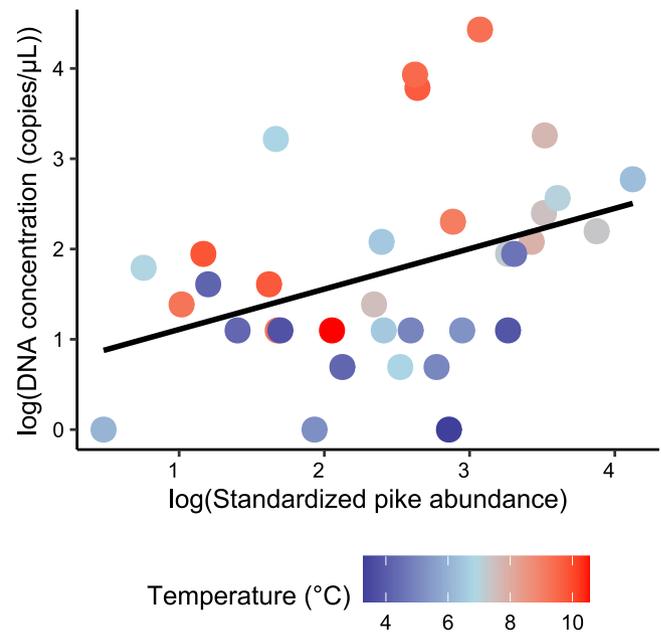


FIGURE 5 Raw data plot (log-log scale) of the relationship between DNA concentration and standardized pike abundance (CPUE) colored by water temperature.

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 ($\bar{x}=60\text{ cm}$, $SD=10\text{ cm}$). 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.

ACKNOWLEDGMENTS

We would like to thank Henrik C. Andersson at the Stockholm County Administrative Board and all the anglers involved in the REFISK-project 2020 for providing the angling data. Dr. Zandra Gerdes (Aquabiota Water Research), Ofir Svensson (Calluna AB) for help during laboratory work, and Ola Renman and John Persson (SLU) for help during the eDNA-data collection. This work was

funded by a grant from the Swedish Environmental Protection Agency, Naturvårdsverket (NV-03728-17).

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.

ORCID

M. Ogonowski  <https://orcid.org/0000-0002-7082-0990>

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

REFERENCES

- Alós, J., Palmer, M., Trías, P., Díaz-Gil, C., & Arlinghaus, R. (2015). Recreational angling intensity correlates with alteration of vulnerability to fishing in a carnivorous coastal fish species. *Canadian Journal of Fisheries and Aquatic Sciences*, 72, 217–225.
- Andruszkiewicz Allan, E., Zhang, W. G., Lavery, A. C., & Govindarajan, A. F. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA*, 3, 492–514.
- Arlinghaus, R., Alós, J., Beardmore, B., Díaz, Á. M., Hühn, D., Johnston, F., Klefoth, T., Kuparinen, A., Matsumura, S., Pagel, T., Pieterek, T., & Riepe, C. (2018). *Recreational piking – Sustainably managing pike in recreational fisheries. Page Biology and Ecology of Pike*. CRC Press.
- 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.
- 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, 123–134.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.
- Bean, N. J. (2010). *An improved bioenergetics model for northern pike (Esox Lucius) of box canyon reservoir, Pend Oreille River*. Eastern Washington University.
- Bergström, U., Larsson, S., Erlandsson, M., Ovegård, M., Ragnarsson Stabo, H., Östman, Ö., & Sundblad, G. (2022). Long-term decline in northern pike (*Esox lucius* L.) populations in the Baltic Sea revealed by recreational angling data. *Fisheries Research*, 251, 106307.
- Bockrath, K. D., Tuttle-Lau, M., Mize, E. L., Ruden, K. V., & Woiak, Z. (2022). Direct comparison of eDNA capture and extraction methods through measuring recovery of synthetic DNA cloned into living cells. *Environmental DNA*, 4, 1000–1010.
- Brehehy, P., & Burchett, W. (2017). Visualization of regression models using visreg. *The R Journal*, 9, 56–71.
- Bylemans, J., Furlan, E. M., Hardy, C. M., McGuffie, P., Lintermans, M., & Gleeson, D. M. (2017). An environmental DNA-based method for monitoring spawning activity: A case study, using the endangered Macquarie perch (*Macquaria australasica*). *Methods in Ecology and Evolution*, 8, 646–655.
- Canales, R. A., Wilson, A. M., Pearce-Walker, J. I., Verhougstraete, M. P., & Reynolds, K. A. (2018). Methods for handling left-censored data in quantitative microbial risk assessment. *Applied and Environmental Microbiology*, 84, e01203-18.
- 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, 152–160.
- Capo, E., Spong, G., Norman, S., Königsson, H., Bartels, P., & Byström, P. (2019). Droplet digital PCR assays for the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) from environmental DNA collected in the water of mountain lakes. *PLoS One*, 14, e0226638.
- Casselman, J. M. (1974). External sex determination of northern pike, *Esox lucius* Linnaeus. *Transactions of the American Fisheries Society*, 103, 343–347.
- Chambert, T., Pilliod, D. S., Goldberg, C. S., Doi, H., & Takahara, T. (2018). An analytical framework for estimating aquatic species density from environmental DNA. *Ecology and Evolution*, 8, 3468–3477.
- Chen, L.-X., & Zeng, L.-Q. (2022). Previous experience alters individual vulnerability to angling of crucian carp (*Carassius auratus*). *Behavioural Processes*, 195, 104565.
- Clark, C. F. (1950). Observations on the spawning habits of the northern pike, *Esox lucius*, in northwestern Ohio. *Copeia*, 1950, 285.
- Cohen, M. A., & Ryan, P. B. (1989). Observations less than the analytical limit of detection: A new approach. *JAPCA*, 39, 328–329.
- Collins, R. A., Baillie, C., Halliday, N. C., Rainbird, S., Sims, D. W., Mariani, S., & Genner, M. J. (2022). Reproduction influences seasonal eDNA variation in a temperate marine fish community. *Limnology and Oceanography Letters*, 7, 443–449.
- Craig, J. F. (2008). A short review of pike ecology. *Hydrobiologia*, 601, 5–16.
- Crane, D. P., Miller, L. M., Diana, J. S., Casselman, J. M., Farrell, J. M., Kapuscinski, K. L., & Nohner, J. K. (2015). Muskellunge and northern pike ecology and management: Important issues and research needs. *Fisheries*, 40, 258–267.
- Curtis, A. N., Tiemann, J. S., Douglass, S. A., Davis, M. A., & Larson, E. R. (2021). High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Diversity and Distributions*, 27, 1918–1931.
- de Souza, L. S., Godwin, J. C., Renshaw, M. A., & Larson, E. (2016). Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLoS One*, 11, e0165273.
- 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, e23398.
- Díaz-Suarez, A., Noreikiene, K., Kisan, V., Burimski, O., Svirgsden, R., Rohtla, M., Ozerov, M., Gross, R., Vetemaa, M., & Vasemägi, A. (2022). Temporally stable small-scale genetic structure of northern pike (*Esox lucius*) in the coastal Baltic Sea. *Fisheries Research*, 254, 106402.
- Dinse, G. E., Jusko, T. A., Ho, L. A., Annam, K., Graubard, B. I., Hertz-Picciotto, I., Miller, F. W., Gillespie, B. W., & Weinberg, C. R. (2014). Accommodating measurements below a limit of detection: A novel application of cox regression. *American Journal of Epidemiology*, 179, 1018–1024.
- Donadi, S., Bergström, L., Bertil Berglund, J. M., Anette, B., Mikkola, R., Saarinen, A., & Bergström, U. (2020). Perch and pike recruitment in coastal bays limited by stickleback predation and environmental forcing. *Estuarine, Coastal and Shelf Science*, 246, 107052.
- 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, e0162277.

- Eberhardt, L. L. (1976). Quantitative ecology and impact assessment. *Journal of Environmental Management*, 4, 1.
- Eklöf, J. S., Sundblad, G., Erlandsson, M., Donadi, S., Hansen, J. P., Eriksson, B. K., & Bergström, U. (2020). A spatial regime shift from predator to prey dominance in a large coastal ecosystem. *Communications Biology*, 3, 1–9.
- Flink, H., Tibblin, P., Hall, M., Hellström, G., & Nordahl, O. (2023). Variation among bays in spatiotemporal aggregation of Baltic Sea pike highlights management complexity. *Fisheries Research*, 259, 106579.
- Forsman, A., Tibblin, P., Berggren, H., Nordahl, O., Koch-Schmidt, P., & Larsson, P. (2015). Pike *Esox lucius* as an emerging model organism for studies in ecology and evolutionary biology: A review: *Esox lucius* as a model in ecology and evolution. *Journal of Fish Biology*, 87, 472–479.
- Frost, W. E., & Kipling, C. (1967). A study of reproduction, early life, weight-length relationship and growth of pike, *Esox lucius* L., in Windermere. *The Journal of Animal Ecology*, 36, 651.
- Gaudet-Boulay, M., García-Machado, E., Laporte, M., Yates, M., Bougas, B., Hernandez, C., Côté, G., Gilbert, A., & Bernatchez, L. (2022). Relationship between brook charr (*Salvelinus fontinalis*) eDNA concentration and angling data in structured wildlife areas. *Environmental DNA*. <https://doi.org/10.1002/edn3.341>
- Glass, D. C., & Gray, C. N. (2001). Estimating mean exposures from censored data: Exposure to benzene in the Australian petroleum industry. *The Annals of Occupational Hygiene*, 45, 275–282.
- Glazier, D. S. (2005). Beyond the “3/4-power law”: Variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews of the Cambridge Philosophical Society*, 80, 611–662.
- Green, R. H. (1979). *Sampling design and statistical methods for environmental biologists*. John Wiley & Sons.
- Hansson, S., Bergström, U., Bonsdorff, E., Härkönen, T., Jepsen, N., Kautsky, L., Lundström, K., Lunneryd, S.-G., Ovegård, M., Salmi, J., Sendek, D., & Vetemaa, M. (2017). Competition for the fish – Fish extraction from the Baltic Sea by humans, aquatic mammals, and birds. *ICES Journal of Marine Science*, 75, 999–1008.
- Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, 2, e616. <https://doi.org/10.7717/peerj.616>
- Hartig, F. (2022). *DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models*. R package version 0.4.6. <http://florianhartig.github.io/DHARMA/>
- Hernandez, C., Bougas, B., Perreault-Payette, A., Simard, A., Côté, G., & Bernatchez, L. (2020). 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in eastern Canada. *Environmental DNA*, 2, 373–386.
- Hervé, A., Domaizon, I., Baudoin, J.-M., Dejean, T., Gibert, P., Jean, P., Peroux, T., Raymond, J.-C., Valentini, A., Vautier, M., & Logez, M. (2022). Spatio-temporal variability of eDNA signal and its implication for fish monitoring in lakes. *PLoS One*, 17, e0272660.
- Holmes, V., Aman, J., York, G., & Kinnison, M. T. (2022). Environmental DNA detects spawning habitat of an ephemeral migrant fish (Anadromous Rainbow Smelt: *Osmerus mordax*). *BMC Ecology and Evolution*, 22, 121.
- Hornung, R. W., & Reed, L. D. (1990). Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene*, 5, 46–51.
- Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, 15, 216–227.
- Jo, T., Murakami, H., Masuda, R., & Minamoto, T. (2020). Selective collection of long fragments of environmental DNA using larger pore size filter. *Science of the Total Environment*, 735, 139462.
- Jo, T., Murakami, H., Yamamoto, S., Masuda, R., & Minamoto, T. (2019). Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. *Ecology and Evolution*, 9, 1135–1146.
- Jo, T., Takao, K., & Minamoto, T. (2022). Linking the state of environmental DNA to its application for biomonitoring and stock assessment: Targeting mitochondrial/nuclear genes, and different DNA fragment lengths and particle sizes. *Environmental DNA*, 4, 271–283.
- Jo, T. S. (2023). Correlation between the number of eDNA particles and species abundance is strengthened by warm temperature: Simulation and meta-analysis. *Hydrobiologia*, 850, 39–50.
- Kačergytė, I., Petersson, E., Arlt, D., Hellström, M., Knappe, J., Spens, J., Žmihorski, M., & Pärt, T. (2021). Environmental DNA metabarcoding elucidates patterns of fish colonisation and co-occurrences with amphibians in temperate wetlands created for biodiversity. *Freshwater Biology*, 66, 1915–1929.
- 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.
- Killen, S. S., Atkinson, D., & Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters*, 13, 184–193.
- Kitchell, J. F., Stewart, D. J., & Weinger, D. (1977). Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). *Journal of the Fisheries Research Board of Canada*, 34, 1922–1935.
- 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, 271–282.
- 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.
- Knudsen, S. W., Ebert, R. B., Hesselsøe, M., Kuntke, F., Hassingboe, J., Mortensen, P. B., Thomsen, P. F., Sigsgaard, E. E., Hansen, B. K., Nielsen, E. E., & Møller, P. R. (2019). Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, 510, 31–45.
- Kuparinen, A., Klefoth, T., & Arlinghaus, R. (2010). Abiotic and fishing-related correlates of angling catch rates in pike (*Esox lucius*). *Fisheries Research*, 105, 111–117.
- 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, 1401–1414.
- Laikre, L., Miller, L. M., Palmé, A., Palm, S., Kapuscinski, A. R., Thoreson, G., & Ryman, N. (2005). Spatial genetic structure of northern pike (*Esox lucius*) in the Baltic Sea. *Molecular Ecology*, 14, 1955–1964.
- 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, 23–33.
- Lesperance, M. L., Allison, M. J., Bergman, L. C., Hocking, M. D., & Helbing, C. C. (2021). A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. *Environmental DNA*, 3, 970–981.
- Li, C., Long, H., Yang, S., Zhang, Y., Tang, F., Jin, W., Wang, G., Chang, W., Pi, Y., Gao, L., Ma, L., Zhao, M., Zheng, H., Gong, Y., Liu, Y., & Jiang, K. (2022). eDNA assessment of pelagic fish diversity, distribution, and abundance in the Central Pacific Ocean. *Regional Studies in Marine Science*, 56, 102661.
- Lindén, A., & Mäntyniemi, S. (2011). Using the negative binomial distribution to model overdispersion in ecological count data. *Ecology*, 92, 1414–1421.

- Lindmark, M., Huss, M., Ohlberger, J., & Gårdmark, A. (2018). Temperature-dependent body size effects determine population responses to climate warming. *Ecology Letters*, *21*, 181–189.
- Littlefair, J. E., Hrenchuk, L. E., Blanchfield, P. J., Rennie, M. D., & Cristescu, M. E. (2021). Thermal stratification and fish thermal preference explain vertical eDNA distributions in lakes. *Molecular Ecology*, *30*, 3083–3096.
- Lucas, M. C. (1992). Spawning activity of male and female pike, *Esox lucius* L., determined by acoustic tracking. *Canadian Journal of Zoology*, *70*, 191–196.
- Majumdar, N., Banerjee, S., Pallas, M., Wessel, T., & Hegerich, P. (2017). Poisson plus quantification for digital PCR systems. *Scientific Reports*, *7*, 9617.
- McCall, M. N., McMurray, H. R., Land, H., & Almudevar, A. (2014). On non-detects in qPCR data. *Bioinformatics*, *30*, 2310–2316.
- Möller, S., Winkler, H. M., Richter, S., & Bastrop, R. (2021). Genetic population structure of pike (*Esox lucius* Linnaeus, 1758) in the brackish lagoons of the southern Baltic Sea. *Ecology of Freshwater Fish*, *30*, 140–149.
- Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M. K., Horiuchi, T., Sawada, H., Kondoh, M., Yamashita, Y., & Masuda, R. (2019). Dispersion and degradation of environmental DNA from caged fish in a marine environment. *Fisheries Science*, *85*, 327–337.
- Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface*, *14*, 20170213.
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: A practical guide for biologists. *Biological Reviews*, *85*, 935–956.
- Neumann, R. M., & Willis, D. W. (1995). Seasonal variation in gill-net sample indexes for northern pike collected from a Glacial Prairie Lake. *North American Journal of Fisheries Management*, *15*, 838–844.
- Nevers, M. B., Byappanahalli, M. N., Morris, C. C., Shively, D., Przybylak-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*, e0191720.
- Ohlberger, J., Mehner, T., Staaks, G., & Hölker, F. (2012). Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. *Oikos*, *121*, 245–251.
- 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*, 615–617.
- 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*, 89.
- Olsson, J., Andersson, M. L., Bergström, U., Arlinghaus, R., Audzijonyte, A., Berg, S., Briekmane, L., Dainys, J., Ravn, H. D., Droll, J., Dziemian, Ł., Fey, D. P., van Gemert, R., Greszkiewicz, M., Grochowski, A., Jakubavičiūtė, E., Lozys, L., Lejk, A. M., Mustamäki, N., ... Östman, Ö. (2023). A pan-Baltic assessment of temporal trends in coastal pike populations. *Fisheries Research*, *260*, 106594.
- Pont, D., Meulenbroek, P., Bammer, V., Dejean, T., Erős, T., Jean, P., Lenhardt, M., Nagel, C., Pekarik, L., Schabuss, M., Stoeckle, B. C., Stoica, E., Zornig, H., Weigand, A., & Valentini, A. (2023). Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR. *Molecular Ecology Resources*, *23*, 396–409.
- Pursiainen, A., Veneranta, L., Kuningas, S., Saarinen, A., & Kallasvuo, M. (2021). The more sheltered, the better – Coastal bays and lagoons are important reproduction habitats for pike in the northern Baltic Sea. *Estuarine, Coastal and Shelf Science*, *259*, 107477.
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Raat, A. J. P. (1988). *Synopsis of Biological Data on the Northern Pike Esox lucius Linnaeus, 1758* (p. 178). Synopsis, FAO.
- Roche, D. G., Kruuk, L. E. B., Lanfear, R., & Binning, S. A. (2015). Public data archiving in ecology and evolution: How well are we doing? *PLoS Biology*, *13*(11), e1002295. <https://doi.org/10.1371/journal.pbio.1002295>
- Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, *4*, 9–33.
- Rourke, M. L., Walburn, J. W., Broadhurst, M. K., Fowler, A. M., Hughes, J. M., Fielder, D. S., DiBattista, J. D., & Furlan, E. M. (2022). Poor utility of environmental DNA for estimating the biomass of a threatened freshwater teleost; but clear direction for future candidate assessments. *Fisheries Research*, *258*, 106545.
- 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*, 1–9.
- Seymour, M., & Smith, A. (2023). Arctic char occurrence and abundance using environmental DNA. *Freshwater Biology*, *68*, 781–789.
- Shelton, A. O., Ramón-Laca, A., Wells, A., Clemons, J., Chu, D., Feist, B. E., Kelly, R. P., Parker-Stetter, S. L., Thomas, R., Nichols, K. M., & Park, L. (2022). Environmental DNA provides quantitative estimates of Pacific hake abundance and distribution in the open ocean. *Proceedings of the Royal Society B: Biological Sciences*, *289*, 20212613.
- Skov, C., & Nilsson, P. A. (Eds.). (2018). *Biology and ecology of pike*. CRC Press.
- Song, J. W., Small, M. J., & Casman, E. A. (2017). Making sense of the noise: The effect of hydrology on silver carp eDNA detection in the Chicago area waterway system. *Science of the Total Environment*, *605–606*, 713–720.
- Spear, M. J., Embke, H. S., Krysan, P. J., & Zanden, M. J. V. (2021). Application of eDNA as a tool for assessing fish population abundance. *Environmental DNA*, *3*, 83–91.
- Stoeckle, B. C., Beggel, S., Cerwenka, A. F., Motivans, E., Kuehn, R., & Geist, J. (2017). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. *PLoS One*, *12*, e0189119.
- Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., & VanMorter, S. M. (2021). Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, *78*, 293–304.
- Sundblad, G., & Bergström, U. (2014). Shoreline development and degradation of coastal fish reproduction habitats. *Ambio*, *43*, 1020–1028.
- Svensson, R. (2021). *Development of northern pike (Esox lucius) populations in the Baltic Sea, and potential effects of grey seal (Halichoerus grypus) predation*. Master thesis in Biology, Swedish University of Agricultural Sciences.
- Takahara, T., Minamoto, T., & Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS One*, *8*, e56584.
- Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS One*, *7*, e35868.
- 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.
- 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*, e41732.

- 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.
- Tsuji, S., & Shibata, N. (2021). Identifying spawning events in fish by observing a spike in environmental DNA concentration after spawning. *Environmental DNA*, *3*, 190–199.
- Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T., & Yamanaka, H. (2017). Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. *PLoS One*, *12*, e0176608.
- Turkson, A. J., Ayiah-Mensah, F., & Nimoh, V. (2021). Handling censoring and censored data in survival analysis: A standalone systematic literature review. *International Journal of Mathematics and Mathematical Sciences*, *2021*, e9307475.
- Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). Springer.
- Villegas-Ríos, D., Alós, J., Palmer, M., Lowerre-Barbieri, S., Bañón, R., Alonso-Fernández, A., & Saborido-Rey, F. (2014). Life-history and activity shape catchability in a sedentary fish. *Marine Ecology Progress Series*, *515*, 239–250.
- Wennerström, L., Olsson, J., Ryman, N., & Laikre, L. (2016). Temporally stable, weak genetic structuring in brackish water northern pike (*Esox lucius*) in the Baltic Sea indicates a contrasting divergence pattern relative to freshwater populations. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*, 1–10.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., ... Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*, 1686.
- Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., Whiteley, A. R., Lowe, W. H., & Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216.
- Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K., & Elser, J. J. (2003). Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology*, *17*, 237–245.
- Wu, L., Wu, Q., Inagawa, T., Okitsu, J., Sakamoto, S., & Minamoto, T. (2023). Estimating the spawning activity of fish species using nuclear and mitochondrial environmental DNA concentrations and their ratios. *Freshwater Biology*, *68*, 103–114.
- Yates, M. C., Cristescu, M. E., & Derry, A. M. (2021). Integrating physiology and environmental dynamics to operationalize environmental DNA (eDNA) as a means to monitor freshwater macro-organism abundance. *Molecular Ecology*, *30*, 6531–6550.
- 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*, 5–13.
- 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*, 3068–3082.
- 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*, 553–560.
- Yates, M. C., Wilcox, T. W., Stoeckle, M. Y., & Heath, D. D. (2022). Interspecific allometric scaling in eDNA production in fishes reflects physiological and surface area allometry. *bioRxiv*.
- Zhang, D. (2022). *rsq: R-squared and related measures*.
- Zhang, J., Ding, R., Wang, Y., & Wen, J. (2022). Experimental study on the response relationship between environmental DNA concentration and biomass of *Schizothorax prenanti* in still water. *Frontiers in Ecology and Evolution*, *10*. <https://doi.org/10.3389/fevo.2022.972680>

SUPPORTING INFORMATION

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

How to cite this article: Ogonowski, M., Karlsson, E., Vasemägi, A., Sundin, J., Bohman, P., & Sundblad, G. (2023). Temperature moderates eDNA–biomass relationships in northern pike. *Environmental DNA*, *5*, 750–765. <https://doi.org/10.1002/edn3.440>