INTRODUCTION

The detection and quantification of environmental DNA (eDNA), genetic material shed by organisms into their surrounding environment (Taberlet et al., 2012), holds promise for the surveillance of rare or difficult to detect organisms (Dougherty et al., 2016; Thomsen et al., 2012) as well as common, commercially valuable organisms in applications like fisheries stock assessment (e.g. Salter et al., 2019). Research to date suggests eDNA may often be more sensitive to detect organismal presence than conventional surveys (Goldberg et al., 2014).
et al., 2013; Jerde et al., 2011), and may also be cost and time efficient relative to some standard sampling methodologies (Evans et al., 2017; Smart et al., 2016). However, uncertainties remain related to the physical, chemical, and biological factors influencing eDNA production, transport, and persistence in natural ecosystems (Barnes & Turner, 2016; Cristescu & Hebert, 2018). As such, substantial research is still needed to define the environmental and organismal contexts for which eDNA does and does not perform well.

Many eDNA studies have been conducted in lotic ecosystems (streams and rivers), where water flow directionality and variability through time (i.e. the hydrograph or flow regime; Poff et al., 1997) may strongly affect the performance of this method (Stoeckle et al., 2017). Yet the effects of stream flow on eDNA remain understudied (but see Akre et al., 2019; Jane et al., 2015; Shogren et al., 2018). High stream flows or floods may affect eDNA concentrations and the overall detectability of target organisms through multiple, potentially opposing mechanisms. High stream flows might be expected to dilute eDNA in the environment, thus decreasing eDNA concentrations and potentially producing false negatives (Jane et al., 2015; Stoeckle et al., 2017). Similarly, transport of sediment or soil at high stream flows could cause false negatives through PCR inhibition (Buxton et al., 2017; Jane et al., 2015). Alternatively, high stream flows could re-suspend buried eDNA from stream or river sediments, consequently increasing detection probabilities and potentially leading to false positives (Turner et al., 2015). Further, at low flows it is expected that eDNA would be quickly removed from the water column as it settles onto the substrate, but during high flows eDNA may persist in the water column longer and be transported farther (Shogren et al., 2018). Researchers and practitioners working in environmental management need guidance on which of these opposing effects of stream flow on eDNA prevail under most conditions in order to design and implement appropriate sampling schemes.

Previous research on the effects of stream flow on eDNA has largely been restricted to artificial streams (Shogren et al., 2018), or short-duration enclosure (Jane et al., 2015) and tracer experiments (Fremier et al., 2019; Shogren et al., 2019). Results of some of these studies have been inconsistent with respect to the effects of stream flow or floods on eDNA concentrations and the detectability of target taxa. For example, Jane et al. (2015) enclosed Brook Trout (Salvelinus fontinalis) in two headwater streams, finding that during high flows eDNA copy number declined in one stream but increased in the other. Several experimental stream studies have provided mechanistic insight into how stream flow and substrate type affect eDNA transport and retention (Shogren et al., 2017, 2018), but it may be difficult to extrapolate from these small-scale, short-duration observations to the effects of large magnitude floods in natural ecosystems, as well as to estimate the relative importance of stream flow in comparison with other abiotic or biotic factors that can simultaneously affect eDNA. Specifically, is the effect of stream flow on eDNA concentration and detectability as important as the abundance or biomass of study organisms across disparate ecosystems (Yates et al., 2019), or as important as other abiotic factors like temperature or UV exposure that may influence eDNA production and persistence (Kessler et al., 2020; de Souza et al., 2016; Strickler et al., 2015)?

We propose that long-duration in situ studies are needed to generalize the effects of stream flow on eDNA concentrations and detectability, as well as to compare these effects concurrently to the role of other abiotic or biotic factors related to eDNA production, transport and persistence (Barnes & Turner, 2016). Here, we assessed how stream flow affects eDNA concentrations and detectability in situ using populations of an invasive freshwater mollusc, the Asian Clam (Corbicula fluminea). We used a longitudinal study to assess the role of stream flow, including high magnitude floods, on eDNA concentrations and detectability over an entire year at two stream sites, as well as a seasonal study (summer, autumn) to evaluate similar effects at eight stream sites over a gradient of low to high C. fluminea abundance. Together, our two studies provide the longest duration and largest-scale investigation of the effects of stream flow on eDNA concentrations and offer direct contrasts between the role of stream flow on eDNA relative to other abiotic or biotic factors. Our results should help researchers and practitioners design better eDNA sampling schemes for lotic environments and inspire subsequent studies on the relationship between stream flow and eDNA in order to replicate and validate our work in different ecosystems and taxonomic groups.

2 METHODS

2.1 Study species

Corbicula fluminea is a small (<5 cm) freshwater clam native to Africa, Asia and Australia that has invaded North America, South America and Europe likely through ballast water, bait releases, and intentional introductions as a food source (Crespo et al., 2015). Due to its rapid sexual maturity, high fecundity, and ability to reproduce both sexually and asexually (Hornbach, 1992), C. fluminea is considered among the most impactful aquatic invasive species globally (Sousa et al., 2008). Invasions of C. fluminea have been reported to alter biogeochemical cycling (Turek & Hollein, 2015), negatively affect native mollusc species (Haag, 2019), clog water intakes and canals (Isom, 1986), and cost over $1 billion/year in the United States (US) to manage (Pimentel et al., 2005). We chose C. fluminea as our focal species due to its prevalence in our study sites, the availability of a genus-specific eDNA assay for Corbicula (Cowart et al., 2018), and our hope that these results will be relevant to the management of this invader. While multiple forms and species of Corbicula have invaded the USA (Haponski & O’ Foighil, 2019; Tiemann et al., 2017), only C. fluminea was found at our study sites.

2.2 Longitudinal eDNA study

We selected two streams in central Illinois (Champaign County, US) equipped with US Geological Survey (USGS) flow gages that
measure stream flow every 15 min. Additionally, these streams represented a contrast in stream flow and density of *C. fluminea*. Copper Slough is an urbanized, flashy, headwater stream (12.8 km² drainage area) with gravel-sand substrate that drains into the Kaskaskia River (Table 1; Figure S1). Salt Fork is a larger (215.7 km² drainage area) rural stream with sand substrate, surrounded by row-crop agricultural fields, that drains into the Vermilion River of the Wabash River watershed (Table 1; Figure S1). We planned to sample Copper Slough bimonthly and Salt Fork monthly (Figure 1). In addition to this planned sampling, we opportunistically collected low-flow and high-flow events, including before rain and during rising and falling limbs of the hydrograph. We sampled these two stream sites from 11 January to 27 December 2018. In total, Copper Slough was sampled 33 times and Salt Fork was sampled 24 times (Figure 1).

At each sampling event, we collected four 250 ml surface water samples across the width of each stream at the location of the USGS flow gage. During high-flow events, when flowing across the stream was not possible, we collected water samples using buckets lowered from the bridge at the location of the USGS flow gage. All bottles had been washed with 50% bleach prior to use. Buckets and bottles were triple-rinsed with stream water at the site prior to water collection. After sample collection, bottles were sealed in a clean plastic bag, placed on ice in a cooler and filtered <2 hr after collection. During one collection, samples from Copper Slough were refrigerated and filtered the following morning (-12 hr after collection) but this delay does not affect the quantity of eDNA recovered (Curtis et al., 2021). For each sampling event, one field blank of distilled water per site was used to assess potential contamination in collection supplies. During each water collection (after water samples were collected), water quality parameters, including pH, temperature, salinity and total dissolved solids (TDS), were recorded using a handheld probe (Oakton®) and turbidity was recorded using a portable metre (Sper Scientific®; Table S1). We wore nitrile gloves to collect all water samples, filter water samples, and during all laboratory procedures, with frequent glove changes.

Water samples were transported to the University of Illinois at Urbana-Champaign (UIUC), where we cleaned the bench space with a 50% bleach solution prior to filtration and used supplies (funnels, forceps) that had been previously washed with 50% bleach (Goldberg et al., 2016). We then vacuum filtered samples onto 0.8 µm cellulose nitrate filters (Whatman™, General Electric Healthcare) and submerged the filters in 900 µl of cetyl trimethylammonium bromide (CTAB) in a 2 ml microcentrifuge tube (Renshaw et al., 2015). We kept these tubes in the dark at room temperature for 1 month to increase cell lysis (Wegleitner et al., 2015) and then placed them into a −80°C freezer until extraction.

### 2.3 Seasonal eDNA study

We examined the relationship between *C. fluminea* density and eDNA concentration and detectability, as well as the dependency of these relationships on stream flow, by expanding our sampling to include six additional streams beyond Copper Slough and Salt Fork (Boneyard Creek, Saline Branch of the Vermilion River, Spoon River, Whitley Creek, Kickapoo Creek and Little Kickapoo Creek). These eight total stream sites were chosen because each had a USGS flow gage and represented a gradient of low to high *C. fluminea* density (Table 1; Figure S1). We conducted conventional sampling of *C. fluminea* density during summer 2018, then collected eDNA water samples during summer low flows (18 June–10 July 2018) and autumn high flows (1 December 2018) at each stream.

Corbicula fluminea density was quantified adjacent to USGS flow gages by sampling 15 random quadrats (0.25 m²) within a stream length that was ten times its wetted width, following a modified version of systematic random sampling for freshwater mussels in the US (Strayer & Smith, 2003). We reduced sampling effort from the 40 recommended quadrat samples of Strayer and Smith (2003) to 15 random quadrat samples because preliminary results demonstrated that equivalent *C. fluminea* density estimates were achieved with the lower sampling effort. Stream substrate was excavated from each quadrat, sieved, and all live *C. fluminea* were counted. After each quadrat was sampled, *C. fluminea* were returned to the same general location in the stream where they had been excavated. *Corbicula fluminea* densities were only quantified during summer because flows

### Table 1 Site descriptions from streams sampled for the longitudinal (Copper Slough and Salt Fork) and seasonal eDNA studies, including the latitude, longitude, USGS flow gage number, estimated drainage area and average *C. fluminea* densities estimated from quadrat sampling.
were too high in autumn for conventional sampling; our analyses assume that relative *C. fluminea* densities did not change seasonally within sites.

Environmental DNA sample collection for the seasonal study followed the same methods as above for the longitudinal study. We collected four 250ml surface water samples per site and recorded water quality parameters (Table S2). We used one field blank (distilled water) per site to assess background contamination in bottles and filtering supplies. We bagged water samples, placed them on ice, transported them to UIUC, and filtered and stored samples consistent with our preceding methods. We collected summer eDNA water samples prior to conventional *C. fluminea* density sampling at every site to minimize risk of contamination that might arise from handling our study organism.

2.4 | *Corbicula* eDNA assay

Initially, we used a *Corbicula* genus-specific assay with the following primers developed by Cowart et al. (2018) to amplify a 208 bp region of the COI gene:

F-primer: 5′-TTTATTAGATGATGGGCAGCTGTA-3′.
R-primer: 5′-TGATCTAACCAACAAAAGCATAGC-3′,

where *C. fluminea* amplification was determined in this primer-only assay by a melt curve temperature of ~76.61°C. However, we noticed in March 2018 that two different peaks were present in the melt curve analysis of some samples. One peak was consistent with *C. fluminea* but the other showed amplification of a non-target organism at a melt curve temperature of ~85.15°C (Figure S2). We used Sanger sequencing to determine that this non-target amplification was a
gut bacterium (Klebsiella spp.) that we postulate was present due to abundant Canada Goose (Branta canadensis) populations at our sites. The dual amplification of C. fluminea and Klebsiella spp. affected our ability to quantify copy number, so we developed a genus-specific probe (5′-FAM-AGTGATGCCAATAATAATGGGTGTTTTG-GMG-NFQ-3′) to eliminate this non-target amplification. Subsequent sequence confirmation indicated that only C. fluminea amplified with this primer–probe assay. We then ran or re-ran all eDNA samples in our study with the new genus-specific probe-based assay. Like the assay developed by Cowart et al., (2018), our assay cannot discriminate between some congeners within the Corbicula genus, all of which are non-native to North America, including undescribed cryptic species or forms (Haponski & Ó Foighil, 2019; Tiemann et al., 2017). Prior to use of this assay, we ran optimization of different primer and probe concentrations, with different annealing temperatures, and selected the combination that produced the earliest Cq values and highest efficiency (%) of serial dilutions. We report specifics on assay performance below.

2.5 | eDNA extraction and qPCR

Prior to extractions or qPCR preparation, the laboratory space was cleaned with a 50% bleach solution and UV treated for 20 min. Following Renshaw et al. (2015), we extracted DNA from filters using a chloroform–isoamyl alcohol extraction procedure in a clean room, free from high-copy DNA and isolated from the PCR laboratory. This extraction method (Renshaw et al., 2015) has been shown to be robust to inhibition from tannins or humic acids (Curtis & Larson, 2020; Schrader et al., 2012) and often produces higher DNA yields than other extraction procedures (Deiner et al., 2015). One extraction blank was used for every ~25 samples.

Quantitative PCR reactions used the following: 10 µl TaqMan Environmental Master Mix 2.0 (Applied Biosystems®), 6.15 µl of sterile water, 0.35 µl of each primer (10 µM), 0.15 µl probe (10 µM) and 3 µl of eDNA. Negative plate controls replaced 3 µl of DNA with 3 µl of the master mix (Cowart et al., 2018). Plates were prepared in an isolated, PCR product-free clean room, then ran on a QuantStudio 3 Real-Time PCR system (Applied Biosystems®) using the following qPCR parameters: 95°C for 10 min denaturation and 40 cycles at 95°C for 15 s and 62°C at 1 min. We used a synthetic COI gBlock fragment (Integrated DNA Technologies) with GenBank accession GQ401362 (base 44 to 543) to develop serial dilutions (1:10) typically from 4.5 x 10⁶ copies/µl (1 x 10⁻³ ng/µl) to 4.5 copies/µl (1 x 10⁻⁹ ng/µl), which we used to create a standard curve (Cowart et al., 2018). We ran all samples in triplicate and considered amplification in 1/3 plate replicates as a positive detection. To confirm that positive amplifications were Corbicula, one randomly selected sample per plate was cleaned with ExoSap-It Express™ (Applied Biosystems®) and Sanger sequenced at the University of Illinois’ W.M. Keck Center. We edited sequences in Geneious® to remove ambiguities, realigned, and then using NCBI’s BLAST confirmed that all sequences were C. fluminea.

No field blanks or negative plate controls amplified. Serial dilutions of positive C. fluminea DNA produced R² values that ranged between 0.991 and 0.999 and efficiencies between 91% and 100%. To calculate copy number in eDNA samples, we used the Thermo Fisher Scientific DNA copy number calculator to determine the number of copies/ng (4,454,142,012 copies/ng for C. fluminea) and multiplied that by the quantity calculated for each well replicate, based on the standard curve. We defined our limit of detection (LOD) as the lowest standard that amplified in 1/3 of replicates and our limit of quantification (LOQ) as the lowest standard that always amplified in 3/3 replicates. Here, our LOQ was 4.5 copies/µl (~1 x 10⁻⁹ ng/µl) and our LOD was 0.45 copies/µl (~1 x 10⁻¹⁰ ng/µl).

2.6 | Statistical analyses

For the longitudinal study, we analysed eDNA copy number and omitted detection probability modelling (below; seasonal study) because C. fluminea was always present in these two study streams and eDNA was almost always detected (56 of 57 sampling events had positive detections). We averaged plate and site replicates to produce one eDNA copy number estimate for each sampling event per site. We then related eDNA copy number to stream flow and a series of additional predictor variables, including temperature, number of daylight hours, and Julian day, via multiple regression. Beyond our primary focus on stream flow, we included temperature because cooler temperatures may allow eDNA to persist longer in the environment (Eichmiller et al., 2016), whereas warmer temperatures are associated with increased reproductive activity of C. fluminea, particularly in the late spring or early summer (Hornbach, 1992). Such reproductive events have been found to have strong effects on eDNA concentration and detectability in other study systems (e.g. de Souza et al., 2016).

We included daylight hours as a measure of potential UV exposure, which can influence eDNA degradation (Kessler et al., 2020; Strickler et al., 2015), and Julian day of the year to control for seasonal variation in C. fluminea eDNA production, persistence, or transport independent of our other predictor variables. Daylight hours and Julian day were obtained from the US Naval Observatory Astronomical Applications Department. We omitted other abiotic measures of stream water chemistry from our models because these generally varied little over time or between our stream sites (Table S1, S2). Streams in our study region have a slightly basic pH with relatively high conductivity, TDS, salinity and turbidity values owing to both the underlying sedimentary geology of central Illinois and intensive human land use in these watersheds (Larimore & Bayley, 1996; Walser & Bart, 1999). Turbidity did vary predictably with stream flow (i.e. higher turbidity during floods), but given the extent to which these prospective predictor variables were confounded with each other, we opted to use stream flow as the primary predictor in our models.

To better meet assumptions of linear regression, we log + 1-transformed eDNA copy number and stream flow. We then fit linear
mixed-effects models using the package "lme4" (Bates et al., 2015) in R (v. 4.0; R Core Team, 2020) to examine relationships between eDNA copy number and all combinations of predictor variables (stream flow, temperature, daylight hours, Julian day), as well as a null model with no predictors. We used mixed-effects models with a random effect for site to account for within-site replication at Copper Slough and Salt Fork. We compared competing regression models of *C. fluminea* eDNA copy number using the Bayesian information criterion (BIC) under an information theoretic approach, in which model fit is evaluated while penalizing for model complexity (Burnham & Anderson, 2002). We used BIC rather than Akaike’s information criterion (AIC) because AIC may lead to over-fitting in multi-model comparisons (Dennis et al., 2019). We calculated BIC using the “MuMIn” package (Bartoń, 2020) in R (v. 4.0; R Core Team, 2020). The most supported model in this approach has the lowest BIC value.

For the seasonal study, we first followed a similar statistical approach as in the longitudinal study, in which we sought to explain log + 1-transformed *C. fluminea* eDNA copy number by several predictor variables. Exploratory data analyses revealed that temperature and stream flow were highly correlated between our summer and autumn sampling events (Pearson’s *r* = −0.70; Figure 2), and consequently, we combined these predictors into a single variable for season (summer or autumn). We also included summer *C. fluminea* density at our sites as a predictor of eDNA copy number, specifically seeking to compare the role of season (and its associated differences in stream flow) to density in explaining eDNA concentrations. We again fit linear mixed-effects models in the package “lme4” with site as a random effect and compared model performance with BIC calculated using the “MuMIn” package (Bartoń, 2020) in R (v. 4.0; R Core Team, 2020).

Next, we investigated the role of season and *C. fluminea* density on eDNA detectability, because we had more frequent non-detections of *C. fluminea* eDNA in the seasonal than longitudinal study. We used occupancy modelling, a hierarchical regression approach common in fisheries and wildlife research, which estimates both the probability that a site is occupied by a species (occupancy, Ψ) and the probability of detecting the species when present (detection, *p*) using repeated observations over either space or time (MacKenzie et al., 2002). Environmental DNA sampling is amenable to this occupancy modelling framework because replicated field or laboratory samples can be used as the unit of response for estimating both occupancy and detection probability of the eDNA of the focal organism (Dorazio & Erickson, 2017; Schmidt et al., 2013). We conducted occupancy modelling using the “unmarked” package (Chandler et al., 2020) in R (v. 4.0; R Core Team, 2020), where the presence or absence of *C. fluminea* eDNA in our four field replicates per season and site was the response variable. We modelled occupancy of *C. fluminea* eDNA using our summer density estimate of this species, anticipating that the eDNA of this species was more likely to be present at higher rather than lower abundances. We modelled the detection probability of *C. fluminea* eDNA using both the summer density estimate and season. We expected that detection probability should increase with increasing abundance, and that the high stream flows associated with our autumn sampling could affect eDNA detection probability by the type of opposing dilution or transportation/mobilization effects explained in our introduction. We compared these competing occupancy models with BIC calculated using the “MuMIn” package (Bartoń, 2020) in R (v. 4.0; R Core Team, 2020). Lastly, we used the most supported occupancy model to estimate the number of water samples required to achieve a 95% probability of detecting *C. fluminea* eDNA when present based on the cumulative probability equation of McArdle (1990).

### 3 | RESULTS

#### 3.1 | Longitudinal eDNA study

Two competing models for *C. fluminea* eDNA copy number were similarly supported (ΔBIC ≤ 2; Table 2). Our most supported model included stream flow and temperature as predictors, with a BIC weight (wi) of 0.44 and fit *C. fluminea* eDNA copy number relatively well (adjusted *r*² = .61). The equivalent well-supported model included only temperature, with a BIC weight (wi) of 0.29 and adjusted *r*² = .57. We found less support for effects of either Julian day or daylight hours on *C. fluminea* eDNA copy number. Based on our most supported model, we found a significant positive relationship between temperature and eDNA copy number (t-value = 6.99, *p* < .001; Figure 3a), and a significant negative relationship between stream flow and eDNA copy number (t-value = −2.21, *p* = .03; Figure 3b).
TABLE 2 All candidate models for eDNA copy number from the longitudinal eDNA sampling using linear mixed-effect modelling with site as a random effect

| Model                        | K | BIC | ΔBIC | BIC wi | adj $r^2$
|------------------------------|---|-----|------|--------|--------
| Temperature + Flow           | 3 | 93.56| 0.00 | 0.44   | .61    |
| Temperature                  | 2 | 94.42| 0.86 | 0.29   | .57    |
| Temperature + Flow + Hours of daylight | 4 | 97.24| 3.68 | 0.07   | .62    |
| Temperature + Hours of daylight | 3 | 97.33| 3.77 | 0.07   | .58    |
| Temperature + Flow + Julian day | 4 | 97.49| 3.93 | 0.06   | .61    |
| Temperature + Julian day     | 3 | 98.41| 4.85 | 0.04   | .57    |
| Temperature + Hours of daylight + Julian day | 4 | 99.92| 6.36 | 0.02   | .59    |
| Flow + Julian day            | 3 | 100.37| 6.81 | 0.01   | .15    |
| Flow + Julian day + Hours of daylight | 4 | 114.85| 21.29 | 0.00 | .43    |
| Global                       | 5 | 115.16| 21.60 | 0.00 | .63    |
| Julian day + Hours of daylight | 3 | 119.53| 25.97 | 0.00 | .31    |
| Hours of daylight            | 2 | 120.14| 26.58 | 0.00 | .24    |
| Flow                         | 2 | 123.36| 29.80 | 0.00 | .13    |
| Null                         | 1 | 129.44| 35.88 | 0.00 | .00    |
| Flow + Hours of daylight     | 3 | 129.74| 36.18 | 0.00 | .37    |
| Julian day                   | 2 | 132.56| 39.00 | 0.00 | .02    |

Note: BIC is Bayesian information criterion; ΔBIC is the difference between the lowest BIC value and the model; BIC wi is the weight of the model compared to all models; and adj $r^2$ is the likelihood-based pseudo $r^2$ values used to assess model fit.

3.2 | Seasonal eDNA study

Summer C. fluminea density at our eight stream sites ranged from 0 individuals/m$^2$ (Boneyard Creek) to -111 individuals/m$^2$ (Copper Slough; Table 1). We did not detect any C. fluminea eDNA in either summer or autumn at the one stream site (Boneyard Creek) where conventional sampling also did not detect this species. In addition, we failed to detect C. fluminea eDNA during summer sampling at Whitley Creek, where this species was present but relatively rare (3.6 individuals/m$^2$; Table 1). Non-detections of C. fluminea eDNA increased in the autumn, as we failed to detect this species from five stream sites (Kickapoo Creek, Little Kickapoo Creek, Saline Branch, Spoon River, Whitley Creek), including one stream site with the second highest observed C. fluminea densities in our study (82.8 individuals/m$^2$ at Little Kickapoo Creek; Table 1).

Two competing models for C. fluminea eDNA copy number were equally supported (ΔBIC ≤2), which included season and C. fluminea density (BIC wi = 0.53; adjusted $r^2 = .59$; Table 3) and only season (BIC wi = 0.43; adjusted $r^2 = .47$). Season had a significant effect on eDNA copy number (t-value = 3.35, p = .01) with higher copy numbers observed in the summer. Conversely, we found a positive but non-significant relationship between C. fluminea density and eDNA copy number (t-value = 1.71, p = .14; Figure 4).

Our most supported model for C. fluminea eDNA occupancy and detection probability included season and C. fluminea density as detection covariates and no covariates for occupancy (BIC wi = 0.62; adjusted $r^2 = .60$; Table 4). A competing model with only season as a detection covariate was equivalent by ΔBIC (BIC wi = 0.35; adjusted $r^2 = .48$). Per the most supported of these two equivalent models, we found a significant effect of season on C. fluminea eDNA detectability (z-value = 3.81, p < .001), and a positive but non-significant effect of density on C. fluminea eDNA detectability (z-value = 1.83, p = .07). We used these results from our overall most supported model to estimate the number of water samples necessary to achieve a 95% cumulative probability of detecting C. fluminea eDNA in both summer and autumn over a density gradient for this species (Figure 5a–d). Corbicula fluminea eDNA is relatively easy to detect in the summer, requiring three water samples at low densities (<15 individuals/m$^2$; Figure 5a,d) and one water sample at high densities (>85 individuals/m$^2$; Figure 5c,d). Conversely, C. fluminea eDNA is considerably more difficult to detect in the autumn, requiring 60 water samples at a density of 1 individual/m$^2$, 30 samples when there are 35 individuals/m$^2$, and six water samples when highly abundant (e.g. 125 individuals/m$^2$; Figure 5a–d).

4 | DISCUSSION

We found that high stream flows diluted eDNA concentrations and decreased detectability for our target organism, the Asian Clam C. fluminea. Our year-long longitudinal study at two sites revealed that this dilution effect of higher stream flows was significant, but weaker than the strong positive effect of temperature on C. fluminea eDNA concentrations and detectability, which we believe is largely attributable to late spring or early summer reproduction by this species (Aldridge & McMahon, 1978). Conversely, our seasonal study at eight sites revealed that the combined effect of both temperature and stream flow was stronger than the relatively weak and non-significant positive effect of C. fluminea density on eDNA concentrations and detectability. This is consistent with emerging consensus that organismal abundance or biomass is often positively correlated with eDNA copy number (t-value = 3.35, p = .01) with higher copy numbers observed in the summer. Conversely, we found a positive but non-significant relationship between C. fluminea density and eDNA copy number (t-value = 1.71, p = .14; Figure 4).

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sampling at low or base stream flows in warm conditions when this species may be reproductively active (late spring and early summer), and we would recommend against opportunistic or ad hoc convenience sampling during high stream flows or floods. Resource managers and practitioners seeking to use eDNA to monitor populations or communities of interest will likely need to optimize their sampling designs to account for the variable natural history of study organisms and their ecosystems.
High stream flows or floods could be expected to affect eDNA concentrations and detectability through a variety of potentially opposing mechanisms. For example, eDNA has been found to travel shorter distances in smaller streams and longer distances in larger rivers (Deiner & Altermatt, 2014; Jane et al., 2015; Pont et al., 2018), and as such, higher stream flows might be expected to transport eDNA farther from its source, potentially increasing detectability (Milhau et al., 2019; Shogren et al., 2018). Alternatively, high stream flows or floods could intuitively be anticipated to dilute eDNA, reducing concentrations and detectability (Klymus et al., 2015; Shogren et al., 2018, 2019). Here we used long-duration and large spatial scale studies to investigate the effects of stream flow on eDNA, concurrently accounting for gradients of target organism abundance and other abiotic and biotic factors. We found that higher stream flows dilute eDNA concentrations and can even produce non-detections at locations where the target species is relatively common. As one extreme example, we observed a decrease in C. fluminea eDNA concentrations from ~316 copies/µl to 0 copies/µl over a 3-hr interval at Copper Slough when stream flow increased from 0.90 m³/s to 1.72 m³/s concurrent with a rain event. Our results demonstrate that convenience or ad hoc sampling during high stream flows could impair monitoring or surveillance programmes for lotic organisms using eDNA. Further, while short-duration or small spatial scale experimental studies have high potential to provide mechanistic understanding of stream flow effects on eDNA transport or retention (e.g. Fremier et al., 2019; Jane et al., 2015; Shogren et al., 2018), we recommend that more long-duration, large spatial scale studies be conducted in situ to contrast the effects of stream flow on eDNA concentrations and detectability relative to other abiotic and biotic factors.

A future priority for studies of stream flow effects on eDNA may be to incorporate more information on stream geomorphology, including underlying soils or geology (Jerde & Mahon, 2015). Differences in stream substrate have been shown to influence transport and resuspension dynamics of eDNA in artificial streams and flow-through columns, where finer sediments (i.e. sand, pea gravel) retain eDNA and result in shorter transport distance relative to larger substrates (Shogren et al., 2016, 2017). Similarly, during rain events, surface runoff flushes terrestrial soil and organic matter into streams, which could potentially bind with eDNA and reduce detectability (van Bochove et al., 2020). Clay soils in particular form a tight bond with DNA and can result in lower yield extractions of eDNA (Yankson & Steck, 2009), although studies of pond sediments have found weak and inconsistent effects of clay on eDNA detection probabilities (Buxton, Groomebridge, & Griffiths, 2017; Buxton et al., 2018). Soils at our eight study streams are classified as relatively similar silty clay loams or silt loams (USDA-NRCS, n.d.), and future studies might investigate whether stream flow effects on eDNA concentrations and detectability are similar for streams of different underlying soil types or geologies (Jerde & Mahon, 2015). For example, a study using streams in adjacent ecoregions of differing geologies or geomorphology could better test the role of sediment size and clay composition on eDNA performance under variable flow regimes, perhaps by sampling at sites within existing stream gage networks (e.g. Pilliod et al., 2019; Sepulveda, Schmidt, et al., 2019).
We found a strong positive effect of temperature on eDNA concentrations and detectability for *C. fluminea*. In past eDNA studies, temperature has often been associated with degradation, as warmer temperatures accelerate the breakdown of eDNA (e.g. Strickler et al., 2015). Alternatively, some in situ studies have instead found increased organismal activity, metabolism, or reproduction associated with warmer water temperatures to increase eDNA concentrations or detectability (Buxton et al., 2017; de Souza et al., 2016; Wacker et al., 2019). We believe that our positive association between *C. fluminea* eDNA and water temperature is a consequence of late spring and early summer reproduction by this species. *Corbicula fluminea* generally releases veligers (larvae) as water temperatures reach ~25°C (Aldridge & McMahon, 1978), although timing of this life history event may be variable between *C. fluminea* populations in the USA (Denton et al., 2012). We observed a 30-fold increase in *C. fluminea* eDNA concentrations relative to winter months at the end of May when water temperatures reached ~25°C in both Copper Slough and Salt Fork, and as such, we believe the strong positive

**Figure 5** Relationship between sampling effort (x-axis) and cumulative detection probability of *C. fluminea* eDNA (y-axis) for autumn and summer seasons with 95% confidence intervals from the most supported model (Table 4) at (a) a density of 1 individual/m², (b) at the average density from our seasonal study of 35 individuals/m², (c) and at 125 individual/m², where the dashed black line indicates 95% probability of detection. (d) The mean predicted number of water samples needed for a 95% probability of detecting *C. fluminea* eDNA across *C. fluminea* density by season, without 95% confidence intervals for clarity of presentation.
relationship between eDNA concentrations and warmer temperatures observed in our study is largely the consequence of *C. fluminea* reproduction. Past studies have emphasized that timing of reproductive events can be used to inform or optimize eDNA sampling for target organisms (de Souza et al., 2016; Spear et al., 2015), and we similarly would identify late spring or early summer temperatures approaching 25°C to be ideal for eDNA monitoring of *C. fluminea* populations.

In our seasonal study, we found weak positive but non-significant relationships between *C. fluminea* density and both eDNA concentrations and detectability. Yates et al. (2019) recently used a meta-analysis to investigate relationships between organismal abundance or biomass and eDNA concentrations, finding that eDNA concentrations were more strongly correlated with abundance or biomass under controlled laboratory experiments than natural field conditions. This reveals the tendency for environmental factors to complicate relationships between organism abundance or biomass and eDNA concentration in situ, as observed in our study and other past work (e.g. Matsuhashi et al., 2016; Wacker et al., 2019). Some researchers have found strong, positive relationships between organism abundance or biomass and eDNA concentrations under field conditions (e.g. Salter et al., 2019), suggesting that this emerging methodology has high potential for accurate estimation of population sizes or community structure rather than just presence/absence or occupancy. Yet our results support that many relationships between eDNA data and population ecology will be highly dependent on understanding the abiotic and biotic context of eDNA sample collection. Finally, Yates et al. (2019) identified small sample sizes or low replication as a barrier to identifying relationships between eDNA concentrations and organismal abundance or biomass in situ, and our study used similarly low replication of field sites (n = 8) relative to those studies included in Yates et al. (2019). Future research may find stronger relationships between *C. fluminea* eDNA concentrations or detectability and organismal density by increasing the number of stream sites sampled, avoiding high stream flow events, and potentially avoiding reproductive events in order to relate eDNA to the density of adult *C. fluminea* sampled by our method, rather than veligers.

Our seasonal study confounded temperature and stream flow between our summer and autumn sampling; however, our year-long longitudinal study at Copper Slough and Salt Fork supports a strong positive effect of temperature, and a weaker but still significantly negative effect of stream flow on *C. fluminea* eDNA concentrations and detectability. Accordingly, both environmental factors should be anticipated to interact synergistically to reduce eDNA concentrations and detectability in the cool, high-flow autumn samples relative to the warm, low-flow summer samples. This prediction from our longitudinal study was supported by models of both eDNA copy number and occupancy from our seasonal study, as *C. fluminea* eDNA concentrations were low and difficult to detect in our autumn samples. We failed to detect *C. fluminea* eDNA during autumn sampling at a site with ~82.8 individuals/m², and our power analysis based on detection probability modelling identified high field replication as necessary to detect this organism in autumn even when abundant. As such, our study supports past work that has found season of eDNA sampling to strongly affect performance of this method (Buxton, Groombridge, Zakaria, et al., 2017; Spear et al., 2015; Wacker et al., 2019). Managers or practitioners should tailor eDNA monitoring programmes to the seasonal natural history of their study organisms and associated environmental conditions. Given that organisms may differ dramatically in their detectability between seasons (i.e. de Souza et al., 2016), the design of multi-taxon eDNA surveys may be particularly challenging, as the best time of the year to sample for one priority taxa may not be optimal for another.

Our results identify several understudied but critical considerations for future eDNA applications in lotic ecosystems, but our findings may also be applicable to marine systems, where similar dilution and transport of eDNA from currents has been documented (e.g. Andruszkiewicz et al., 2019). First, high stream flows can dilute eDNA concentrations and produce false negatives, even in cases where study organisms are relatively abundant. We recommend that researchers and managers or practitioners avoid eDNA sampling during high stream flows or floods. It may not be feasible or biologically relevant to always collect eDNA samples during low stream flows. For example, some target species may be most active or reproduce during higher flows of the autumn or winter (de Souza et al., 2016). Accordingly, if researchers must take eDNA samples during periods of high stream flows or floods, we recommend increased sample replication to improve detection probabilities (Sepulveda, Schabacker, et al., 2019). Second, understanding the natural history of target species, and relationships to seasonal variability in abiotic and biotic conditions, should lead to improved eDNA sampling programmes. In our longitudinal study, apparent reproduction by *C. fluminea* in late spring and early summer resulted in high eDNA copy numbers at this time of the year, and monitoring programmes for this invasive species might seek to sample at temperatures associated with reproduction to improve detection probabilities. Alternatively, studies seeking to relate adult *C. fluminea* densities to eDNA concentrations might instead strive to avoid this pulse of veliger-associated eDNA. Lastly, occupancy modelling is a useful tool for quantifying detection probabilities in eDNA studies, and associated power analyses can provide guidance on sampling effort necessary to detect target organisms under environmental conditions (Dorazio & Erickson, 2017; Schmidt et al., 2013). Researchers and managers should continue to apply occupancy estimation with detection probability frameworks to improve the design and implementation of eDNA sampling programmes for specific taxa and ecosystems, including for lotic ecosystems where variable stream flow and floods may strongly affect performance of this methodology.

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BIOSKETCH
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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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