Received: 16 February 2024 | Revised: 1 July 2024 | Accepted: 29 July 2024

DOI: 10.1111/fwb.14320

Freshwater Biology WILEY

ORIGINAL ARTICLE

Aquatic invasive species exhibit contrasting seasonal detectability patterns based on environmental DNA: Implications for monitoring

Christopher I. Rounds[1] | Todd W. Arnold[1] | Chan Lan Chun[2,3] | Josh Dumke[2] | Anna Totsch[2] | Adelle Keppers[2] | Katarina Edblad[2] | Samantha M. García[4] | Eric R. Larson[4] | Jenna K. R. Nelson[1,5] | Gretchen J. A. Hansen[1]

[1]Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota Twin Cities, St. Paul, Minnesota, USA

[2]Natural Resources Research Institute, University of Minnesota Duluth, Duluth, Minnesota, USA

[3]Department of Civil Engineering, University of Minnesota Duluth, Duluth, Minnesota, USA

[4]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois, USA

[5]Minnesota Department of Natural Resources, St. Paul, Minnesota, USA

Correspondence
Christopher I. Rounds, Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota Twin Cities, St. Paul, MN, USA.
Email: round060@umn.edu

Funding information
Minnesota Environment and Natural Resources Trust Fund

Abstract

1. Aquatic invasive species (AIS) are a global threat to freshwater biodiversity and ecosystem services. Documenting AIS prevalence at broad spatial scales is critical to effective management and early detection. However, conventional monitoring for AIS is costly and is rarely applied at the resolution and scale required for effective management. Monitoring of AIS using environmental DNA (eDNA) has the potential to enable broadscale surveillance at a fraction of the cost of conventional methods, but key questions must first be addressed related to how eDNA detection probability varies among environments, seasons, and multiple species with different life histories.
2. To quantify spatiotemporal variation in the detection probability of AIS using eDNA sampling, we surveyed 20 lakes with known populations of four aquatic invasive species: common carp (*Cyprinus carpio*), rusty crayfish (*Faxonius rusticus*), spiny waterflea (*Bythotrephes longimanus*), and zebra mussels (*Dreissena polymorpha*). We collected water samples at 10 locations per lake, five times throughout the open water season resulting in a total of 1,000 water samples. Quantitative polymerase chain reaction was used with species-specific assays to determine the presence of each species' eDNA in water samples. With Bayesian occupancy models, we quantified the effects of lake and site characteristics and Julian date on eDNA detection probability.
3. The probability of eDNA detection varied seasonally, and the seasonal variation was species-specific and related to species life histories. Zebra mussel eDNA was generally the most detectable among the species we targeted, and detection probability peaked in midsummer when only six water samples were required to achieve a 95% probability of detection (80% Bayesian credible interval: 3–12 samples). Spiny waterflea eDNA detections also peaked in mid to late summer, but were overall the most difficult species to detect, requiring 160 samples for

a 95% probability of detection (80% Bayesian credible interval: 67–1,616 samples). Common carp eDNA was most detectable in the spring and rusty crayfish eDNA was most detectable in the early autumn, corresponding to key life history events.
4. Sampling for eDNA during the optimal time of the year for each species decreased the number of samples required to reach a 95% probability of detection by an order of magnitude or more.
5. Our results are relevant for decision makers interested in using eDNA as a multi-species monitoring tool and highlight the importance of life history in the efficacy of eDNA monitoring.

KEYWORDS
Bayesian hierarchical models, *Bythotrephes longimanus*, *Cyprinus carpio*, *Dreissena polymorpha*, *Faxonius rusticus*, life history, multi-species monitoring, occupancy model, seasonality

1 | INTRODUCTION

Aquatic invasive species (AIS) can alter the ecosystems they invade and cause significant ecological and economic impacts (Ehrenfeld, 2010). Species invasions can decrease native biodiversity; decrease the abundance, growth, and survival of recreationally important native species; and shift ecosystems to undesirable conditions (Gallardo et al., 2016). To mitigate these negative effects, billions of dollars have been spent on AIS management in the U.S.A. (Cuthbert et al., 2021), primarily on reactive management once a species has become established. Prevention of species invasions is a more effective and efficient use of resources (Simberloff et al., 2013), which requires monitoring to determine if prevention methods are working and to identify new invasions. Early detection of new invasives can be a fail-safe when prevention efforts fail (Reaser et al., 2020). Although broadscale monitoring of AIS presence is important for early detection of new invasions, widespread, coordinated monitoring programmes are uncommon (Lodge et al., 2016; Vander Zanden et al., 2010). Most regions lack information on the true prevalence and distribution of AIS due to a lack of statistically valid sampling across the landscape, instead relying on opportunistic reporting, which almost certainly underestimates prevalence (Latzka et al., 2016; Vander Zanden et al., 2010).

Effective and inexpensive monitoring techniques capable of detecting multiple AIS are needed to establish a baseline of AIS prevalence and to rapidly respond to new invasions (Reaser et al., 2020; Vander Zanden et al., 2010). However, physical surveys using conventional collection and identification methods require considerable effort and expertise (Bonar et al., 2009; Trebitz et al., 2017), and physical detections may still lag many years behind a species' first arrival to a new habitat (Branstrator et al., 2017). These surveys often miss species when they are at low abundances, are time and resource intensive, and are species or taxa-specific (Borrell et al., 2017; Evans, Li, et al., 2017; Evans, Shirey, et al., 2017). Invasive species frequently occur at low abundances, particularly early in their invasion status (Spear et al., 2021), making them difficult to detect (McCarthy et al., 2013). However, the detection of invasions when the species is at a low abundance provides a greater chance of successful management or eradication (Hulme, 2006; Myers et al., 2000; Simberloff, 2009). Robust and inexpensive monitoring procedures capable of detecting diverse AIS are therefore required to establish baselines of AIS prevalence and monitor for new invasions (Lodge et al., 2016).

Environmental DNA (eDNA) is a promising focus for AIS monitoring (Ficetola et al., 2008; Jerde et al., 2011). Environmental DNA is DNA collected and identified from environmental samples such as soil, water, or air, without requiring collection of target organisms (Barnes & Turner, 2016). In aquatic systems, eDNA is usually collected by taking water samples, filtering the water to capture DNA, extracting DNA, and then using genetic tools to detect and quantify the target DNA (for more see Ruppert et al., 2019). The use of eDNA has numerous advantages over conventional sampling methods. For instance, samples can be analysed using molecular methods to detect multiple species from broad taxonomic groups (Dysthe et al., 2018; West et al., 2020), which may only be possible using multiple sampling methods with conventional monitoring. Furthermore, eDNA can detect species at low abundances (Goldberg et al., 2015; Jerde et al., 2011). Additionally, eDNA sampling methods are inexpensive relative to traditional sampling (Davy et al., 2015; Evans, Li, et al., 2017), particularly when sampling for multiple species (Andres et al., 2023; McColl-Gausden et al., 2021). Collectively, these advantages mean eDNA is conducive to effective and widespread multi-species monitoring efforts (Jeremy et al., 2015).

Although eDNA monitoring offers many potential benefits, its application requires an understanding of factors that influence species detectability (Bylemans et al., 2019; Troth et al., 2021). Seasonal trends of eDNA concentrations can have a large impact on detection probability (Erickson et al., 2017), which can vary due to a species' life history or annual cycle (Collins et al., 2022;

de Souza et al., 2016; Dunn et al., 2017; Milhau et al., 2021). A well-documented example of seasonally varying eDNA detectability is greater detection probability during spawning events (Bayer et al., 2019; Takahashi et al., 2018; Takeuchi et al., 2019; Tsuji & Shibata, 2021). When sampling for more than one species, particularly species with vastly different life histories, the effects seasonality and species life history have on eDNA detection probability may obfuscate optimal sample timing. The effect of species life history on eDNA detectability is particularly important for metabarcoding studies, where detection probabilities are typically lower than single species detection methods (McColl-Gausden et al., 2023), therefore timing samples to correspond with periods of highest detection probabilities is critical. Environmental conditions (e.g., water clarity, temperature, pH) also influence eDNA detection probability, and the occurrence of unfavourable conditions may require increased sampling effort for species detections (Barnes et al., 2014; Jo & Minamoto, 2021; Wang et al., 2021). Understanding how life history and environmental factors affect eDNA detections can inform allocation of sampling efforts to infer species presences.

The objective of our research was to quantify the influence of seasonality and environmental conditions on the detectability of multiple species using eDNA. We sought to determine the optimal timing of eDNA sampling for four important AIS: common carp (*Cyprinus carpio*), rusty crayfish (*Faxonius rusticus*), spiny waterflea (*Bythotrephes longimanus*), and zebra mussel (*Dreissena polymorpha*). We estimate the eDNA detection probability of these four species over the open water season and the number of samples required to detect each species at a given probability threshold using eDNA. Additionally, we show how the clarity of a lake, stratification status, and sampling site water depth affect eDNA detection probabilities. Although our work focuses on four species, our methodology is relevant for designing effective eDNA sampling for diverse assemblages of organisms across any landscape.

2 | METHODS

2.1 | Study species

We sampled 20 lakes in Minnesota, U.S.A. with known populations of common carp, spiny waterflea, rusty crayfish, and zebra mussels (Figure 1a; Table 1). These species were chosen because they are widespread high-priority invasive species in Minnesota with different life histories that we hypothesised would lead to different seasonal patterns of eDNA detections (Figure 1b). The lakes were chosen based on the known occupancy of one or more of the target species, and to span a range of physical and chemical characteristics (i.e., lake size, maximum depth, ecoregion; Table 1). The in-lake occupancy of common carp was determined using previous Minnesota Department of Natural Resources (MNDNR) fisheries surveys, spiny waterflea and zebra mussel occupancy was determined using the MNDNR Infested Waters List (Minnesota Department of Natural Resources, 2023), and rusty crayfish occupancy was determined by prior EDDMapS detections (EDDMapsS, 2023).

We expected that the eDNA of common carp, a littoral and highly fecund fish, would be most detectable in spring and early summer based on the formation of large spawning aggregations (Bajer & Sorensen, 2010; Di Muri et al., 2022), and that eDNA detectability would decrease later in the year. We predicted that peak eDNA detectability of rusty crayfish, a benthic decapod, would coincide with moulting events or ovigery in the late summer to early autumn (Crocker & Barr, 1968; Dunn et al., 2017; Somers & Green, 1993), and that eDNA detectability would be lower in the spring and early summer. Spiny waterflea, a large cladoceran zooplankton, were expected to be most detectable with eDNA in late summer to early autumn when they reach peak abundance (Walsh et al., 2019; Yan et al., 2001). Zebra mussel, a small freshwater mussel, was expected to reach peak eDNA detectability in mid-summer coinciding with release of their planktonic veligers (Haag & Garton, 1992).

2.2 | Water sampling

We sampled each lake during a single year throughout the ice-free season. For each lake, we collected water samples from 10 sampling points. Eight points were split evenly between pelagic and littoral areas of the lake and were randomly selected based on existing state agency water sampling points. The remaining two points were at a boat launch and the deepest point of the lake. We visited each lake five times throughout the open water season (ice on to ice off) in 2021 or 2022. Lakes were first sampled within 4 weeks of ice-off. The remaining sampling events occurred every 4–6 weeks, with the final sampling event occurring as close as possible to anticipated lake freeze-up.

During each lake visit, we collected 250 ml of surface water in high-density polyethylene bottles at each of the 10 sampling points. To prevent contamination, we rinsed bottles with 10% bleach pre-sampling, wore gloves while taking samples, and switched gloves between samples. We brought a 250-ml field control to each lake as an indicator of field contamination (Morisette et al., 2021). The field control was filled with deionised water prior to sampling and opened for 5 s prior to the start of sampling. At the deepest point of each lake, we measured Secchi depth, pH, and conductivity at the surface and recorded a temperature and dissolved oxygen profile from the surface to the bottom of the lake at 1-m increments.

2.3 | Molecular analyses

Water samples were kept in the dark and on ice between sample collection and filtration (Curtis, Larson, & Davis, 2021). Water samples were brought back to the lab for filtration and preservation, which usually occurred within 12 hr but in some cases 36 hr of sample collection, limiting eDNA degradation (Curtis, Larson, & Davis, 2021). Each water sample was prefiltered through a 75-μm stainless steel

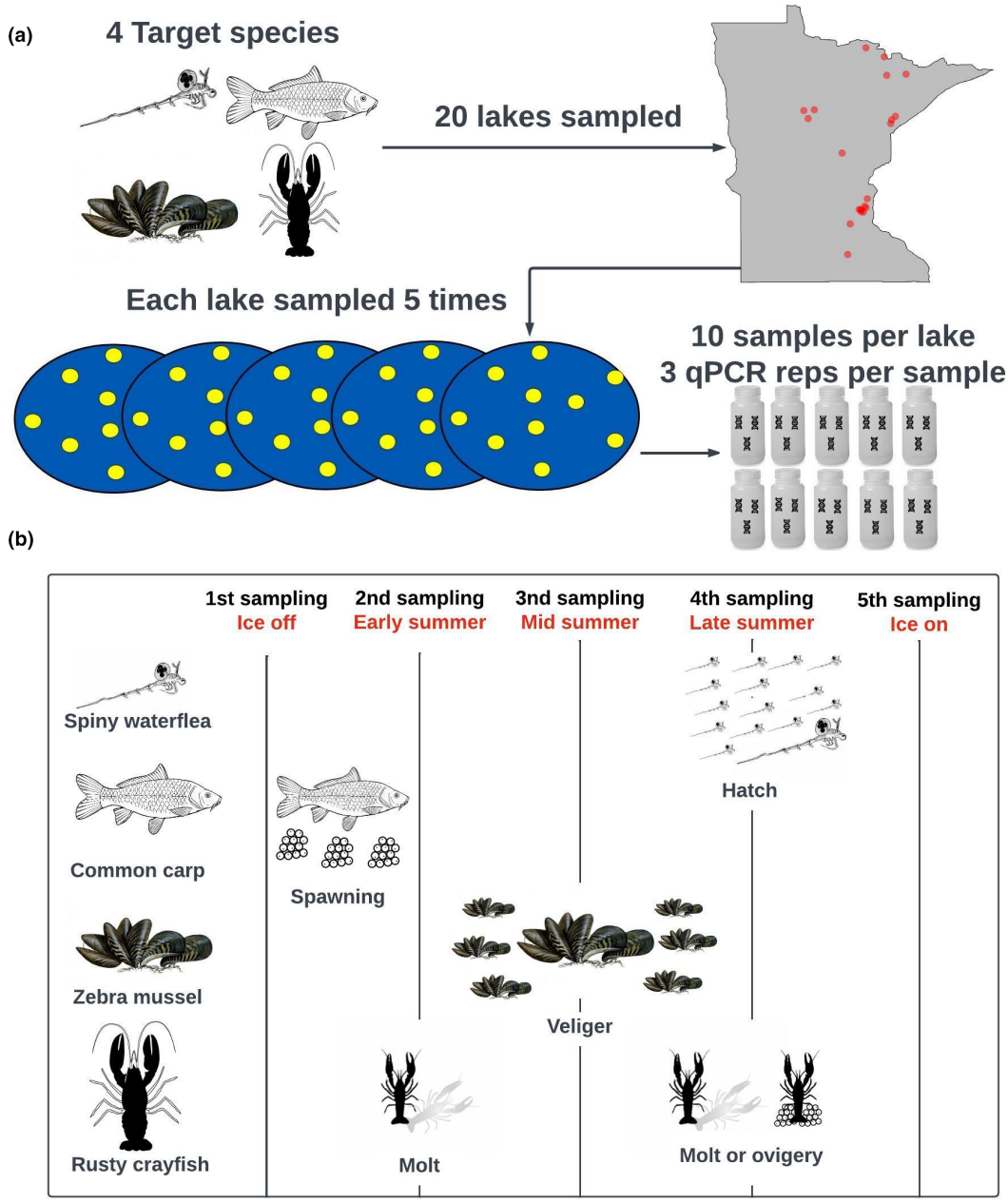

FIGURE 1 Conceptual model of environmental DNA sampling and life history events expected to correlate with peak environmental DNA detections. (a) We selected four aquatic invasive species with known populations in 20 different lakes throughout Minnesota. We sampled each lake five times by taking 10 water samples at fixed locations within lakes and analysed three quantitative polymerase chain reaction replicates from each water sample. (b) Species icon positions correspond to when peak detections were predicted to occur. Spiny waterflea detections should peak when they reach peak abundance and hatch in late summer. Common carp detections should peak between ice-off and early summer when carp spawn. Zebra mussel detections should peak near mid-summer when they release veligers. Rusty crayfish detections should peak in the summer or early autumn due to moults or ovigery. Icons were provided by the Minnesota Department of Natural Resources.

mesh filter to remove large debris and reduce filter clogging (Wilson et al., 2014). The remaining water was filtered through a 1-μm, 47-mm diameter cellulose-nitrate filter (Whatman Cytivia #7190004) using a vacuum pump. All water samples were able to filter without clogging. Filters were preserved in 95% ethanol and stored at −20°C until DNA extraction. For each step of laboratory processing, we wore clean gloves and used equipment that was soaked in 10% bleach prior to use. Any physical workspace was cleaned with 10% bleach between samples. Downstream laboratory processes were split between two separate academic labs, with both labs having demonstrated repeatable and reproducible results (García et al., 2024; Appendix S1: Section S1).

At the time of DNA extraction, filters were removed from their vials and cut in half with sterile razors. Half of the filter was used for

TABLE 1 Summary of characteristics of our 20 study lakes, including geographic coordinates (lat, long), surface area (km^2), and maximum depth (m) clarity 9 m, as well as species detected historically and with environmental DNA (eDNA) of the 20 lakes included in this study.

Lake	Lat	Long	Species present	Species missed with eDNA	New species detected with eDNA	Area (km^2)	Max depth (m)	Clarity (m)
Bald Eagle	45.113	−93.015	CC, ZM	ZM	RC	42	11	2.3
Benedict	47.140	−94.695	RC, ZM	–	CC	19	27.7	3.2
Clear	44.088	−93.485	CC	–	–	26	10.4	1.4
Crane	48.289	−92.473	RC, SWF	SWF	–	118	24.4	1.7
Fish	46.994	−92.275	SWF	SWF	–	132	11	1.5
Forest	45.273	−92.952	CC, ZM	–	RC	92	11.3	2.6
Island	47.018	−92.178	SWF	SWF	–	324	28.7	1.2
Johanna	45.044	−93.171	CC, ZM	ZM	RC	9	13.1	3.1
Kabetogama	48.474	−92.996	SWF	SWF	CC, RC	973	24.4	2.1
Leech	47.160	−94.443	RC, ZM	–	CC	4170	45.7	2.7
McCaron's	44.998	−93.113	ZM	CC	RC	3	17.4	3.7
Mille Lacs	46.251	−93.646	CC, SWF, ZM	SWF	RC	5190	12.8	3.0
Owasso	45.036	−93.123	CC, ZM	RC	SWF	15	11.3	3.0
Phalen	44.989	−93.055	CC, RC	–	–	8	27.7	2.7
Pike	46.866	−92.302	ZM	–	–	20	18.3	2.9
Lower Prior	44.735	−93.414	CC, ZM	–	RC	39	18.3	5
Shagwa	47.916	−91.886	RC, SWF	SWF	CC	95	14.6	2.4
Ten Mile	46.970	−94.577	ZM	ZM	RC	206	27.7	5.1
Vermillion	47.861	−92.333	RC, SWF	–	ZM	1589	23.2	2.7
White Bear	45.083	−92.084	CC, ZM	–	RC	98	25.3	5.7

Note: The species present refers to which of the four AIS have been previously recorded in a lake. Species missed is the species not detected with environmental DNA sampling. New species detected is the species detected using environmental DNA sampling that were not previously known to exist in a lake.

Abbreviations: CC, common carp; RC, rusty crayfish; SWF, spiny waterflea; ZM, zebra mussel.

extraction and analysis, and the remaining half was stored in 95% ethanol at −20°C as an archive. To isolate DNA from filters, we used the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's spin-column protocols (Eichmiller et al., 2016), modified by running the final elute through the column twice and only using 50 μl of Solution C6. The PowerSoil Pro kit was chosen because it can reduce common polymerase chain reaction (PCR) inhibitors (Lear et al., 2018; Pearman et al., 2020) anticipated to occur in some of our study lakes (e.g., organic matter, algae, iron) although we did not test for inhibition explicitly. We conducted all DNA extractions in rooms separate from spaces with high copy DNA from PCR. Between extractions, we sterilised workspaces with 50% bleach and UV radiation for 30 min (Goldberg et al., 2016). Between samples, we switched gloves and used sterile equipment (Goldberg et al., 2016). Extractions were performed one lake visit at a time, and for each extraction, we used a lab blank (a sterile filter unexposed to any biological material) to assess lab contamination. The extracted genomic materials were stored at −80°C until quantitative PCR (qPCR) analysis.

To determine eDNA presence and concentration, we used species-specific triplicate qPCRs. We used primers targeting mitochondrial cytochrome oxidase subunit regions for each species based on existing assays developed and validated for the region they are used (Appendix S1: Table S1; common carp, Eichmiller et al., 2014; rusty crayfish, Coster et al., 2021; García et al., 2024; spiny waterflea, Walsh et al., 2019; zebra mussel, Amberg et al., 2019). One laboratory used a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and a QuantStudio™ 3 Real-Time qPCR machine (Thermo Fisher Scientific, Waltham, U.S.A.), whereas the other laboratory used a QuantStudio™ 3 Real-Time qPCR (for more information see García et al., 2024). We ran all samples from a lake-visit together. Detailed qPCR conditions can be found in Appendix S1: Section S1 and Appendix S1: Table S1. qPCR reactions were done in separate rooms from extractions, and we followed best practices to minimise contamination between samples (Goldberg et al., 2016). Each plate contained four non-template controls (1 μl molecular grade water). For each plate, we used species-specific gBlock fragments to create standard curves with 10-fold serial dilutions ranging from 0.1 to 100,000 copies per reaction (Integrated DNA Technologies, Coralville, IA, U.S.A.). We determined whether amplification occurred using qPCR software from each machine. We defined plate-level limits of detection (LOD) calculated following

Klymus et al. (2020). The LOD was based on three replicates, and we considered any qPCR replicate from a given site above the LOD as a positive detection. The mean qPCR efficiency, r^2, and LOD can be found in Table 2. Since we were interested in whether the detection probability changed over time (and not the copy numbers of eDNA), we do not report the limit of quantification. Additionally, we excluded samples from any lake-visit that had field or lab blank qPCR amplification above the LOD.

2.4 | Data analyses

We used a Bayesian multi-species occupancy model to quantify how the probability of detection in a water sample of four AIS using eDNA was influenced by seasonality and environmental conditions. Multi-species occupancy models are often hierarchical, which allows data from all species to contribute to estimates of species-specific occupancy and detection probability (Dorazio & Royle, 2005; White et al., 2020). Given our primary focus on factors affecting eDNA detection probabilities, and that lake-level occupancy of each species was known a priori, we did not estimate occupancy directly, but instead focused on detection probability by conditioning on known occupancy or eDNA detections from this study.

We focused our modelling efforts on covariates of detection because determining optimal eDNA sampling schemes requires determining when and where to sample for each species, and how much to sample based on the detectability of the organism. However, we also included other site-level covariates hypothesised to influence the availability of eDNA. These covariates were water clarity, which we thought would decrease sample level detectability due to UV DNA degradation (Kessler et al., 2020; Strickler et al., 2015); percentage of the water column mixed, which we assumed would decrease detectability in a sample due to a dilution effect (Curtis, Tiemann, et al., 2021; Klobucar et al., 2017); and sampling site depth, which would decrease sample level detectability, especially for common carp and rusty crayfish that live in primarily littoral areas. The model allows the estimation of relationships between covariates and response:

$$\begin{aligned}\text{logit}\left(\rho_{s,i,j,v}\right) = {} & \beta_{0,s} + \beta_{s,i} + \beta_{s,i,j} + \beta_{1,s}\text{JulianDay}_{i,v} \\ & + \beta_{2,s}\text{JulianDay}^2{}_{i,v} + \beta_{3,s}\text{Clarity}_{i,v} \\ & + \beta_{4,s}\text{PercentMixed}_{i,v} + \beta_{5,s}\text{Depth}_{i,j}\end{aligned} \quad (1)$$

where p is detection probability, s indexes species, i indexes lake, j indexes sites within a lake, and v represents lake-visit. The detection model has species-specific intercepts $\beta_{0,s}$, with nested random intercepts for lake and site-within-lake, $\beta_{s,i}$ and $\beta_{s,i,j}$, respectively. Depth is the depth of the water column from lake i at site j. Julian day is the numeric day of year at the time of sampling, Clarity is the Secchi depth for the lake at the time of sampling, and PercentMixed is the percentage of the water column at the maximum depth that is within 1°C of the surface of the lake. All covariates were standardised to have a mean of 0 and an *SD* of 1. For site depth, we standardised after log transformation because of a strongly right-skewed distribution. We quantified the detection or non-detection of three qPCR replicates. The sub-sample detection probability is given by

$$\rho_{s,i,j,v,k} = 1 - \left(1 - \rho_{s,i,j,v}\right)^{-3} \quad (2)$$

using the same notation defined in Equation (1) and where k indicates qPCR replicate and 3 is the total number of qPCR replicates. Therefore, the observation model per sub-sample is

$$y_{s,i,j,v,k} \sim \text{Binomial}\left(\rho_{s,i,j,v,k}, z_{s,i}\right) \quad (3)$$

where $z_{s,i}$ describes the known presence ($z_{s,i}=1$) or absence ($z_{s,i}=0$) of species s in lake i. The observed variable, $y_{s,i,j,v,k}$ describes the detection of the species s at lake i, site j, lake visit v, and qPCR replicate k, which is a random variable determined by the detection probability $\rho_{s,i,j,v,k}$.

For model parameters, we used diffuse priors. Specifically, we used half-Cauchy hyperpriors for standard deviations (Outhwaite et al., 2018). Priors for regression coefficients (β), including intercepts were estimated by treating species as a random effect and drawing species-specific regression coefficients from a shared normal distribution, e.g., $\beta \sim N(\mu, \sigma)$, where $\mu \sim N(0, 1.75)$ and $\sigma \sim \text{Unif}(0,1)$ (Boone et al., 2023; Mordecai et al., 2011; White et al., 2020). For example, the species-specific effect of clarity, $\beta_{3,s}$, is derived from a normal distribution with a mean ($\mu_{\beta 3}$) and variance ($\sigma^2{}_{\beta 3}$) corresponding to $\beta_{3,s} \sim N(\mu_{\beta 3}, \sigma^2{}_{\beta 3})$. This provides a vague prior after transforming values from the logit to the real scale.

The model was fit in JAGS using the package rjags (Su & Yajima, 2015). For each model, we used 500,000 iterations with a burn-in of 100,000 on three chains with a thinning rate of one in 10 yielding 120,000 posterior samples. To assess model convergence, we visually inspected all trace plots and ensured the Gelman–Rubin criterion r-hat values <1.01 (Gelman & Rubin, 1992). We also

TABLE 2 Quantitative polymerase chain reaction (qPCR) efficiency, r^2, and limits of detection (LOD) for the four target species.

Species	qPCR efficiency	r^2	LOD (copies/μL)
Common carp	97.1 (95.5–98.8)	0.986 (0.984–0.989)	4.27 (2.90–5.65)
Rusty crayfish	98.2 (94.6–101.9)	0.982 (0.978–0.985)	6.73 (3.66–9.79)
Spiny waterflea	96.9 (93.3–100.5)	0.985 (0.982–0.988)	11.42 (3.99–18.86)
Zebra mussel	105.9 (103.8–107.9)	0.980 (0.976–0.985)	2.40 (1.60–3.20)

Note: Mean quantitative PCR efficiency, r^2, and mean LOD values for the four target species with 95% confidence intervals in parentheses. The LOD is the three-replicate LOD calculated from Klymus et al. (2020).

calculated the Bayesian *p*-value (Kéry & Schaub, 2012) to assess goodness-of-fit. All covariates included in the model had variance inflation factors below 2, suggesting no evidence of multicollinearity (Fieberg, 2024; Zuur et al., 2009). We made inferences on covariate effect sizes based on the posterior mean and 80% Bayesian credible intervals (BCIs). We plotted these effects over the observed range of covariate values while holding other covariates at their mean and setting random effects to zero. Further, we used the mean posterior eDNA detection probability in a water sample (ρ) to calculate the number of water samples required to detect a species given its presence.

$$N = \log(1-\alpha)/\log(1-\rho) \quad (4)$$

where we used α equal to 0.95 (McArdle, 1990). All modelling was done in R version 4.1.2 (R Core Team, 2021) and visualisations were made using ggplot2 (Wickham, 2016). All data and code are stored at the Data Repository for the University of Minnesota (Rounds et al., 2023).

3 | RESULTS

We detected common carp eDNA in 13 of the 20 lakes sampled, rusty crayfish eDNA in 15 of the 20 lakes, spiny waterflea eDNA in two of 20 lakes, and zebra mussel eDNA in 10 of 20 lakes. The eDNA of all four species was detected in at least one lake where they were previously unknown to occur. Common carp eDNA was detected in four lakes where they were not known to occur with an average of 6.5 positive qPCR replicates in each new lake detected of 150 qPCR replicates possible (five sampling events × 10 samples × three qPCR replicates). Rusty crayfish eDNA was detected in nine lakes where they were not known to occur with an average of 4.75 positive qPCR replicates in each new lake detected of 150 qPCR replicates possible. Spiny waterflea eDNA was detected in one lake where they were not known to occur with three positive qPCR replicates of 150 possible, and zebra mussel eDNA was detected in one lake where they were not known to occur with two positive qPCR replicates of 150 qPCR replicates possible. In lakes where AIS were known to occur based on previous surveys, we failed to detect the eDNA of common carp in one lake, rusty crayfish in two lakes, spiny waterflea in six lakes, and zebra mussels in three lakes. We excluded all samples from lake visits that had field or lab blank contamination. This eliminated samples from one lake visit for common carp (1% contaminated; lab blank), two lake visits for rusty crayfish (2% contaminated; both field blanks), one lake visit for spiny waterflea (1% contaminated; lab blank), and eight lake visits for zebra mussels (8% contaminated; four lab and four field blanks).

3.1 | Occupancy model results

Our model converged, and goodness-of-fit tests indicated no discernible problems (Bayesian *p*-value = 0.15). Based on posterior distributions, detection probabilities varied among sites within a lake to a greater extent than they varied among lakes (Appendix S1: Figure S1). However, detection probabilities varied among species to the greatest extent.

Environmental DNA detection probability decreased throughout the open-water season for common carp, peaked near August–September for rusty crayfish, and peaked in July–August for spiny waterflea and zebra mussels (Figure 2a). Zebra mussel eDNA was most detectable throughout the open water season compared to other species and required six samples (80% BCI: 3–12 samples) for a 95% probability of detection at peak detectability. Environmental DNA detection probability for spiny waterflea was consistently the lowest (below 0.02). The optimal time to sample to detect all four species using eDNA was therefore determined by the detection probability of spiny waterflea and occurred in July–August, requiring 160 samples for a 95% probability of detection (Figure 2b). The best-case scenario (10% credible interval) for eDNA detection probability for spiny waterflea during this period was 67 samples, while the worst-case scenario (the 90% credible interval) was 1,616 samples.

Environmental DNA detection probability generally increased with increasing water clarity (Figure 3a), but water clarity negatively affected eDNA detection probability of common carp (Figure 4). Similarly, the percentage of the water column mixed increased the eDNA detection probability for three of the four species, but again the effect was negative for common carp, and the 80% BCI overlapped zero for common carp and rusty crayfish (Figures 3c and 4). Sampling site depth had a negative effect on the eDNA detection probability for all four species (Figure 3e), but the 80% BCI overlapped zero for spiny waterflea (Figure 4). Our estimates of species-specific covariates of eDNA detection had variability not represented in Figure 3; for graphical estimates of variability see Figure 4.

4 | DISCUSSION

We sampled eDNA from 20 lakes, five different times throughout the open water season resulting in a total of 1,000 water samples to determine factors influencing the detection probability of four common AIS. Environmental effects (clarity, site depth, and lake mixing) on eDNA detection probabilities were species-specific. The Julian date strongly affected eDNA detection probabilities, with different species showing different patterns of peak eDNA detectability. The common carp eDNA detection probability decreased throughout the year, whereas rusty crayfish eDNA detection probability peaked in late summer and early autumn, and spiny waterflea and zebra mussels eDNA detection probability peaked in mid-summer. For our suite of species, optimal sampling timing and effort is driven by the hardest-to-detect species, spiny waterflea, and we suggest an effort of 160 field samples in July–August to achieve a 95% detection probability of all four species (80% BCI: 67–1,616 samples). Excluding spiny waterflea decreases the required effort substantially and changes the optimal timing of eDNA sampling (optimal timing in between June and July with an

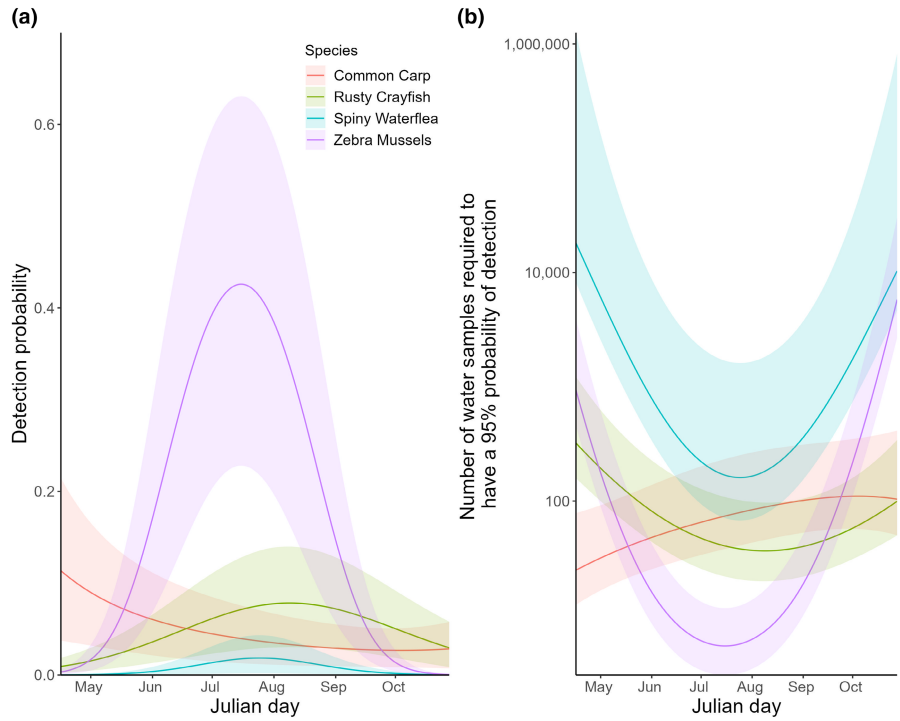

FIGURE 2 Seasonal trends in environmental DNA detection probability per water sample (a) and number of environmental DNA water samples required to have a 95% probability of detecting a species given its presence on the log scale (b). Shaded bands indicate 80% credible intervals. Shaded bands and lines are evaluated for predicted effects when other covariates are at their mean and random effects for lake and site are set to zero.

effort of 57 samples for a 95% probability of detection; 80% BCI: 31–154 samples).

Our results indicate that different species have distinct periods of peak detectability by eDNA sampling, and species generally followed our hypotheses about the seasonality of detections (Figures 1b and 2a). Common carp detectability with eDNA decreased over the season, following our expectations that detectability would decrease after spring spawning (Figure 2a). It is possible that common carp detection probability was greatest in the spring due to large spring spawning events (Bajer & Sorensen, 2010; Banet et al., 2022). For rusty crayfish, the role of seasonality effects were less pronounced (Figure 2a). However, it is likely that peak detectability during the summer–autumn months can be attributed to a combination of increases in crayfish density, activity, and the onset of ovigery (Kvistad et al., 2021, 2023; Somers & Green, 1993). We found that spiny waterflea eDNA was the most difficult to detect. Similar to Walsh et al. (2019), we found peak (albeit still low) detection probability of spiny waterflea eDNA during July–August. Previous research has found that zebra mussel eDNA detections are greatest in mid-summer (Sepulveda et al., 2019) and probably relate to the release of veligers (Haag & Garton, 1992). Our results indicate that zebra mussel eDNA is highly detectable in mid-summer but decreases markedly in spring and autumn.

Multiple studies have documented that eDNA detectability can vary seasonally or with life history events for target taxa (Dunn et al., 2017; Takahashi et al., 2018; Takeuchi et al., 2019), but few have evaluated seasonal eDNA detectability between multiple species (Collins et al., 2022; de Souza et al., 2016; Erickson et al., 2017) or integrated this seasonality into sampling recommendations for multi-species communities of AIS. In our case, optimal sampling for eDNA surveillance of AIS was determined by the hardest-to-detect species, the spiny waterflea. However, different managers or policymakers could have different priorities for monitoring that might omit this species, and our other three focal taxa evidence tradeoffs in sampling design dependent on species-specific eDNA detectability by season. For example, if sampling in mid-July, zebra mussel eDNA could be detected with high confidence (95%) using only six samples per lake (80% BCI: 3–11 samples) and rusty crayfish eDNA could be detected with only 40 samples per lake (80% BCI: 21–106 samples), but common carp would require 75 samples per lake (80% BCI: 39–239 samples). Conversely, if common carp was the highest priority species for an AIS monitoring programme, eDNA sampling in early May would detect this species with high confidence (95%) with only 32 sample per lake (80% BCI: 17–93 samples), but zebra mussel would require 177 samples per lake (80% BCI: 81–548 samples) and rusty crayfish would require 188 samples per lake (80% BCI: 97–600 samples). Accordingly, eDNA sampling will require choices by researchers or managers to scale their sampling effort to seasonal detectability of their highest priority species, while recognising that this will result in some lower priority or secondary species being less detectable. These trade-offs should be considered when repurposing stored eDNA samples for secondary taxa (e.g., Dysthe et al., 2018), and will be even more acute when

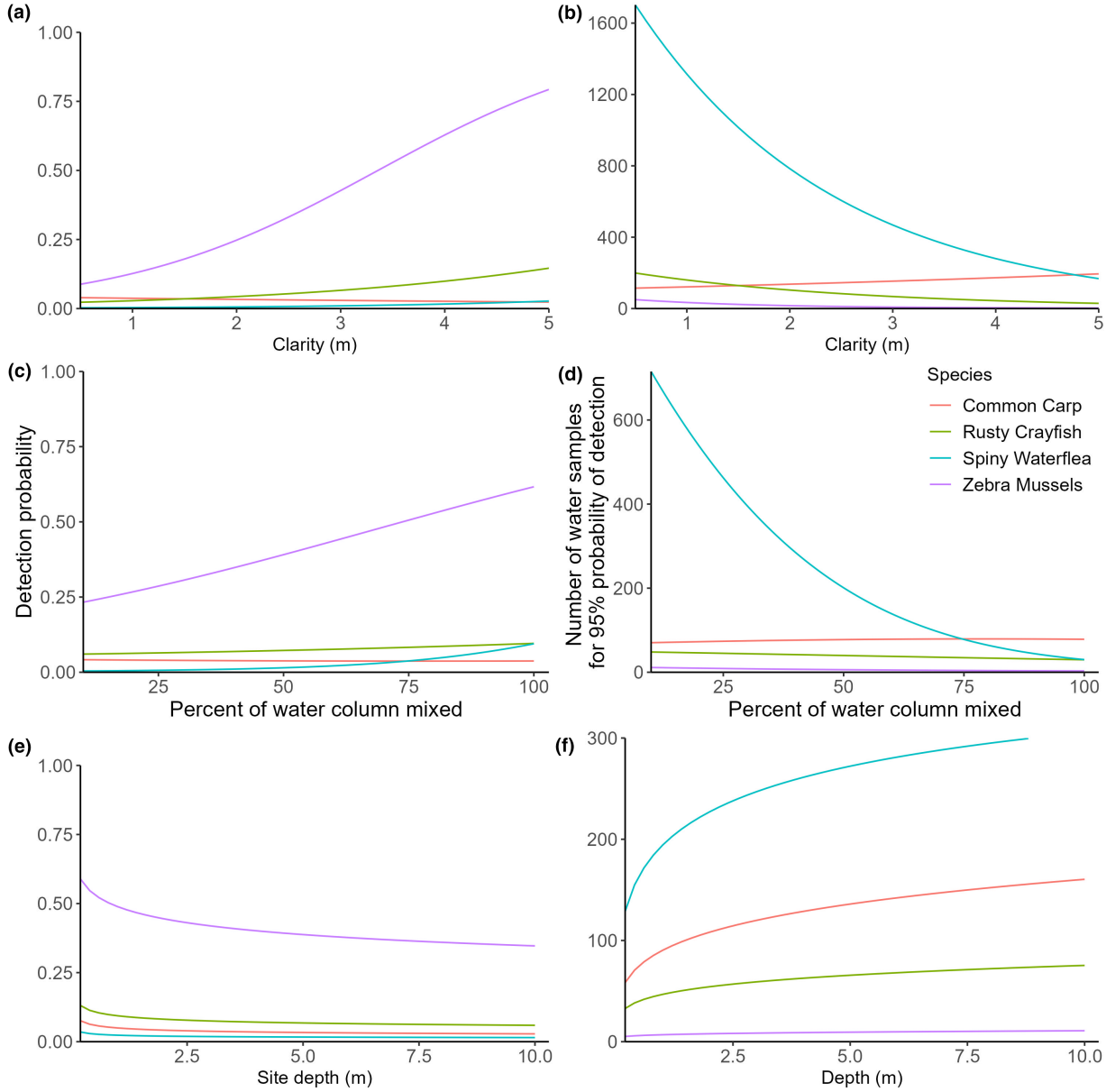

FIGURE 3 Trends of environmental DNA detection probability over water clarity (a), percentage of water column mixed (c) and sampling site depth (e), and the number of environmental DNA water samples required to have a 95% probability of detecting a species given its presence for the range of covariate values (b, d, f). Lines shown indicate effects when other covariates are at their mean and random effects for lake and sampling site are set to zero.

monitoring entire communities with eDNA metabarcoding because this method is generally less sensitive to organism presence at low abundance than single-species approaches such as qPCR (Blackman et al., 2020; Deiner et al., 2017; Moss et al., 2022). We encourage users of eDNA to consider phenology or seasonality of focal taxa as explanations for inferred false negatives along with more common attribution to laboratory or field methodologies (Burian et al., 2021; Jerde, 2021; Rees et al., 2014).

To control the impacts of environmental factors on detection probability, we included three environmental covariates in our occupancy model that we hypothesised would influence detection probability: water clarity, stratification status, and sampling depth. Environmental DNA detectability for rusty crayfish and zebra mussels increased with increasing water clarity, but the credible intervals overlapped zero for common carp and spiny waterflea. Previous research has shown detection probabilities can be higher in clearer lakes (Dougherty et al., 2016), probably due to a lower amount of PCR inhibitory substances such as humic acids (Stoeckle et al., 2017), for which we did not test. Counteracting this effect, water clarity might negatively affect eDNA detection probability due to the DNA degradation effects of UV radiation (Kessler et al., 2020; Strickler et al., 2015), but the importance of this phenomenon has been contested in natural conditions (Mächler et al., 2018). The percentage of a lake's water column

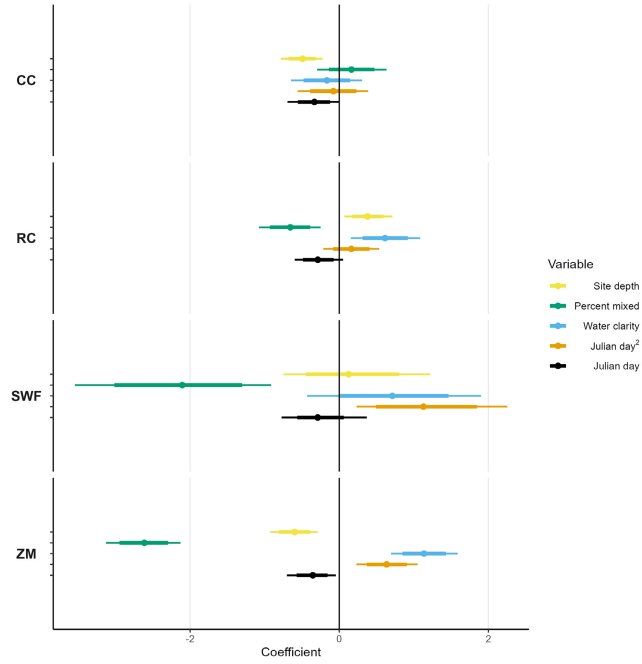

FIGURE 4 Covariate effect sizes for the environmental DNA detection probability of all species. The point represents the median covariate effect for each species-covariate combination. The thick bars represent 80% Bayesian credible interval and the thin bars represent 95% Bayesian credible interval for each species-covariate combination. The label CC is used for the covariate effects for common carp, RC corresponds to rusty crayfish, SWF denotes spiny waterflea, and ZM is zebra mussel.

that was within 1°C from the surface increased eDNA detection probability, indicating that detections were more likely when lakes were well mixed. Previous work has shown lake mixing can increase the amount of DNA collected from hypolimnetic organisms with surface water sampling (Klobucar et al., 2017; Littlefair et al., 2021). Interestingly, the effect of mixing was more certain for the organisms with a planktonic life stage (spiny waterflea and zebra mussels; 80% BCI do not overlap zero) than for organisms without planktonic stages (common carp and rusty crayfish; 80% BCI overlap zero; Figure 4). Detection probability decreased as sampling site depth increased, similar to previous work showing a weak negative effect of sampling depth in small lakes on plant eDNA diversity metrics (Drummond et al., 2021). Depth did not influence detection probability of spiny waterflea, a result that makes sense given that the species is pelagic throughout its life.

Environmental DNA was well suited for detecting most species in our study, although it was least effective for spiny waterflea, which went undetected with eDNA in six lakes where it was thought to exist. However, we relied on previous traditional surveys to help us understand the occupancy status of our study lakes. The eDNA and traditional surveys were mismatched in time and could have resulted in prior positive detections that failed to establish or that were at an invasion stage when the species were more abundant and thus more detectable using traditional methods (Strayer et al., 2017). In total, using eDNA we missed 11 AIS infestations where they were presumed to exist but documented 15 cases of eDNA detections at lakes where AIS had not been previously detected. We believe this highlights the sensitive nature of eDNA as well as its utility as a rapid detection tool for new AIS infestations. We suggest that to verify a lake has been invaded, eDNA detections of new invasives should also be paired with targeted sampling to collect voucher specimens (Beng & Corlett, 2020; Sepulveda et al., 2023).

We estimated strong seasonal and environmental effects on eDNA detection rates despite methodological choices that may have reduced the detectability of our organisms. Detectability of our species might have been higher if we filtered larger volumes of water (Hunter et al., 2019), had not prefiltered our samples (Li et al., 2018), or used alternative extraction methods that might recover higher eDNA yields (García et al., 2024; Hinlo et al., 2017; Renshaw et al., 2015). Further, our study was also limited by a moderate rate of contamination for zebra mussel (Hutchins et al., 2022; Sepulveda et al., 2020). However, we anticipate that our results are robust to these limitations. First, we believe that seasonal differences in detectability would persist between our four focal species even if overall eDNA detectability was improved by different laboratory or field methods, as our findings were consistent with the biology and life history of the focal species and past studies of individual taxa (Dunn et al., 2017; Sepulveda et al., 2019; Walsh et al., 2019). The number of samples required to detect spiny waterflea eDNA with high confidence, for example, might be reduced with alternative methods, but we anticipate that common carp eDNA would remain more detectable than this species in the spring or early summer by these same methods. Second, our low level of field and lab contamination was not structured by season, indicating little impact on the results of seasonal timing of sampling. Researchers should continue to work to optimise laboratory and field methods that improve sensitivity of eDNA approaches to organism occupancy, especially at low relative abundances, while being mindful that environmental and biological factors may still constrain the benefits of this optimisation (Barnes & Turner, 2016).

Conventional methods for multi-species surveillance at a landscape scale are difficult and costly (Borrell et al., 2017; Evans, Li, et al., 2017), which inhibits AIS management over large areas. This difficulty manifests in AIS early detection. For example, more than 850 waterbodies in Minnesota are designated as infested with one or more AIS (MN DNR list of infested waters, April 2023). However, this number is almost certainly a gross underestimate due to a lack of statistically valid sampling across the landscape of lakes (Latzka et al., 2016). Environmental DNA may allow for an efficient monitoring network facilitating both sampling across a landscape of lakes and standardised monitoring that is infeasible with traditional methods (Jeremy et al., 2015; King et al., 2022), especially if timed to coincide with life history events that increase probability of detection. Based on our multi-lake, multi-species sampling effort, we found the probability of eDNA detection follows species-specific trends. By randomly surveying a larger sample of lakes, without regard to known infestation status, our sampling and modelling approaches could be readily adapted to estimate infestation probabilities (i.e.,

occupancy) across broader landscapes ultimately resulting in more effective multi-species monitoring and AIS management.

AUTHOR CONTRIBUTIONS

Conceptualisation: C.L.C., E.R.L., J.D., G.J.A.H. Developing methods, conducting the research: A.T., A.L., C.I.R., C.L.C., E.R.L., J.D., J.K.R.N., K.E., S.G., G.J.A.H. Data analysis, interpretation: C.I.R., G.J.A.H., T.W.A. Writing: C.I.R. Editing: A.T., A.L., C.I.R., C.L.C., E.R.L., J.D., J.K.R.N., K.E., G.J.A.H., S.G., T.W.A.

ACKNOWLEDGEMENTS

We thank the lab and field teams that helped with sampling including Mayra Velasquez, Denver Link, Amanda Van Pelt, Kate Rounds, and Jasmine Blomgren as well as John Manske and Justin Townsend (Ramsey County Public Works) for assistance in sampling, boat decontamination, and partner discussions. Additional thanks to employees of the Minnesota Department of Natural Resources (Carl Pederson, Eric Jensen) and Voyageurs National Park (Ryan Maki) for assistance with sampling. We thank the Collaborative Conservation Genetics Laboratory of the Illinois Natural History Survey for facility use at the University of Illinois. Finally, we thank two anonymous reviewers for constructive feedback on an earlier version of this manuscript. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Minnesota Aquatic Invasive Species Research Center (MAISRC) and the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Data Repository for the University of Minnesota at https://doi.org/10.13020/xvb3-2672.

ORCID

Christopher I. Rounds https://orcid.org/0000-0003-1346-5707
Eric R. Larson https://orcid.org/0000-0002-9232-5907
Jenna K. R. Nelson https://orcid.org/0000-0003-2960-8485
Gretchen J. A. Hansen https://orcid.org/0000-0003-0241-7048

REFERENCES

Amberg, J. J., Merkes, C. M., Wendylee, S., Rees, C. B., & Erickson, R. A. (2019). Environmental DNA as a tool to help inform zebra mussel, *Dreissena polymorpha*, management in inland lakes. *Management of Biological Invasions*, *10*(1), 96–110. https://doi.org/10.3391/mbi.2019.10.1.06

Andres, K. J., Lambert, T. D., Lodge, D. M., Andrés, J., & Jackson, J. R. (2023). Combining sampling gear to optimally inventory species highlights the efficiency of eDNA metabarcoding. *Environmental DNA*, *5*(1), 146–157. https://doi.org/10.1002/edn3.366

Bajer, P. G., & Sorensen, P. W. (2010). Recruitment and abundance of an invasive fish, the common carp, is driven by its propensity to invade and reproduce in basins that experience winter-time hypoxia in interconnected lakes. *Biological Invasions*, *12*(5), 1101–1112. https://doi.org/10.1007/s10530-009-9528-y

Banet, N. V., Fieberg, J., & Sorensen, P. W. (2022). Migration, homing and spatial ecology of common carp in interconnected lakes. *Ecology of Freshwater Fish*, *31*(1), 164–176. https://doi.org/10.1111/eff.12622

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827. https://doi.org/10.1021/es404734p

Bayer, S. R., Countway, P. D., & Wahle, R. A. (2019). Developing an eDNA toolkit to quantify broadcast spawning events of the sea scallop *Placopecten magellanicus*: Moving beyond fertilization assays. *Marine Ecology Progress Series*, *621*, 127–141. https://doi.org/10.3354/meps12991

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, *29*(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Blackman, R. C., Ling, K. K. S., Harper, L. R., Shum, P., Hänfling, B., & Lawson-Handley, L. (2020). Targeted and passive environmental DNA approaches outperform established methods for detection of quagga mussels, *Dreissena rostriformis bugensis* in flowing water. *Ecology and Evolution*, *10*(23), 13248–13259. https://doi.org/10.1002/ece3.6921

Bonar, S. A., Hubert, W. A., & Willis, D. W. (2009). *Standard methods for sampling North American freshwater fishes*. https://pubs.er.usgs.gov/publication/70046706

Boone, M. L., Evans, E., Arnold, T., & Cariveau, D. P. (2023). Increasing sampling efficiency of Bombus communities with rare and endangered species by optimizing detection probabilities: A multi-species occupancy modelling approach using roadsides as a case study. *Biological Conservation*, *283*, 110122. https://doi.org/10.1016/j.biocon.2023.110122

Borrell, Y. J., Miralles, L., Do Huu, H., Mohammed-Geba, K., & Garcia-Vazquez, E. (2017). DNA in a bottle—Rapid metabarcoding survey for early alerts of invasive species in ports. *PLoS One*, *12*(9), e0183347. https://doi.org/10.1371/journal.pone.0183347

Branstrator, D. K., Beranek, A. E., Brown, M. E., Hembre, L. K., & Engstrom, D. R. (2017). Colonization dynamics of the invasive predatory cladoceran, *Bythotrephes longimanus*, inferred from sediment records: Bythotrephes colonization dynamics. *Limnology and Oceanography*, *62*(3), 1096–1110. https://doi.org/10.1002/lno.10488

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., & Sweet, M. (2021). Improving the reliability of eDNA data interpretation. *Molecular Ecology Resources*, *21*(5), 1422–1433. https://doi.org/10.1111/1755-0998.13367

Bylemans, J., Gleeson, D. M., Duncan, R. P., Hardy, C. M., & Furlan, E. M. (2019). A performance evaluation of targeted eDNA and eDNA metabarcoding analyses for freshwater fishes. *Environmental DNA*, *1*(4), 402–414. https://doi.org/10.1002/edn3.41

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*(5), 443–449. https://doi.org/10.1002/lol2.10271

Coster, S. S., Dillon, M. N., Moore, W., & Jr, G. T. M. (2021). The update and optimization of an eDNA assay to detect the invasive rusty crayfish (*Faxonius rusticus*). *PLoS One*, *16*(10), e0259084. https://doi.org/10.1371/journal.pone.0259084

Crocker, D. W., & Barr, D. W. (1968). *Handbook of the crayfishes of Ontario*. Royal Ontario Museum.

Curtis, A. N., Larson, E. R., & Davis, M. A. (2021). Field storage of water samples affects measured environmental DNA concentration and

detection. *Limnology*, *22*(1), 1–4. https://doi.org/10.1007/s10201-020-00634-y

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*(10), 1918–1931. https://doi.org/10.1111/ddi.13196

Cuthbert, R. N., Pattison, Z., Taylor, N. G., Verbrugge, L., Diagne, C., Ahmed, D. A., Leroy, B., Angulo, E., Briski, E., Capinha, C., Catford, J. A., Dalu, T., Essl, F., Gozlan, R. E., Haubrock, P. J., Kourantidou, M., Kramer, A. M., Renault, D., Wasserman, R. J., & Courchamp, F. (2021). Global economic costs of aquatic invasive alien species. *Science of the Total Environment*, *775*, 145238. https://doi.org/10.1016/j.scitotenv.2021.145238

Davy, C. M., Kidd, A. G., & Wilson, C. C. (2015). Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. *PLoS One*, *10*(7), e0130965. https://doi.org/10.1371/journal.pone.0130965

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*(10), e0165273. https://doi.org/10.1371/journal.pone.0165273

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Di Muri, C., Lawson Handley, L., Bean, C. W., Benucci, M., Harper, L. R., James, B., Li, J., Winfield, I. J., & Hänfling, B. (2022). Spatio-temporal monitoring of lake fish spawning activity using environmental DNA metabarcoding. *Environmental DNA*, *5*, 849–860. https://doi.org/10.1002/edn3.343

Dorazio, R. M., & Royle, J. A. (2005). Estimating size and composition of biological communities by modeling the occurrence of species. *Journal of the American Statistical Association*, *100*(470), 389–398. https://doi.org/10.1198/016214505000000015

Dougherty, M. M., Larson, E. R., Renshaw, M. A., Gantz, C. A., Egan, S. P., Erickson, D. M., & Lodge, D. M. (2016). Environmental DNA (eDNA) detects the invasive rusty crayfish *Orconectes rusticus* at low abundances. *Journal of Applied Ecology*, *53*(3), 722–732. https://doi.org/10.1111/1365-2664.12621

Drummond, J. A., Larson, E. R., Li, Y., Lodge, D. M., Gantz, C. A., Pfrender, M. E., Renshaw, M. A., Correa, A. M. S., & Egan, S. P. (2021). Diversity metrics are robust to differences in sampling location and depth for environmental DNA of plants in small Temperate Lakes. *Frontiers in Environmental Science*, *9*. https://doi.org/10.3389/fenvs.2021.617924

Dunn, N., Priestley, V., Herraiz, A., Arnold, R., & Savolainen, V. (2017). Behavior and season affect crayfish detection and density inference using environmental DNA. *Ecology and Evolution*, *7*(19), 7777–7785. https://doi.org/10.1002/ece3.3316

Dysthe, J. C., Rodgers, T., Franklin, T. W., Carim, K. J., Young, M. K., McKelvey, K. S., Mock, K. E., & Schwartz, M. K. (2018). Repurposing environmental DNA samples—Detecting the western pearlshell (*Margaritifera falcata*) as a proof of concept. *Ecology and Evolution*, *8*(5), 2659–2670. https://doi.org/10.1002/ece3.3898

EDDMapS. (2023). *Early detection & distribution mapping system*. The University of Georgia – Center for invasive species and ecosystem health. http://www.eddmaps.org/

Ehrenfeld, J. G. (2010). Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, *41*(1), 59–80. https://doi.org/10.1146/annurev-ecolsys-102209-144650

Eichmiller, J. J., Bajer, P. G., & Sorensen, P. W. (2014). The relationship between the distribution of common carp and their environmental DNA in a small Lake. *PLoS One*, *9*(11), e112611. https://doi.org/10.1371/journal.pone.0112611

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

Erickson, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R., & Amberg, J. J. (2017). Seasonal trends in eDNA detection and occupancy of bigheaded carps. *Journal of Great Lakes Research*, *43*(4), 762–770. https://doi.org/10.1016/j.jglr.2017.06.003

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

Evans, N. T., Li, Y., Renshaw, M. A., Olds, B. P., Deiner, K., Turner, C. R., Jerde, C. L., Lodge, D. M., Lamberti, G. A., & Pfrender, M. E. (2017). Fish community assessment with eDNA metabarcoding: Effects of sampling design and bioinformatic filtering. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(9), 1362–1374. https://doi.org/10.1139/cjfas-2016-0306

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Fieberg, J. R. (2024). *Statistics for ecologists: A frequentist and Bayesian treatment of modern regression models*. University of Minnesota Libraries Publishing. Retrieved from the University of Minnesota Digital Conservancy, https://hdl.handle.net/11299/260227

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151–163. https://doi.org/10.1111/gcb.13004

García, S. M., Chun, C. L., Dumke, J., Hansen, G. J., Quebedeaux, K. B., Rounds, C., Totsch, A., & Larson, E. R. (2024). Environmental DNA storage and extraction method affects detectability for multiple aquatic invasive species. *Environmental DNA*, *6*(3), e557. https://doi.org/10.1002/edn3.557

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science*, *7*(4), 457–472.

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, *183*, 1–3. https://doi.org/10.1016/j.biocon.2014.11.040

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Haag, W. R., & Garton, D. W. (1992). Synchronous spawning in a recently established population of the zebra mussel, *Dreissena polymorpha*, in western Lake Erie, USA. *Hydrobiologia*, *234*(2), 103–110. https://doi.org/10.1007/BF00010865

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS One*, *12*(6), e0179251. https://doi.org/10.1371/journal.pone.0179251

Hulme, P. E. (2006). Beyond control: Wider implications for the management of biological invasions. *Journal of Applied Ecology*, *43*(5), 835–847. https://doi.org/10.1111/j.1365-2664.2006.01227.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

Hutchins, P. R., Simantel, L. N., & Sepulveda, A. J. (2022). Time to get real with qPCR controls: The frequency of sample contamination and the informative power of negative controls in environmental DNA

studies. *Molecular Ecology Resources*, *22*(4), 1319–1329. https://doi.org/10.1111/1755-0998.13549

Jerde, C. L. (2021). Can we manage fisheries with the inherent uncertainty from eDNA? *Journal of Fish Biology*, *98*(2), 341–353. https://doi.org/10.1111/jfb.14218

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. *Conservation Letters*, *4*(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Jeremy, B., Naomi, E., Alice, V., Coline, G., Tony, D., Griffiths, R. A., Jim, F., Wilkinson John, W., Andy, A., Peter, B., Penny, W., & Francesca, D. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, *183*, 19–28. https://doi.org/10.1016/j.biocon.2014.11.029

Jo, T., & Minamoto, T. (2021). Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses. *Molecular Ecology Resources*, *21*(5), 1490–1503. https://doi.org/10.1111/1755-0998.13354

Kéry, M., & Schaub, M. (2012). *Bayesian population analysis using WinBUGS: A hierarchical perspective* (1st ed.). Academic Press.

Kessler, E. J., Ash, K. T., Barratt, S. N., Larson, E. R., & Davis, M. A. (2020). Radiotelemetry reveals effects of upstream biomass and UV exposure on environmental DNA occupancy and detection for a large freshwater turtle. *Environmental DNA*, *2*(1), 13–23. https://doi.org/10.1002/edn3.42

King, A. C., Krieg, R., Weston, A., & Zenker, A. K. (2022). Using eDNA to simultaneously detect the distribution of native and invasive crayfish within an entire country. *Journal of Environmental Management*, *302*, 113929. https://doi.org/10.1016/j.jenvman.2021.113929

Klobucar, S. L., Rodgers, T. W., & Budy, P. (2017). At the forefront: Evidence of the applicability of using environmental DNA to quantify the abundance of fish populations in natural lentic waters with additional sampling considerations. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(12), 2030–2034. https://doi.org/10.1139/cjfas-2017-0114

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

Kvistad, J. T., Buckley, J. T., Robinson, K. M., Galarowicz, T. L., Claramunt, R. M., Clapp, D. F., O'Neill, P., Chadderton, W. L., Tucker, A. J., & Herbert, M. (2021). Size segregation and seasonal patterns in rusty crayfish *Faxonius rusticus* distribution and abundance on northern Lake Michigan spawning reefs. *Journal of Great Lakes Research*, *47*(4), 1050–1064. https://doi.org/10.1016/j.jglr.2021.03.009

Kvistad, J. T., Galarowicz, T. L., Clapp, D. F., Chadderton, W. L., Tucker, A. J., Annis, G., & Herbert, M. (2023). Evidence of a compensatory response in invasive rusty crayfish (*Faxonius rusticus*) following intensive harvest removal from northern Lake Michigan fish spawning reefs. *Biological Invasions*, *25*, 2831–2847. https://doi.org/10.1007/s10530-023-03076-6

Latzka, A. W., Hansen, G. J. A., Kornis, M., & Vander Zanden, M. J. (2016). Spatial heterogeneity in invasive species impacts at the landscape scale. *Ecosphere*, *7*(3). https://doi.org/10.1002/ecs2.1311

Lear, G., Dickie, I., Banks, J., Boyer, S., Buckley, H. L., Buckley, T. R., Cruickshank, R., Dopheide, A., Handley, K. M., Hermans, S., Kamke, J., Lee, C. K., MacDiarmid, R., Morales, S. E., Orlovich, D., Smissen, R., Wood, J., & Holdaway, R. (2018). Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. *New Zealand Journal of Ecology*, *42*(1), 10–50A.

Li, J., Lawson Handley, L., Read, D. S., & Hänfling, B. (2018). The effect of filtration method on the efficiency of environmental DNA capture and quantification via metabarcoding. *Molecular Ecology Resources*, *18*(5), 1102–1114. https://doi.org/10.1111/1755-0998.12899

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*(13), 3083–3096. https://doi.org/10.1111/mec.15623

Lodge, D. M., Simonin, P. W., Burgiel, S. W., Keller, R. P., Bossenbroek, J. M., Jerde, C. L., Kramer, A. M., Rutherford, E. S., Barnes, M. A., Wittmann, M. E., Chadderton, W. L., Apriesnig, J. L., Beletsky, D., Cooke, R. M., Drake, J. M., Egan, S. P., Finnoff, D. C., Gantz, C. A., Grey, E. K., … Zhang, H. (2016). Risk analysis and bioeconomics of invasive species to inform policy and management. *Annual Review of Environment and Resources*, *41*(1), 453–488. https://doi.org/10.1146/annurev-environ-110615-085532

Mächler, E., Osathanunkul, M., & Altermatt, F. (2018). Shedding light on eDNA: Neither natural levels of UV radiation nor the presence of a filter feeder affect eDNA-based detection of aquatic organisms. *PLoS One*, *13*(4), e0195529. https://doi.org/10.1371/journal.pone.0195529

McArdle, B. H. (1990). When are rare species not there? *Oikos*, *57*(2), 276–277. https://doi.org/10.2307/3565950

McCarthy, M. A., Moore, J. L., Morris, W. K., Parris, K. M., Garrard, G. E., Vesk, P. A., Rumpff, L., Giljohann, K. M., Camac, J. S., Bau, S. S., Friend, T., Harrison, B., & Yue, B. (2013). The influence of abundance on detectability. *Oikos*, *122*(5), 717–726. https://doi.org/10.1111/j.1600-0706.2012.20781.x

McColl-Gausden, E. F., Weeks, A. R., Coleman, R. A., Robinson, K. L., Song, S., Raadik, T. A., & Tingley, R. (2021). Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Molecular Ecology*, *30*(13), 3111–3126. https://doi.org/10.1111/mec.15644

McColl-Gausden, E. F., Weeks, A. R., Coleman, R., Song, S., & Tingley, R. (2023). Using hierarchical models to compare the sensitivity of metabarcoding and qPCR for eDNA detection. *Ecological Informatics*, *75*, 102072. https://doi.org/10.1016/j.ecoinf.2023.102072

Milhau, T., Valentini, A., Poulet, N., Roset, N., Jean, P., Gaboriaud, C., & Dejean, T. (2021). Seasonal dynamics of riverine fish communities using eDNA. *Journal of Fish Biology*, *98*(2), 387–398. https://doi.org/10.1111/jfb.14190

Minnesota Department of Natural Resources. (2023). Infested waters list. https://www.dnr.state.mn.us/invasives/ais/infested

Mordecai, R. S., Mattsson, B. J., Tzilkowski, C. J., & Cooper, R. J. (2011). Addressing challenges when studying mobile or episodic species: Hierarchical Bayes estimation of occupancy and use: Hierarchical Bayes multi-scale occupancy. *Journal of Applied Ecology*, *48*(1), 56–66. https://doi.org/10.1111/j.1365-2664.2010.01921.x

Morisette, J., Burgiel, S., Brantley, K., Daniel, W. M., Darling, J., Davis, J., Franklin, T., Gaddis, K., Hunter, M., Lance, R., Leskey, T., Passamaneck, Y., Piaggio, A., Rector, B., Sepulveda, A., Smith, M., Stepien, C. A., & Wilcox, T. (2021). Strategic considerations for invasive species managers in the utilization of environmental DNA (eDNA): Steps for incorporating this powerful surveillance tool. *Management of Biological Invasions: International Journal of Applied Research on Biological Invasions*, *12*(3), 747–775. https://doi.org/10.3391/mbi.2021.12.3.15

Moss, W. E., Harper, L. R., Davis, M. A., Goldberg, C. S., Smith, M. M., & Johnson, P. T. J. (2022). Navigating the trade-offs between environmental DNA and conventional field surveys for improved amphibian monitoring. *Ecosphere*, *13*(2). https://doi.org/10.1002/ecs2.3941

Myers, J. H., Simberloff, D., Kuris, A. M., & Carey, J. R. (2000). Eradication revisited: Dealing with exotic species. *Trends in Ecology & Evolution*, *15*(8), 316–320. https://doi.org/10.1016/S0169-5347(00)01914-5

Outhwaite, C. L., Chandler, R. E., Powney, G. D., Collen, B., Gregory, R. D., & Isaac, N. J. B. (2018). Prior specification in Bayesian occupancy

modelling improves analysis of species occurrence data. *Ecological Indicators*, *93*, 333–343. https://doi.org/10.1016/j.ecolind.2018.05.010

Pearman, J. K., Keeley, N. B., Wood, S. A., Laroche, O., Zaiko, A., Thomson-Laing, G., Biessy, L., Atalah, J., & Pochon, X. (2020). Comparing sediment DNA extraction methods for assessing organic enrichment associated with marine aquaculture. *PeerJ*, *8*, e10231. https://doi.org/10.7717/peerj.10231

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Reaser, J. K., Burgiel, S. W., Kirkey, J., Brantley, K. A., Veatch, S. D., & Burgos-Rodríguez, J. (2020). The early detection of and rapid response (EDRR) to invasive species: A conceptual framework and federal capacities assessment. *Biological Invasions*, *22*(1), 1–19. https://doi.org/10.1007/s10530-019-02156-w

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). REVIEW: The detection of aquatic animal species using environmental DNA – A review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, *15*(1), 168–176. https://doi.org/10.1111/1755-0998.12281

Rounds, C., Arnold, T. W., Chun, C. L., Dumke, J., Totsch, A., Keppers, A., Edbald, K., García, S. M., Larson, E. R., Nelson, J. K. R., & Hansen, G. J. A. (2023). *Seasonal influence on detection probabilities for multiple aquatic invasive species using environmental DNA*. Data Repository for the University of Minnesota (DRUM). https://doi.org/10.13020/XVB3-2672

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sepulveda, A., Amberg, J., & Hanson, E. (2019). Using environmental DNA to extend the window of early detection for dreissenid mussels. *Management of Biological Invasions*, *10*(2), 342–358. https://doi.org/10.3391/mbi.2019.10.2.09

Sepulveda, A. J., Dumoulin, C. E., Blanchette, D. L., McPhedran, J., Holme, C., Whalen, N., Hunter, M. E., Merkes, C. M., Richter, C. A., Neilson, M. E., Daniel, W. M., Jones, D. N., & Smith, D. R. (2023). When are environmental DNA early detections of invasive species actionable? *Journal of Environmental Management*, *343*, 118216. https://doi.org/10.1016/j.jenvman.2023.118216

Sepulveda, A. J., Hutchins, P. R., Forstchen, M., Mckeefry, M. N., & Swigris, A. M. (2020). The elephant in the lab (and field): Contamination in aquatic environmental DNA studies. *Frontiers in Ecology and Evolution*, *8*. 440. https://doi.org/10.3389/fevo.2020.609973

Simberloff, D. (2009). We can eliminate invasions or live with them. Successful management projects. *Biological Invasions*, *11*(1), 149–157. https://doi.org/10.1007/s10530-008-9317-z

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., & Vilà, M. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution*, *28*(1), 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Somers, K. M., & Green, R. H. (1993). Seasonal patterns in trap catches of the crayfish *Cambarus bartoni* and *Orconectes virilis* in six south-central Ontario lakes. *Canadian Journal of Zoology*, *71*(6), 1136–1145. https://doi.org/10.1139/z93-155

Spear, M. J., Walsh, J. R., Ricciardi, A., & Zanden, M. J. V. (2021). The invasion ecology of sleeper populations: Prevalence, persistence, and abrupt shifts. *Bioscience*, *71*(4), 357–369. https://doi.org/10.1093/biosci/biaa168

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*(12), e0189119. https://doi.org/10.1371/journal.pone.0189119

Strayer, D. L., D'Antonio, C. M., Essl, F., Fowler, M. S., Geist, J., Hilt, S., Jarić, I., Jöhnk, K., Jones, C. G., Lambin, X., Latzka, A. W., Pergl, J., Pyšek, P., Robertson, P., von Schmalensee, M., Stefansson, R. A., Wright, J., & Jeschke, J. M. (2017). Boom-bust dynamics in biological invasions: Towards an improved application of the concept. *Ecology Letters*, *20*(10), 1337–1350. https://doi.org/10.1111/ele.12822

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

Su, Y. S., & Yajima, M. (2015). R2jags: Using R to run 'JAGS'. R package version 0.5-7, 34.

Takahashi, M. K., Meyer, M. J., Mcphee, C., Gaston, J. R., Venesky, M. D., & Case, B. F. (2018). Seasonal and diel signature of eastern hellbender environmental DNA. *The Journal of Wildlife Management*, *82*(1), 217–225. https://doi.org/10.1002/jwmg.21349

Takeuchi, A., Iijima, T., Kakuzen, W., Watanabe, S., Yamada, Y., Okamura, A., Horie, N., Mikawa, N., Miller, M. J., Kojima, T., & Tsukamoto, K. (2019). Release of eDNA by different life history stages and during spawning activities of laboratory-reared Japanese eels for interpretation of oceanic survey data. *Scientific Reports*, *9*(1), 6074. https://doi.org/10.1038/s41598-019-42641-9

Trebitz, A. S., Hoffman, J. C., Darling, J. A., Pilgrim, E. M., Kelly, J. R., Brown, E. A., Chadderton, W. L., Egan, S. P., Grey, E. K., Hashsham, S. A., Klymus, K. E., Mahon, A. R., Ram, J. L., Schultz, M. T., Stepien, C. A., & Schardt, J. C. (2017). Early detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating advanced technologies, and identifying research needs. *Journal of Environmental Management*, *202*, 299–310. https://doi.org/10.1016/j.jenvman.2017.07.045

Troth, C. R., Sweet, M. J., Nightingale, J., & Burian, A. (2021). Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. *Science of the Total Environment*, *768*, 144466. https://doi.org/10.1016/j.scitotenv.2020.144466

Tsuji, S., & Shibata, N. (2021). Identifying spawning events in fish by observing a spike in environmental DNA concentration after spawning. *Environmental DNA*, *3*(1), 190–199. https://doi.org/10.1002/edn3.153

Vander Zanden, M. J., Hansen, G. J. A., Higgins, S. N., & Kornis, M. S. (2010). A pound of prevention, plus a pound of cure: Early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *36*(1), 199–205. https://doi.org/10.1016/j.jglr.2009.11.002

Walsh, J. R., Spear, M. J., Shannon, T. P., Krysan, P. J., & Vander Zanden, M. J. (2019). Using eDNA, sediment subfossils, and zooplankton nets to detect invasive spiny water flea (*Bythotrephes longimanus*). *Biological Invasions*, *21*(2), 377–389. https://doi.org/10.1007/s10530-018-1862-5

Wang, S., Yan, Z., Hänfling, B., Zheng, X., Wang, P., Fan, J., & Li, J. (2021). Methodology of fish eDNA and its applications in ecology and environment. *Science of the Total Environment*, *755*, 142622. https://doi.org/10.1016/j.scitotenv.2020.142622

West, K. M., Stat, M., Harvey, E. S., Skepper, C. L., DiBattista, J. D., Richards, Z. T., Travers, M. J., Newman, S. J., & Bunce, M. (2020). eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical Island ecosystem. *Molecular Ecology*, *29*(6), 1069–1086. https://doi.org/10.1111/mec.15382

White, S., Faulk, E., Tzilkowski, C., Weber, A., Marshall, M., & Wagner, T. (2020). Predicting fish species richness and habitat relationships

using Bayesian hierarchical multispecies occupancy models. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(3), 602–610. https://doi.org/10.1139/cjfas-2019-0125

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer New York. isbn:978-3-319-24277-4 https://ggplot2.tidyverse.org

Wilson, C. C., Bronnenhuber, J., Boothroyd, M., Smith, C., & Wozney, K. (2014). *Environmental DNA (eDNA) monitoring and surveillance: Field and laboratory standard operating procedures*. https://doi.org/10.13140/RG.2.1.4724.5041

Yan, N. D., Blukacz, A., Sprules, W. G., Kindy, P. K., Hackett, D., Girard, R. E., & Clark, B. J. (2001). Changes in zooplankton and the phenology of the spiny water flea, *Bythotrephes*, following its invasion of Harp Lake, Ontario, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(12), 2341–2350. https://doi.org/10.1139/f01-171

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer New York. https://doi.org/10.1007/978-0-387-87458-6

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: Rounds, C. I., Arnold, T. W., Chun, C. L., Dumke, J., Totsch, A., Keppers, A., Edblad, K., García, S. M., Larson, E. R., Nelson, J. K. R., & Hansen, G. J. A. (2024). Aquatic invasive species exhibit contrasting seasonal detectability patterns based on environmental DNA: Implications for monitoring. *Freshwater Biology*, *69*, 1479–1493. https://doi.org/10.1111/fwb.14320