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Research Article

Assessing grass carp (*Ctenopharyngodon idella)* occupancy and detection probability within Lake Erie from environmental DNA

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Abstract

Grass carp (*Ctenopharyngodon idella*), an invasive cyprinid within the Laurentian Great Lakes, is naturally reproducing in several Lake Erie tributaries, which has raised concerns of the species' spread throughout Lake Erie and the other Great Lakes. Knowledge of the recent invasion extent outside of the western basin of Lake Erie, particularly in eastern tributaries and nearshore waters, is limited. Understanding the invasion extent would improve the efficacy of ongoing coordinated multi-agency control efforts. Molecular tools, such as environmental DNA (eDNA), have shown promise for early detection of aquatic invasive species. In this study, water samples (N = 476) were collected for grass carp eDNA monthly between May and November in 2018 and 2019, at three sites in the Michigan waters of Lake Erie and the Detroit River. We fit Bayesian multi-scale occupancy models to determine differences in eDNA capture and detection probability among grass carp qPCR assays, sampling sites, and across time. To determine whether grass carp were physically present, and to validate eDNA samples, we quantified recent grass carp presence in sampled areas using an existing acoustic telemetry and field sampling framework. Our results indicate that grass carp eDNA capture probability differed among sites, but there was no difference among months. Positive grass carp eDNA detections were observed across multiple months at each site, with 69% of site-specific sampling events testing positive for grass carp eDNA on at least one assay and replicate. The majority (65%) of weeks where positive eDNA sampling detections occurred also concurrently had one or more grass carp detected via acoustic telemetry 1–6 days prior. Our results highlight the potential utility of using eDNA to monitor the invasion extent of grass carp within the nearshore waters of Lake Erie. However, further evaluation of the factors that influence grass carp eDNA characteristics among sites within Lake Erie are needed to determine its efficacy for surveillance protocols by natural resource management agencies.

Key words: invasive carp, Great Lakes, qPCR, monitoring, acoustic telemetry, hierarchical model

Introduction

Monitoring and early detection are essential components of control efforts for invasive species. Early detection of invasive species is critical for effective

control because once an invasive species becomes widespread, the cost and effort of removal, control, and eradication increases exponentially until establishment (Haubrock et al. 2022). In aquatic environments, molecular tools provide a promising means to detect the presence of invasive species (Thomsen et al. 2012; Sard et al. 2019; Pukk et al. 2021; Wang et al. 2021). Specifically, the use of environmental DNA (eDNA; e.g. whole cells or fragments of DNA released by an organism into the environment) has garnered increased attention among ecologists and resource managers for the ability to detect rare or low abundance aquatic organisms, including invasive species (Dejean et al. 2012; Dougherty et al. 2016). In recent years, eDNA has become an integral tool for detection and non-detection in invasive species monitoring in aquatic systems and can be as or more sensitive than conventional capture-based gear types (Sard et al. 2019; Pukk et al. 2021), especially in early invasion stages where species detection probability is often low with conventional sampling efforts (Jerde et al. 2011; Chucholl et al. 2021). In particular, eDNA could be imperative for rapid implementation of U.S. policy on Early Detection and Rapid Response (EDRR) frameworks to minimize the impacts of invasive species and enhance monitoring capacities (Reaser et al. 2020).

Although eDNA can be an effective invasive species monitoring tool in aquatic environments, it is important to be cognizant of factors that may influence its utility as a proxy for target species presence/absence. The influence of sensitivity of the eDNA assays (e.g., specificity of primer pairs and/or probes) on detection probability has often been overlooked (Xia et al. 2021). The detection probability of eDNA can further be affected by the concentration of DNA present in water samples (Schabacker et al. 2020), as well as being absorbed by sediment (Lorenz et al. 1996), potentially altering DNA presence at a given time. For example, sediment containing eDNA can be re-suspended (Stoeckle et al. 2017) and may lead to false positives (the detection of a species that is no longer present in the environment) (Stoeckle et al. 2017). While this uncertainty can raise concerns for eDNA use in management, there are ways to reduce false positives and false negatives (e.g. increased replication of sampling across spatial and temporal scales) (Rees et al. 2014; Nagarajan et al. 2022). The detection probability of eDNA can also change seasonally based on different patterns of animal behavior, such as temporal shifts in spatial distribution, the release of gametes during spawning (Erickson et al. 2016; de Souza et al. 2016), and the effects of temperature on eDNA production and decay rates (Eichmiller et al. 2016; Harrison et al. 2019; Jo et al. 2020). Therefore, estimating detection probability of eDNA for invasive species can provide insight into our ability to use this tool for early detection and rapid response.

Grass carp (*Ctenopharyngodon idella*) is an invasive cyprinid that was introduced in the United States in the mid-20th century in landlocked

waterbodies as a biocontrol resource for mitigating macrophyte growth (Hanlon et al. 2000). Recent evidence of grass carp reproduction in several tributaries of Lake Erie has led to concerns among resource management agencies about their invasion potential within the Laurentian Great Lakes (Embke et al. 2016; Whitledge et al. 2021). Based on bioenergetics and risk assessment studies, grass carp could reduce nearshore aquatic vegetation by as much as 50% in Lake Erie through direct consumption, as has been observed in other inland waterbodies (Cassani et al. 2008; Cudmore et al. 2017; Van der Lee et al. 2017). Given the potential threats grass carp pose within the Great Lakes, coordinated response efforts have been in place among natural resource management agencies and academic partners within Lake Erie and its tributaries since 2014 (Herbst et al. 2021). However, the effectiveness of the most common gear types used (electrofishing and trammel nets) for direct capture of grass carp remains limited (Fischer et al. 2022a). Effort has mainly been concentrated in a few select tributaries within Lake Erie, primarily within the western basin. Additionally, recent telemetry studies have revealed that grass carp are highly mobile and can travel over 50 km in one year (Harris et al. 2021). Given their vagility, grass carp may be present in other tributaries within Lake Erie unbeknownst to natural resource agencies. Grass carp removal efforts are beginning to expand to other sub-basins within Lake Erie, and understanding where grass carp are present is necessary for improving the success of targeted control efforts. Additionally, the current removal methods provide limited utility for surveillance due to low detection probabilities (Gu and Swihart 2004). Therefore, eDNA is one potential tool that could be used to understand the presence/absence patterns of grass carp within a vast system, such as the Great Lakes, and has been demonstrated to be successful in detecting other invasive carps, silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carps, in lotic (flowing) systems (Jerde et al. 2011; Mize et al. 2019).

Grass carp are known to exhibit seasonal differences in distribution within Lake Erie. Most fish migrate upstream in tributaries in May–August, and then migrate downstream near river mouths or to other tributaries within Lake Erie outside the spawning season (Harris et al. 2021; Fischer et al. 2022b). This migration may affect the temporal detection probability of eDNA. Additionally, changes in seasonal hydrology (e.g., river flow) may affect the distribution of grass carp and the distribution of eDNA. For example, Thalinger et al. (2021) observed that detection probability of eDNA of four fish species housed in stationary cages declined with increasing discharge levels within a fish-free glacial river, presumably due to dilution of DNA. Therefore, evaluating the detection probability of grass carp eDNA assays across seasons is important for understanding implications for monitoring this invasive species.

The primary objectives of this study were to: 1) quantify grass carp eDNA detection probability across time, sites, and among eDNA molecular assays,

2) validate eDNA detections with acoustic telemetry data, and 3) compare results to capture-based gears. Results from this study are important for informing natural resource management agencies on the utility of eDNA for invasive carp detection and understanding the implications for monitoring the invasion extent of grass carp within the Laurentian Great Lakes.

Materials and methods

Study site

Three locations (Hot Ponds / River Raisin, North Maumee Bay, and Detroit River) within the Michigan waters of Lake Erie were targeted for eDNA water sampling in 2018 and 2019 (Figure 1). The Western Basin of Lake Erie is considered the epicenter of grass carp invasion in the Great Lakes because of evidence of reproductively viable diploid grass carp spawning in multiple tributaries confirmed by the presence of eggs (Embke et al. 2016). Our study sites were chosen due to previous confirmation of grass carp presence from acoustic telemetry and direct removal efforts (Harris et al. 2021; Lang 2022).

The Hot Ponds site was an area of thermal effluent from a power plant near the mouth of the River Raisin in Monroe, MI. The sampling area included the thermal pond and the embayment just south of the entrance to the pond (approximately 77 ha). North Maumee Bay is an area of nearshore marsh at the mouth of the Ottawa River that includes shallow vegetative habitat (approximately 495 ha, though samples were only taken near the vegetative habitat). The Detroit River site was located in the Trenton Channel (the portion of the Detroit River that flows to the west of Grosse Ile, MI) with specific sampling locations in a small channel that runs behind Elizabeth Park as well as the nearshore areas next to Humbug Marsh National Wildlife Refuge (approximately 50 ha). Although flow characteristics were not recorded, we note that there was generally very little flow in the Hot Ponds and North Maumee Bay, but the Detroit River system experienced a much greater flow.

eDNA field sampling procedure

Standardized collection of water samples (1 L volume and filtered within 14 hours of collection using 1.2 µm PES filters) for grass carp eDNA was conducted monthly, May–November in 2018 (n = 10 per site per month, N = 233 total in 2018) and May–October in 2019 (n = 10 per site per month, N = 243 total in 2019), at all three sites (Table 1). In each event, 10 sample sites were randomly selected from the sampling area, with all sites located adjacent to shore to represent the same habitat type sampled by conventional gears. One liter water samples were collected at the surface with 1-L sterile Nalgene bottles (Thermo-Fisher, Waltham, MA), prior to any sampling with capture-based gears, and placed on ice in a sterilized cooler until samples

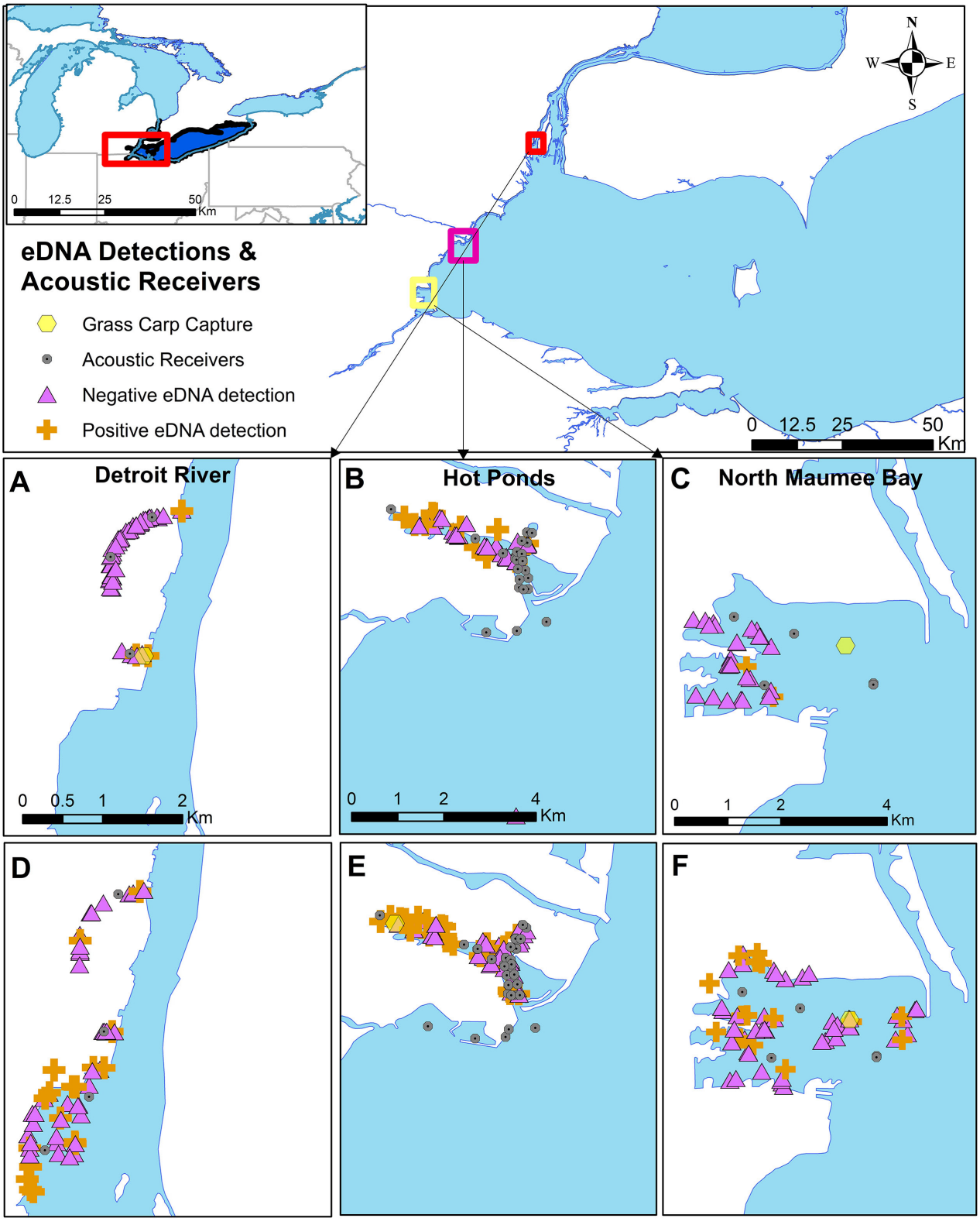

Figure 1. Map denoting all monthly grass carp eDNA sampling events in 2018 (A–C) and 2019 (D–F) aggregated at each sampling location (Hot Ponds, Detroit River, and North Maumee Bay) and acoustic receiver locations (grey circles) in the western basin of Lake Erie. Positive and negative eDNA detections, defined as at least one positive qPCR detection on one replicate among all markers (GCTM10, GCTM22, GCTM32) are denoted by orange crosses and pink triangles, respectively. The 3 grass carp captured from conventional gear (total sampling events = 451) in the Detroit River (October 2018), Hot Pond (July 2019) and North Maumee Bay (July 2019) are denoted by a yellow hexagon.

Table 1. Number of field samples (including controls) for each qPCR assay at each site sampled for eDNA in 2018 and 2019 in western Lake Erie. DR = Detroit River, HP = Hot Ponds, MB = Maumee Bay. Note that samples are site-specific.

Site	Year			
	2018		2019	
	Assay	Samples	Assay	Samples
DR	GCTM10	78	GCTM10	81
	GCTM22	78	GCTM22	81
	GCTM32	78	GCTM32	81
HP	GCTM10	77	GCTM10	82
	GCTM22	77	GCTM22	82
	GCTM32	77	GCTM32	82
MB	GCTM10	78	GCTM10	80
	GCTM22	78	GCTM22	80
	GCTM32	78	GCTM32	80

were filtered. Two control ("no-DNA") samples were used to quantify contamination during field collections during each sampling event – distilled water (no DNA/ddH20) was poured into a sterile 1-L Nalgene bottle and filtered prior to and after the entire batch collection of water samples were processed. All monthly water samples were collected at least 3 weeks apart at each site. In 2018, the November Hot Ponds sample was technically collected on October 30, 2018, but represented our November sample given that it met our 3-week duration between sampling events.

To avoid contamination during field collections, we implemented decontamination processes that were conducted for each sampling effort. Prior to sample collection, all Nalgene bottles were sterilized by soaking in a 20% bleach solution for 10 minutes (Prince and Andrus 1992; Pukk et al. 2021). Bottles were then rinsed with distilled water and air dried for at least 48 hours prior to sample collection. The cooler, as well as any equipment used to collect data (e.g., clipboard, pencils, supplies container), were sterilized by wiping with Sani-Cloth bleach disposable wipes (Professional Disposables International, Inc., Woodruff, NJ), and nitrile gloves were changed between each water sample collection and filtration event.

Ten water samples were collected during each sampling event at each site. All samples were filtered with a Smith-Root ANDe backpack eDNA sampler using sterile, single-use filter housings and 1.2µm polyether sulfone (PES) filters (Thomas et al. 2018). All samples were filtered in the same day (on land during or shortly after sampling or in the evening in the lab, < 14 hours after collection). Filters were removed from the housing with sterile, single-use forceps and stored in 95% ethanol in pre-labeled tubes. From each water sample, 4 aliquots of water were taken for qPCR processing (4 multiplexed replicates of the three assays).

eDNA extraction and quantitative PCR

Environmental DNA collected on filters in 2018 and 2019 was extracted as described in Sard et al. (2019), based on a protocol developed by Laramie et al.

Table 2. Gene region, primer, and probe sequences used to amplify GCTM10 ,GCTM22, and GCTM32 for grass carp.

Gene	Primers and Probes	Sequence
ND2	Forward	5′- CCYTACGTACTCGCAATTCTAC -3′
ND2	Reverse	5′- GTGGTGGTGTTGGGCTATTA -3′
ND2	Probe	5′- VIC- ACCCTAACCTTTGCTAGCTCCCAC -MGBNFQ-3′
COII	Forward	5′- CCGACTCCTAGAAACAGATCAC -3′
COII	Reverse	5′- GGGACAGCTCAGGAATGTAATA -3′
COII	Probe	5′- 56-FAM- CCAGTTCGT/ZEN/GTCCTAGTATCTGCCGA -3IABkFQ -3′
COIII	Forward	5′- CCACGGACTACACGTCATTATT -3′
COIII	Reverse	5′-GATGTTCGGATGTAAAGTGGTATTG -3′
COIII	Probe	5′-NED- TTCCTAGCTGTTTGCCTTCTCCGT -MGBNFQ-3′

(2015). Briefly, we used Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc., Germantown, MD) supplemented with a Qiagen QIAshredder column to extract DNA from PES filters. The extracted DNA was then treated with a Zymo OneStep PCR Inhibitor Removal column (Zymo, Irvine, CA). Some water samples filtered with difficulty; in these cases, two filters instead of one were used to process the samples (N = 20, < 5% of samples). For these samples, the filters were extracted separately and then the elutions were combined into one tube following extraction. Extraction negatives, consisting of clean, unused filters, were included with each set of extractions.

The eDNA samples were tested for the presence of grass carp DNA using real-time quantitative PCR (qPCR). The reagent sets target three mitochondrial loci: GCTM10 amplifies a 141 base pair region of the NADH dehydrogenase subunit 2 (ND2) gene, GCTM22 amplifies a 99 base pair region of the cytochrome *c* oxidase subunit II (COII) gene, and GCTM32 amplifies an 86 base pair region of the cytochrome *c* oxidase subunit III (COIII) gene (Table 2). All primers and the probe for GCTM22 were synthesized by Integrated DNA Technologies, (Coralville, IA) and the probes for GCTM10 and GCTM32 were synthesized by Applied Biosystems (Waltham, MA). Reactions were run in multiplex on four replicates of each sample. Two replicates of a standard curve produced by 5-fold serial dilution of the 464 base pair GCTM1/3 gBLOCK gene fragment (Integrated DNA Technologies, Coralville, IA) in 100 ng/µL yeast tRNA (Sigma Life Science, St. Louis, MO) were included on each plate, with copy numbers ranging from 10 to 31,250. This gBLOCK gene fragment contains the DNA sequences targeted by all three reagent sets. In addition, two no template control reactions containing UV treated sterile nuclease free water (Millipore Sigma, Burlington, MA) and two positive reactions containing grass carp genomic DNA at a target copy range of 500 to 6000 were also included on each plate. Positive controls were added to the plates last to minimize the chance of contamination of samples. PCR reaction volume was 20 µL, and reactions consisted of 10 µL of 2X TaqMan Environmental Master Mix 2.0 (Life Technologies, Carlsbad, CA), 1 µL of a 10 µM GCTM primer mix, 2 µL of a 2.5 µM GCTM probe mix, 3 µL of eDNA template or UV treated sterile nuclease free water for no template control reactions, and 4 µL of UV treated sterile nuclease free water. Alternatively, standard curve and positive

control reactions contained the same amounts of 2X TaqMan Environmental Master Mix 2.0, primer mix and probe mix, and 6 µL of UV treated sterile nuclease free water, but standard curve reactions contained 1 µL of GCTM1/3 gBLOCK DNA, and positive control reactions contained 1 µL of grass carp genomic DNA.

Reactions were carried out on a QuantStudio™ 6 Flex Real-Time PCR System (Thermo-Fisher, Waltham, MA) using the Fast 96-well block, standard cycling conditions and the following cycling parameters: an initial denaturation step of 95 °C for 10 minutes; then 45 cycles of 95 °C for 15 seconds and 65 °C for 1 minute, with image collection at 65 °C. Data were analyzed using QuantStudio™ Real-Time PCR Software v 1.2 (Thermo-Fisher, Waltham, MA), using automatic baseline and manual threshold settings. A positive detection was determined when an amplification curve crossed the fluorescence threshold (above background fluorescence) for at least one assay in one of the four replicates.

Species specificity

The grass carp qPCR assays were tested for species specificity using genomic DNA isolated from three other invasive carp species with similar sequences (black carp [*Mylopharyngodon piceus*], silver carp, and bighead carp), as well as grass carp DNA. Template DNA isolations from each of the 4 invasive carp were subjected to 10-fold serial dilution, producing 7 samples with concentrations ranging from 1 ng/µL to 0.000001 ng/µL, and 1 µL of DNA was used as template in each qPCR reaction. Two replicates were used for each concentration of each species and, as described above, the GCTM1/3 gBLOCK standard curve and grass carp positive control DNA were also included. We evaluated the assays using the above qPCR conditions with 3 different annealing temperatures (63.4 °C, 65.4 °C and 65.9 °C).

Statistical analyses

To determine assay performance at low target eDNA concentrations, we quantified the limits of detection (LOD) and quantification (LOQ) from the qPCR standards for each assay (52 replicates for each standard concentration across the 26 plates). Within qPCR, LOD is defined as the lowest copy number where 95% of the replicates per concentration were positive. LOQ represents the lowest concentration of target DNA that can be quantified within an assay. All data used to create the standard curves were used to estimate LOD and LOQ. Curve-fitting analyses followed the methodology of Klymus et al. (2020). Sigmoidal models were used to determine the LOD, and model variants consisted of using all available logarithmic functions, and the best fitting model was chosen based on log likelihood values. LOQ was quantified by modeling the coefficient of variation (CV) of Cq (cycle number above fluorescence threshold) for linear, exponential decay, and polynomial models

and used a threshold of 35% CV (Klymus et al. 2020). For the purpose of this study, we considered a sample positive for grass carp eDNA if at least one replicate for one of the three assays exceeded the fluorescence threshold after the first 15 cycles, similar to the methods of DeHaan et al. (2023).

Bayesian multi-scale hierarchical models were used to estimate eDNA detection probabilities across month, site, and assay. Hierarchical models are beneficial for estimating occupancy from eDNA samples given the detection of eDNA is conditional on several dependent factors, and hierarchical models factorize joint probability distributions into simpler expressions of conditional probabilities (Mize et al. 2019). Specifically, these models can accommodate the estimation of several parameters in the context of eDNA data: 1) probability of species eDNA occurrence at a given site (ψ), 2) conditional probability of grass carp eDNA collected in the water sample at a site given that grass carp were present at the site (Θ), and 3) conditional probability of detection from qPCR in replicates collected at a sampling site given that the species eDNA was present in the water sample *(p)*. The occupancy probabilities are presented as medians with 95% credible intervals.

Model covariates included sampling location (site), eDNA assay, and time (month and season). Site and time were included as covariates for ψ given that grass carp express seasonal distributions within Lake Erie and its tributaries and may not always be present at each site within each month or season of sampling (Harris et al. 2021). The probability of eDNA capture (Θ) can also be influenced by different environmental conditions, such as water flow rates and temperature, across sites (e.g., lotic vs. lentic) and within sites over time (Fukumoto et al. 2015; de Souza et al. 2016; Curtis et al. 2021). Detection probability depends on the ability to detect eDNA among replicates through molecular techniques, such as qPCR; therefore, assay was used as covariate for detection probability (*p*). We included two time covariates (month and season) for ψ and Θ to determine if there were differences in parameter estimates and model support among different temporal resolutions. We also include a null (intercept only) model without covariates in model selection analyses (all parameters assumed constant). Overall, hierarchical model variants included all possible combinations of covariates among the three parameters in the model candidate set.

All hierarchical models were fit with the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm, with 8,000 iterations, and a burn-in of 1,000. For each model, convergence was assessed by evaluation of trace plots for steady state distributions (Supplementary material Figure S1). All hierarchical modelling was conducted in the *eDNAoccupancy* R package (Dorazio and Erickson 2018) and performed in R version 4.2.1 (R Core Team 2018). Autocorrelation functions were applied to the Markov chain for each of the parameters in the multi-scale occupancy model to determine the number of iterations of the MCMC algorithm that were needed to reduce error generated by correlations among Monte Carlo variables (Dorazio and

Erickson 2018). For model selection we used Widely Applicable Information Criterion (WAIC), a Bayesian criterion that uses the posterior distribution of models to quantify predictive variance and goodness of fit (Watanabe 2013). Similar to AIC, lower WAIC values indicate the models better fit the data.

Telemetry and Removal Efforts

In addition to eDNA sampling, we used acoustic telemetry data and data collected during grass carp removal efforts as a form of ground truthing to verify if grass carp were at the sites prior to eDNA sampling. While neither dataset was robust enough to incorporate into modeling efforts, they nevertheless provided a worthwhile ground truthing comparison to the presented eDNA results. Limitations and caveats associated with these data are discussed in subsequent sections.

To determine whether grass carp were present within the same week of eDNA sampling, we opportunistically tagged 27 individual grass carp with acoustic transmitters that were available in Lake Erie from acoustic receivers deployed as part of the multi-agency collaborative Great Lakes Acoustic Telemetry Observation System (Hayden et al. 2014; Harris et al. 2021). Acoustic receivers were present concurrently with eDNA sampling sites: Detroit River (N = 2), Hot Ponds (N = 23), and Maumee Bay (N = 4, present in 2019 only). For additional information on grass carp acoustic tagging and telemetry procedures please refer to Harris et al. (2021). Receivers were located within 2.5 km of all eDNA sampling locations within each site (Figures 1 and S2). To obtain insight on average distances from acoustic receiver locations and eDNA sample collections, nearest neighbor analysis was employed in the R package *nngeo* (Dorman 2022). Nearest neighbor distance analysis determines the shortest distance among a set of points (i.e., between eDNA samples and acoustic receivers).

Grass carp response efforts from natural resource agencies were conducted immediately after water samples were collected at the same sites of eDNA sampling in this study (March–November; Figure S2). Traditional gear methods that were deployed for grass carp response efforts consisted of mounted electrofishing and large-mesh trammel nets (183 × 4.3 m; 10.2 cm inner-square monofilament and 45.7 cm twisted multifilament nylon outer mesh panels) (Fischer et al. 2022a). Electrofishing sampling consisted of pulsed DC waveform (60 Hz, 24% duty cycle) for a cumulative shock time averaging 15 minutes per sample, for a total of 45 hours throughout the study for electrofishing only events (N = 173). These gear types were either deployed solo (electrofishing only) or in combination (electrofishing + trammel nets). Combination sampling events (N = 183) consisted of deploying trammel net sets in a "U-shaped" shore-to-shore net configuration, with electrofishing inside and outside of the nets for 15 minutes to drive fish into shallow water or the trammel nets. For more information on the capture-based gear response strategies and methods, please refer to Fischer et al. (2022a) and Lang (2022).

Results

Assay sensitivity

All standard curves met the requirements of slope ~ −3.3, $r^2 > 0.95$ and efficiency = 80–120% (Figure S3), and no extraction negatives or no-template qPCR control (NTC) reactions showed amplification. All qPCR positive controls were positive. On all 26 plates, positive amplification was observed for all three assays in both replicates of the standard curve 1/3 gBLOCK copy numbers of 50, 250, 1250, 6250, and 31250 (Figure S4). In the reactions containing 10 copies of the GCTM1/3 gBLOCK gene fragment, failure to amplify was seen in 8 wells for GCTM10 (8/52 = 15.3%), 5 wells for GCTM22 (5/52 = 9.6%), and 6 wells for GCTM32 (6/52 = 11.5%). The LOD was 50 copies of DNA per reaction for each assay based on the lowest standard concentration tested with 95% or greater positive detections among all replicates tested. The modeled LOD for each GCTM10, GCTM22, GCTM32 was 15.6, 12.8, and 17.1 copies per replicate, respectively. The estimated LOQ for GCTM10, GCTM22, GCTM32 was 73, 52, and 18, respectively.

Assay specificity

At the lower 2 annealing temperatures tested (63.4 °C and 65.4 °C), neither bighead nor silver carp amplified at any DNA concentration for any of the 3 assays, and so were not tested at the highest annealing temperature (65.9 °C). Black carp showed no amplification for GCTM32 at any of the 3 annealing temperatures. However, amplification products were observed for GCTM10 and GCTM22 in black carp at higher DNA concentrations at the two lower temperatures evaluated. Elevating the annealing temperature to 65.9 °C resulted in the loss of amplification in black carp, except in one replicate at the highest DNA concentration tested (1 ng/µL) for both GCTM10 and GCTM22. Of note, black carp are not thought to be present in Lake Erie at this time, and cross-amplification should not affect the results.

qPCR of empirical field samples

Grass carp DNA was detected in 82 samples (19%) and no eDNA detections were present in negative control samples. A total of 38 samples (8.7%) had positive grass carp DNA detections on more than one assay and more than one replicate. Grass carp DNA was detected in almost every month and site, with the exception of North Maumee Bay and Detroit River in 2018. Regardless of assay, the Hot Ponds site had the greatest proportion of samples with positive detections (25.8%), while both North Maumee Bay and Detroit River had nearly identical proportions of detections with 14.6% and 14.4% of samples testing positive.

Positive detection rates differed slightly among DNA assays. For GCTM10, GCTM22, and GCTM32 the number of samples with a positive detection (at least one qPCR replicate with positive detection) was 52 (11.8%), 56 (12.7%),

Table 3. Candidate set of hierarchical occupancy models used to estimate probability of grass carp eDNA occurrence among sites (ψ), the conditional probability of grass carp eDNA occurrence at a sampling locality within a site given that grass carp were present at the site (Θ), and the conditional probability of eDNA detection on replicate filters collected at a sampling locality given that the species is present at the sampling locality *(p)* from three sites in western Lake Erie sampled in 2018 and 2019. Covariates included location (site), time (Month) and probe type (GCTM10, GCTM22, GCTM32). Model comparison was evaluated with the Widely Applicable Information Criterion (WAIC).

Model	WAIC	Δ WAIC	Lack of fit	Predicted Variance
ψ(Site)Θ(Site)p(.)	309.44	-	298.70	18.63
ψ(.)Θ(Site)p(.)	309.50	0.06	298.99	10.73
ψ(.)Θ(Month)p(.)	317.61	8.18	299.05	18.56
ψ(Season)Θ(.)p(.)	317.67	8.24	299.01	18.65
ψ(Month)Θ(.)p(.)	317.72	8.28	299.04	18.67
ψ(Season)Θ(Season)p(.)	317.84	8.40	299.04	18.79
ψ(.)Θ(Season)p(.)	317.74	8.31	299.07	18.66
ψ(Site)Θ(.)p(.)	317.94	8.51	299.04	18.90
ψ(.)Θ(.)p(.)	325.00	15.57	305.50	20.21
ψ(Season)Θ(Site)p(.)	325.41	15.98	305.49	19.91
ψ(Site + Season)Θ(.)p(.)	325.59	16.16	305.44	20.15
ψ(Site)Θ(Season)p(.)	325.72	16.29	305.48	20.23
ψ(Site + Season)Θ(Site + Season)p(.)	325.92	16.49	305.49	20.43
ψ(.)Θ(Site + Season)p(.)	326.37	16.94	305.52	20.84
ψ(Site)Θ(Month)p(.)	329.57	20.14	308.65	20.92
ψ(Month)Θ(Month)p(.)	330.14	20.71	308.66	21.84
ψ(Site + Season)Θ(Site)p(Probe)	377.63	68.20	276.90	100.72
ψ(Site + Season)Θ(.)p(Probe)	378.35	68.92	277.00	101.34
ψ(Site + Season)Θ(Site + Season)p(Probe)	378.42	68.99	277.00	101.41
ψ(Site + Season)Θ(Season)p(Probe)	378.55	69.12	277.09	101.46
ψ(Season)Θ(Site + Season)p(Probe)	379.41	69.98	277.28	102.10
ψ(Site)Θ(Site + Season)p(Probe)	379.62	70.19	277.40	102.21
ψ(.)Θ(Site + Season)p(Probe)	379.88	70.45	277.31	102.57
ψ(Site + Month)Θ(.)p(.)	383.56	74.13	282.86	100.69
ψ(Site + Month)Θ(Site + Month)p(.)	385.47	76.04	283.38	102.09
ψ(Site + Month)Θ(Site + Month)p(Probe)	386.95	77.52	282.90	104.05
ψ(.)Θ(Site + Month)p(.)	396.44	87.01	292.14	104.29
ψ(.)Θ(.)p(Probe)	396.45	87.01	292.14	104.29

and 62 (15.5%), respectively. Please see Table S1 for a detailed summary of qPCR replicate detections and non-detections among site, year, and month for each grass carp eDNA assay.

Occupancy Modelling

Of the 28 candidate models we compared, the most parsimonious model with the lowest WAIC score included site as a covariate of both eDNA occupancy and eDNA capture probability ([ψ(Site)Θ(Site)p(.)], Table 3). The next best-fitting model [ψ(.)Θ(Site)p(.)] included site as a predictor of eDNA capture probability (Θ) with all other parameters held constant. Models with an assay covariate on the detection probability (p) and temporal covariates included in the capture probability (Θ) and site occupancy (ψ) parameters were not as supported according to information criteria (Table 3). We also observed no difference in sample eDNA capture probability (Θ) among months in the third best fitting model [ψ(.)Θ(Month)p(.)], (Table 3)] based on overlapping 95% credible intervals among estimates (Figure S5). The mean probability of grass carp site occupancy (ψ) was 1.0 (95% CrIs: 0.99–1.0). eDNA capture probability (Θ) was higher in Detroit River (0.18;

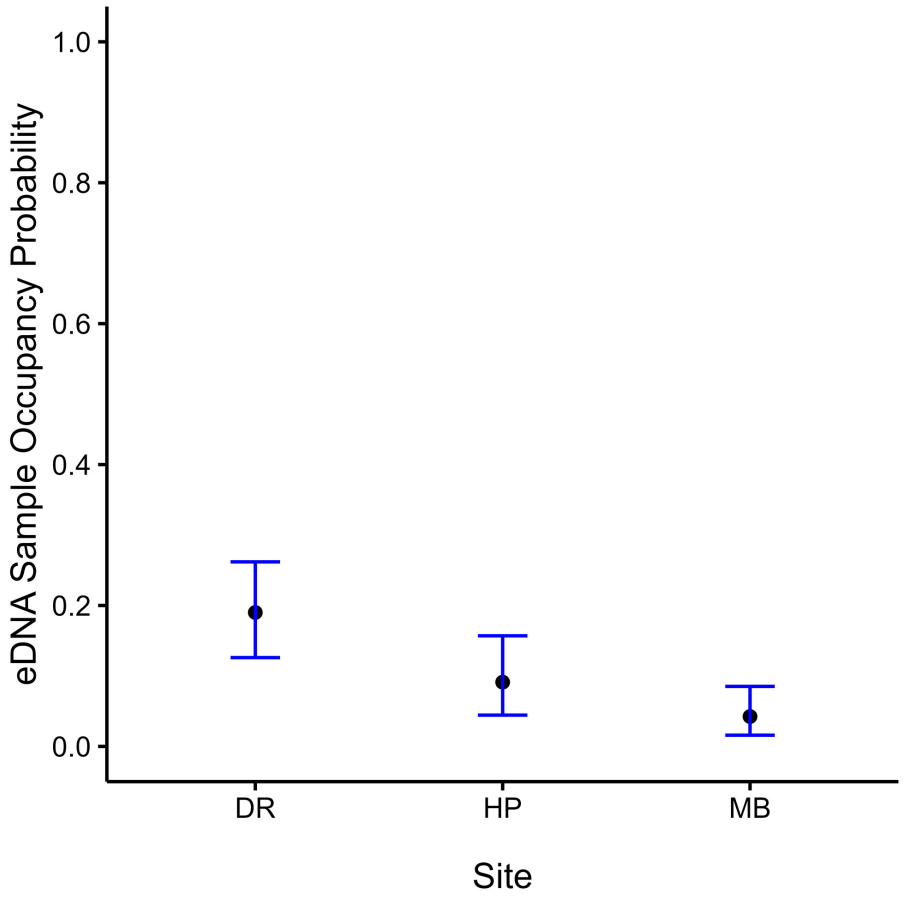

Figure 2. Mean posterior estimates of the probability of capturing grass carp eDNA from a site in a sample among sites (θ) from the model with the lowest WAIC score [ψ(Site)Θ(Site)p(.)]. Error bars represent 95% credible intervals. DR = Detroit River, HP = Hot Ponds, MB = Maumee Bay. All sites are located in western Lake Erie.

95% CrIs: 0.12–0.26) relative to Maumee Bay (0.039; 95% CrIs: 0.01–0.08), but Hot Ponds (0.08, 95% CrIs: 0.04–0.15) was not different from Detroit River or Maumee Bay (Figure 2). The overall detection probability (p) was 0.343 (95% CrIs: 0.31–0.37).

Acoustic telemetry

Across sites, the mean distances between eDNA sampling locations and acoustic receiver locations were 46.7 m (range =10–119 m) and 78.0 m (range = 5 m–122 m) in 2018 and 2019, respectively. Positive acoustic telemetry detections (500 m = maximum receiver detection radius) from at least 1 tagged grass carp occurred in 21 of the eDNA sampling event weeks and sites (N = 32) (Table 4). Overall, positive telemetry detections of grass carp occurred 1–6 days (mean = 2.3 days, median = 1 day) prior to 29 eDNA sampling events with telemetry data available. Of those positive telemetry detection events within 1 week of the eDNA sampling events, 17 (81%) resulted in a positive eDNA detection (at least one detection across replicates) while the other 4 (19%) resulted in negative eDNA detections. The number of tagged grass carp detected from acoustic telemetry within the same week

Table 4. Percentage of positive eDNA replicate detections in each month and site in 2018 and 2019 in western Lake Erie (based on at least one positive detection on at least one marker and one replicate). All markers (GCTM10, GCTM 22, GCTM32) were used to calculate these proportions. Samples were collected monthly from June to November for each site. The number of telemetered grass carp detected within 7 days before sampling for eDNA is denoted in parentheses. Acoustic telemetry receivers in MB in 2018 were not available. DR = Detroit River, HP = Hot Ponds, and MB = Maumee Bay. NA denotes when acoustic telemetry receivers were not in operation.

Site	Year											
	2018						2019					
	June	July	Aug	Sept	Oct	Nov	May	June	July	August	Oct	Nov
DR	0.0% (1)	2.3% (1)	2.3% (2)	0.0% (1)	0.0% (2)	0.0% (0)	4.5% (1)	10.6 % (1)	14.1 % (1)	17.4% (2)	14.1% (2)	2.2% (0)
HP	0.0% (1)	0.1% (1)	4.1% (2)	2.2% (2)	15.8% (3)	0.0% (NA)	9.0% (2)	1.5% (2)	34.8 % (2)	1.5% (3)	15.8% (2)	22.7% (2)
MB	12.0% (NA)	0.0% (NA)	0.0% (NA)	0.0% (NA)	0.0% (NA)	0.0% (NA)	0.0% (0)	6.1% (0)	37.1 % (0)	8.3% (0)	8.3% (0)	0.0% (0)

of eDNA sampling events ranged from 1 to 3 across sites (Figure S6). Within Maumee Bay in 2019, there was no confirmed presence of grass carp from telemetry, but 4 out of 6 eDNA sampling events yielded positive eDNA detections. It is important to note that only a small number of grass carp are tagged (N = 27) and thus, grass carp may still have been present but not detected.

Grass carp removal sampling events

A total of 3 grass carp were captured out of 356 gear sampling events. Only one grass carp was captured concurrently with an eDNA sampling event (Detroit River on October 11, 2018, with electrofishing only method) with a positive detection and two grass carp were captured two weeks prior to eDNA sampling events in the Maumee Bay (July 3) and Hot Ponds (July 7) in 2019 with the combination gear sampling.

Discussion

We quantified eDNA capture probabilities and detection rates of grass carp eDNA in three sites within the western basin of Lake Erie. Across a two-year period, positive grass carp eDNA samples were present at all locations. Sensitivity and specificity results demonstrated that the qPCR assays were able to detect low levels of DNA, and were effective at targeting grass carp, indicating that they are reliable and accurate (Figures S3, S4). Compared to the LOD values in Klymus et al. (2020) for the same grass carp assays, our LOD values were 5.1–7.0 times higher, indicating our qPCR assays required higher DNA concentration levels for detection. However, the LOQ values in our study were 2.6–13.3 times lower for GCTM22 and GCTM32 and 2.6 times higher for GCTM10 relative to Klymus et al. (2020), suggesting that the copy number needed for precise quantification of eDNA concentrations varied across our assays (lower values suggest lower copy numbers needed for precise quantification and vice versa). Additionally, no other invasive carp species were known to be present in our study sites and therefore, cross-amplification of non-target carp eDNA is not likely to influence our results.

Our modeling efforts found that our per sample detection probability was 34% and eDNA capture probabilities varied by site (Figure 2). Acoustic telemetry detections occurred within the same week of the majority of eDNA sampling events (58%), increasing the likelihood that grass carp DNA was present and reducing the chance for false positives. In contrast, only one grass carp was physically captured with conventional gears concurrently with one eDNA sampling event, indicating that conventional gears used could have lower efficacy for detecting grass carp presence/absence compared to eDNA over a longer temporal scale. Our study was the first to successfully collect invasive carp eDNA in Lake Erie and our findings suggest the qPCR assays evaluated here will be useful for future presence/absence grass carp monitoring.

Detection probabilities

Our estimated detection probabilities (one marker detected across at least one sample replicate) were slightly higher (34%) than previous invasive carp eDNA studies, indicating that the assays used here may be beneficial for early detection monitoring. For example, average bighead carp and silver carp detection probabilities in several habitats within the Upper Mississippi Basin did not exceed 27% (Mize et al. 2019). This could reflect greater sampling frequency within our study (monthly and annually) instead of three-month interval sampling efforts in Mize et al. (2019). It is also important to note that site characteristics in Mize et al. (2019) may have partially resulted in lower detection rates relative to this study because most sites sampled here had slower water flow (due to a complex mosaic of backwaters and islands), with the exception of the Detroit River, and may have led to locally higher concentrations of eDNA (Jane et al. 2015; Klymus et al. 2015). Several other factors, such as likely higher bigheaded carp densities in Mize et al. (2019) within the Mississippi River, USA compared to grass carp abundance estimates in Lake Erie (Gouveia et al. 2023), differences in DNA sampling and extraction methods, primer designs, and eDNA concentrations may have resulted in different detection probabilities among studies (Piggott 2016). Given these differences among studies, it may be prudent to investigate how sampling and methodological factors influence eDNA detection probabilities.

Site-specific patterns of DNA occupancy

The probability of grass carp eDNA capture differed across sites, with the Detroit River site exhibiting higher probability relative to Maumee Bay (Figure 2), which may be partially explained by site-specific differences in hydrology and grass carp behavior. For instance, the Maumee Bay and Hot Ponds areas are lentic environments, while the Detroit River is a lotic environment with considerably more flow. In high-velocity environments,

eDNA is more likely to become widely dispersed, and eDNA transport distance increases with increased flow (Thalinger et al. 2021) but can lead to a dilution of DNA, and subsequently engender lower capture probabilities, despite being distributed more broadly (Jane et al. 2015). On the other hand, an investigation on the rare crayfish (*Faxonius eupunctus*) in the lotic Eleven Point River drainage system (Missouri, USA) demonstrated that eDNA detections increased further downstream despite lower observed *F. eupunctus* densities further downriver. Increased eDNA transport and concentrations contributed to this pattern downstream as a result of slow DNA degradation from cooler water temperatures, the presence of local and upstream crayfish, and low dilution (Rice et al. 2018).

DNA transport in lotic environments is often variable within and among streams given that it is contingent on primarily abiotic characteristics. Factors such as flow velocity, depth, turbulence, stream size, sediment composition, and the retention of DNA in the sediment can affect DNA dispersion (Pont et al. 2018; Harrison et al. 2019; Nevers et al. 2020, Chucholl et al. 2021), making generalizations on DNA transport in lotic systems difficult. In our study, one of the lentic environments (Hot Ponds) did not exhibit differences in occupancy relative to Detroit River. This was surprising considering that DNA in lentic systems is often patchy and eDNA concentrations rapidly decline with increasing distance from the source, often requiring many water samples collected in close proximity to each other (Bedwell and Goldberg 2020). However, we did observe longer sustained presence of grass carp (one grass carp was detected consistently from September 2018 to October 2019) in this area compared to other sites based on acoustic telemetry detections, which may have contributed to this pattern. Differences in physical mixing properties could influence the dispersion of eDNA from the focal fish populations within open lentic environments such as the Maumee Bay site. For example, seasonal thermal stratification in warmer months can lead to limited transport of DNA from reduced water flow (MacIntyre and Melack 1995), and reduced vertical mixing could lead to a higher density of DNA containing particles, such as fecal matter, near the sediment surface instead of being distributed more homogenously throughout the water column (Harrison et al. 2019).

Seasonal variation in temperature may also influence eDNA concentrations across sites. For instance, more frequent collection of eDNA samples at higher temperatures is required because eDNA degrades faster at warmer temperatures, thereby decreasing detectability (Pilliod et al. 2014) unless the duration between fish presence and sampling is low (Takahara et al. 2012). Decay rates of eDNA across many fish species are positively correlated with temperature, and thus, could confound the relationship between eDNA concentration and biomass in the field (Andruszkiewicz et al. 2021). Of note, the Hot Ponds area water temperature profile was 5.4 °C–11.0 °C

warmer than other sites in any given month during sampling presumably due to effluent from a nearby power plant, which could have influenced the shedding and degradation rates of eDNA. On the other hand, temperature has been demonstrated to be a driver of grass carp vagility and activity, and thus, may potentially counteract higher rates of eDNA degradation during warmer summer temperatures (Weberg et al. 2020). Grass carp also exhibit seasonal distribution patterns within Lake Erie that may influence the availability of their DNA. For instance, the majority of grass carp reside in tributaries from May–June for spawning, while outside of their spawning season, some grass carp either migrate among nearshore environments or tributaries throughout the western basin of Lake Erie or remain in multiple reaches of their spawning tributaries (Harris et al. 2021). Despite no observed differences in eDNA capture probabilities among months within this study, high parameter uncertainty was present in some months and the influence of seasonal environmental factors on grass carp DNA characteristics (e.g., shedding, decay) could continue to be evaluated to identify eDNA sampling implications and limitations (Barnes et al. 2014). Given the relatively low estimates of eDNA capture probability (Θ) in our study, substantially larger sample sizes may be required for robust detection and estimation of occupancy model parameters (Erickson et al. 2019; Mize et al. 2019). While our sample sizes differ from recommendations in Mize et al. (2019), it may not be applicable in the context of this study as we used different eDNA sampling approach (filtrating of large volume water samples rather than centrifugation of small volumes) and sampling occurred prior to the Mize et al. (2019). However, DeHaan et al. (2023) exhibited higher eDNA capture probability (Θ) > 0.95 at sites within the nearby Sandusky River, OH, with the same assays used in this study across all four seasons. Differences observed between studies could be a result of contrasting water sampling volume, the amount of samples collected at each site and sampling period, habitat type (lotic vs. lentic), and grass carp densities/duration of occupancy. Current sampling efforts are underway within Lake Erie to compare the impact of sampling methodologies used from this study and DeHaan et al. (2023) on eDNA capture probability.

Routine grass carp eDNA sampling in the Great Lakes could be useful for early detection and enhancing rapid response efforts. Habitats outside of the western basin of Lake Erie, including embayments, nearshore areas, and tributaries, are currently being explored with conventional gear, but eDNA could aid in quantifying the efficiency of current removal efforts and improve our understanding of their expansion. Limited knowledge of grass carp presence exists in areas outside of the western basin due to infrequent captures, but acoustic telemetry studies have demonstrated that grass carp can exhibit extensive migrations (> 100 km) among tributaries and nearshore Lake Erie waters (Harris et al. 2021). Based on our study,

eDNA could be promising for detecting grass carp in nearshore waters when present in low densities given the detection probabilities estimated here and frequent positive detections from eDNA when grass carp were known to be present in the system within 1-week of sampling. The application of eDNA has been successful in vast environments for detecting sparsely abundant or rare species, such as the marine winter skate (*Leucoraja ocellata*) in estuarine harbors (Weltz et al. 2017), and several rare species in nearshore Atlantic waters (Stoeckle et al. 2021). Moreover, eDNA could be one of the few available techniques that could be used to detect the presence of juvenile grass carp. Within U.S. reservoirs, juvenile grass carp typically do not exhibit long or out-of-reservoir migrations (Weberg et al. 2020). In their native range, Gorbach and Krytkhtin (1988) reported juvenile grass carp may feed for years in the lower reaches of the Amur River and exhibit limited movement. Once they reach sexual maturity (~ ages 3–6), they begin upstream movement to spawning grounds and can travel up to 500 km in their first 2 years. Within the Great Lakes, juvenile habitat use is largely elusive considering that conventional gears rarely capture smaller age classes (< 4 years of age) (Lang 2022), and eDNA could potentially identify juvenile presence/absence and new spawning tributaries (Hayer et al. 2020).

Overall, this study underscores that eDNA and the assays developed by the USFWS (DeHaan et al. 2023) could be an important exploratory monitoring tool that could be used to determine grass carp presence/absence in waterbodies more efficiently or conveniently than capture-based approaches. Environmental DNA monitoring has shown promise to detect invasive species, is often less time-consuming and expensive than using capture-based approaches, and can be used to explore previously inaccessible aquatic environments or circumvent depth limitations of some fisheries gear types (Pikitch 2018; Jerde 2021). Overall, we found varying capture probabilities of grass carp eDNA among the sites sampled in this study. This is important for managers and others who are interpreting eDNA results because this suggests that detecting eDNA is not consistent across space and indicates further research is needed to identify environmental factors that underpin this pattern. One potential pathway is to use fixed-station field cage studies to help optimally design grass carp eDNA monitoring programs, by contributing to our understanding of the level of replication necessary to ensure reliable detection, spatial transport of eDNA (lentic vs. lotic systems), and the influences of fish density and local environmental conditions on eDNA detection probabilities (Mize et al. 2019; Spence et al. 2021). While this study provides evidence for detection of live grass carp, we acknowledge that uncertainties remain to be resolved with the application of eDNA for monitoring. Secondary vectors of eDNA transport and introduction could occur through various means, such as vessel transport, animal movement, and decaying carcasses, and thus, provide false evidence of a target species presence. We also had limited understanding of the density of fish present concurrently with

sampling which can influence the DNA concentration, and subsequently, eDNA capture probability (Tillotson et al. 2018). Evaluating grass carp density and comparing detection probability of occupancy models with other techniques (e.g. other gear types) may help understand further limitations from estimation approaches as was observed in Ulibarri et al. (2017) and Randall et al. (2023). While this study demonstrates the potential utility of eDNA for early detection and monitoring of grass carp, further evaluation of grass carp eDNA characteristics in the environment could help improve eDNA monitoring within the Great Lakes.

Author's contribution

Research conceptualization and sample design and methodology were conducted by KFR, JDR, KTS, and SH. Field data were collected by KFR, LRN, JDR, and SH, and laboratory data were collected by LRN and JK. Data analysis and interpretation were conducted by JB, with input from all co-authors. Funding provision was conducted by KFR. The manuscript was drafted by JB, and all co-authors contributed to review and editing of the drafts.

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Supplementary material

The following supplementary material is available for this article:

Table S1. qPCR detections of grass carp eDNA across replicates for each assay (GCTM10, GCTM22, GCTM32) at each site and month.
Figure S1. Autocorrelation factor and MCMC trace plots for the alpha coefficient (corresponds to the Θ parameter) from the best-fit hierarchical occupancy model.
Figure S2. Map denoting the 2018 and 2019 eDNA grass carp sampling locations, acoustic receivers, and removal capture gear deployment in the western basin of Lake Erie.
Figure S3. Amplification plots for grass carp standard curves for all three loci.
Figure S4. qPCR standard curves for all three grass carp loci.
Figure S5. Average probability of capturing grass carp eDNA at a sampling location (Θ).
Figure S6. Plot denoting the number of unique grass carp detections and individual grass carp counts from acoustic telemetry with eDNA sampling dates across the Hot Ponds and Detroit River.

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