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Application of eDNA as a tool for assessing fish population abundance

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Abstract

Estimating the abundance of organisms is fundamental to the study and management of ecological systems. However, accurately and precisely estimating organism abundance is challenging, especially in aquatic systems where organisms are hidden underwater. Estimating the abundance of fish is critical for the management of fisheries which relies on accurate assessment of population status to maximize yield without overharvesting populations. Monitoring population status is particularly challenging for inland fisheries in which populations are distributed among many individual waterbodies. Environmental DNA (eDNA) may offer a cost-effective way to rapidly estimate populations across a large number of systems if eDNA quantity correlates with the abundance of its source organisms. Here, we test the ability of quantities of eDNA recovered from surface water to estimate the abundance of walleye (*Sander vitreus*), a culturally and economically important sportfish, in lakes in northern Wisconsin (USA). We demonstrate a significant, positive relationship between traditional estimates of adult walleye populations (both number of individuals and biomass) and eDNA concentration (R^2 = .81; n = 22). Our results highlight the utility of eDNA as a population monitoring tool that can help guide and inform inland fisheries management.

KEYWORDS

environmental DNA, fisheries management, lakes, population monitoring

1 | INTRODUCTION

Estimating the abundance of organisms in the environment is fundamental to the study and management of ecological systems (Krebs, 1972). However, estimating organism abundance with accuracy and precision is often difficult or costly, particularly in aquatic systems where organisms are hidden underwater. Environmental DNA (eDNA) may provide a solution to this problem. By analyzing the DNA in an environmental sample, we can infer which organisms inhabit that environment without directly observing them (Jerde, Mahon, Chadderton, & Lodge, 2011). By quantifying that DNA, we may even infer organism abundance (Yates, Fraser, & Derry, 2019).

Monitoring organism abundance is especially critical in fisheries, wherein managers must balance fish harvest against available fish production to maximize yield without overharvesting the population. Achieving this balance relies on accurate and precise monitoring of the population and reacting to change with management strategies. Assessing population size is especially challenging for

inland fisheries in which populations are distributed among many individual water bodies across a landscape, adding a layer of spatial complexity to monitoring and management. Inland fish populations are often estimated with expensive and personnel-intensive mark–recapture surveys involving capturing and counting individuals with nets and electroshocking boats. Mark–recapture surveys can be difficult to deploy across large numbers of lakes that may comprise a fishery. Therefore, population status data for inland fisheries is generally poor. Overharvest, habitat loss, climate change, and invasive species (Lynch et al., 2016) are driving declines across many inland fisheries (Embke et al., 2019; Post et al., 2002; Rypel, Goto, Sass, & Vander Zanden, 2018). In light of these management challenges, there is a pressing need for new population monitoring approaches, particularly approaches that would enable rapid assessment of fishery status across large numbers of lakes.

Walleye (*Sander vitreus*) are an iconic sportfish in the inland fisheries of central North America. In the state of Wisconsin (USA), walleye are a centerpiece of the state's $2 + billion a year fishing economy (Wisconsin Department of Natural Resources, 2019). However, multiple stressors are contributing to the decline of natural recruitment and production of walleye in many lakes (Embke et al., 2019; Hansen, Read, Hansen, & Winslow, 2017; Rypel et al., 2018). In the mid-1980s, courts reaffirmed the treaty rights of native Ojibwe tribes to spear walleye in the lakes of Wisconsin's Ceded Territory (roughly the northern third of the state; Nesper, 2003). Since then, the walleye fishery has been fraught with cultural and economic tension surrounding harvest pressure from tribal spearing and recreational angling, which drives the region's tourism industry (Nesper, 2003). To conserve the fishery, a joint state-tribal task force has been charged with monitoring walleye populations across Wisconsin's Ceded Territory fishery to inform safe harvest limits for spearing and angling (Cichosz, 2016). This population monitoring effort across a large number of systems provides an opportunity to test the utility of eDNA as a population monitoring tool by comparing quantitative eDNA-derived population estimations to those of mark–recapture surveys.

The Ceded Territory of Wisconsin contains over 900 lakes with walleye populations. State and tribal biologists currently monitor only ~5% of walleye populations annually using costly mark–recapture surveys (Cichosz, 2016). Roughly, half of the 900 + lakes are revisited over time to monitor population trends, but often 5–10 years pass between sampling events on these lakes (Embke et al., 2019). For the remaining half of walleye lakes, only a handful are surveyed each year, and so many lakes are never visited. This infrequent sampling means that management strategies are generally not responsive to changing populations. Given recent declines in walleye populations, expanding population monitoring would be a critical step toward understanding the drivers of regional walleye declines and managing for the fishery's recovery and long-term sustainability (Embke et al., 2019; Rypel et al., 2018).

Improving population assessment may be possible with eDNA surveys. Sampling DNA shed into the environment allows rapid and cost-effective detection of organisms (Goldberg, Strickler, & Pilliod, 2014; Jerde et al., 2011; Rees et al., 2014; Thomsen & Willerslev, 2014), and the ability to infer organism abundance from eDNA quantity has recently been explored for several aquatic species (Pilliod, Goldberg, & Arkle, 2013; Doi et al., 2015; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Doi et al., 2017; Klobucar, Rodgers, & Budy, 2017; Salter, Joensen, Kristiansen, Steingrund, & Vestergaard, 2019). In contrast to the costly and personnel-intensive netting and electroshocking required by traditional mark–recapture surveys to capture individuals, eDNA surveys only require the collection of environmental samples (*e.g.*, water) from the field. Indirectly sampling organisms with eDNA does not provide valuable sex, size, and age data (Evans & Lamberti, 2018), so an eDNA survey would not replace the direct capture or observation of individuals. Rather, it could expand the number of systems for which population size can be monitored. However, our ability to accurately and precisely estimate organism abundance from quantified eDNA remains largely uncertain outside of mesocosms (Yates et al., 2019). Previous quantitative eDNA studies found that eDNA copy number explains on average ~80% of the variability in organism abundance in controlled mesocosms, but only ~50% across natural systems (Yates et al., 2019). Improving on that explanatory power in natural systems is critical to establishing eDNA as a useful tool for monitoring populations.

Here, we test the ability of eDNA to estimate walleye abundance in lakes across Wisconsin's Ceded Territory. Leveraging mark–recapture population estimates currently used to monitor walleye populations, we tested for relationships between walleye eDNA recovered from lake surface water with traditional adult walleye population estimates for 24 lakes collected over two years. Finding a robust correlation would strengthen our understanding of eDNA as a tool for monitoring organism abundance and move toward expanding the data that inform the management of an important inland fishery.

2 | METHODS

2.1 | Mark–recapture sampling

Adult walleye (all fish ≥ 381 mm and all sexable fish) were surveyed in the Ceded Territory of Wisconsin shortly after lake ice had melted (*i.e.*, "ice-off") in May of 2017 and 2018 by the Wisconsin Department of Natural Resources (WDNR) and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC). Both agencies performed mark–recapture surveys to estimate adult walleye populations, but their methods differed slightly. WDNR marked fish caught in fyke nets with a Floy® tag or fin clip and used boat electroshocking to recapture individuals 1–4 days after marking (Cichosz, 2016). GLIFWC both marked and recaptured via boat electroshocking. For most collected walleye, total length (TL, mm) was recorded. Weight was also recorded (kg) for some individuals. Adult population estimates were calculated as:

$$N = \frac{(M+1)(C+1)}{(R+1)} \quad (1)$$

where N = population estimate, M = number of fish caught, marked, and released in the first effort, C = total number of fish caught in the second effort, and R = number of marked fish recaptured in the second effort (Cichosz, 2016). This method includes a Chapman modification (the addition of 1 to M, C, and R) to reduce bias in small sample sizes (Ricker, 1975). Coefficients of variation (CV) for each population estimate were calculated as:

$$\mathrm{CV}(N) = \sqrt{\frac{N^2(C-R)}{(C+1)(R+2)}} \div N \quad (2)$$

We combined mark–recapture population estimates and fish length and weight data to estimate adult walleye biomass for each lake using methods adapted from Embke et al. (2019). For full biomass calculation methods, see Appendix. Although only adult walleye were sampled in these mark–recapture surveys, hereafter we will use the terms "walleye density" and "walleye biomass" to refer to the number and biomass of adult walleye standardized by area (individuals/ha and kg/ha). When generalizing about number and biomass metrics, we will use the term "walleye abundance."

A subset (n = 24) of lakes receiving traditional mark–recapture population estimates were selected for eDNA sampling—12 lakes in July 2017 and 12 lakes in July 2018. Lakes were chosen to span a wide range of walleye densities (0.8–52.4 individuals/ha) and lake surface areas (84–1,617 ha).

2.2 | eDNA sampling

We collected 1 L samples of surface water at nine sites in each lake. Four offshore and five nearshore sites were selected in advance to be representative of dominant lake bottom types. We stored samples in a cooler for no more than 6 hr, vacuum filtered the water onto 47-mm-diameter, 0.7 μm glass microfiber filter paper, and incubated filters overnight in 900 μl of Longmire's cell lysis buffer (Longmire, Maltbie, & Baker, 1997) at room temperature (ca. 25°C) in 2-ml snap-top microcentrifuge tubes. We then extracted DNA from the filters with phenol:chloroform:isoamyl (25:24:1), precipitated DNA from the aqueous phase with 500 μl of ice cold isopropanol and 250 μl of room temperature 5 M NaCl, washed the DNA pellets twice in 70% ethanol, and allowed pellets to air dry for 20 min before rehydrating them in 200 μl of a buffer solution of 10 mM Tris-HCl and 1 mM Na-EDTA. This extraction protocol is adapted from Renshaw, Olds, Jerde, Mcveigh, and Lodge (2015). Extracted samples were stored at −20°C until amplification (1–30 days later).

We performed real-time quantitative polymerase chain reaction (qPCR) on extracted DNA using a BIO-RAD C1000 Thermal Cycler equipped with a CFX96 Real-Time System (Bio-Rad, Hercules, CA). Reactions used Ex Taq DNA Polymerase Hot-Start Version (TaKaRa Bio) and a PCR primer pair and minor groove binding probe (Integrated DNA Technologies) specific for *Sander vitreus* (F: 5'-CTATTATACTATTTACCCTCGGGCTCG-3'; R: 5'-GTC GATTGAACAATGAAGTATTTTGC-3'; Probe: 5'-FAM-TAATTGCCTG AATGGGTC-MGBNFQ-3'; amplicon length: 175 bp) targeting the mitochondrial NADH dehydrogenase subunit 2 (ND2) region. The following PCR profile was used: 55 cycles, T_d: 15 s at 95°C, T_a: 30 s at 60°C, T_e: 30 s 60°C. Each reaction was 20 μl and contained 600 nM of each primer, 250 nM of probe, 7.5 μl of Ex Taq DNA Polymerase Hot-Start, 2.75 μl of Nanopure water, and 4 μl of template DNA. Primers, probe, and protocols were developed by Dysthe et al. (2017).

We quantified eDNA using a serial dilution of gBlocks positive controls (31,250, 6,250, 1,250, 250, 50, 10, and 0 copies/μl; Integrated DNA Technologies). Negative controls were included at the sample collection, filtration, extraction, and qPCR steps. Samples and negative controls were run in quadruplicate qPCRs. Inhibition was tested by spiking a fifth reaction of each sample with 250 copies/μl of gBlocks target DNA and comparing amplification timing to that of the 250 copies/μl standard. Inhibition was characterized by >1 C_t change. Inhibition reaction volumes were balanced by omitting a volume of water equivalent to the gBlocks spike.

Comparison of gBlock-spiked samples to gBlocks positive controls showed negligible inhibition in all samples (difference < 1 Cq). No negative control wells amplified, indicating no discernible contamination. Mean standard qPCR efficiency was 93.37%, and the mean standard R^2 value was .99.

The value chosen to represent each lake's eDNA concentration was calculated from the qPCR starting quantity (see supplementary material, Spear, Embke, Krysan, & Vander Zanden, 2020), defined by the estimated number of DNA copies in each qPCR well at the beginning of the qPCR. Dividing the qPCR starting quantity by the 4 μl of sample DNA in each reaction, then multiplying by the 200 μl in which each sample's DNA extraction was suspended produced an estimated number of DNA copies in each sample. Because each sample was 1 L of lake water, this value is expressed as an eDNA concentration (copies/L of lake water). To arrive at one eDNA concentration value for each lake, we averaged the four qPCR replicates within each of the lake's nine samples and then averaged those values across the nine within-lake samples. eDNA concentration was square-root transformed to achieve normality for linear regression analysis.

2.3 | Linear modeling

Before model selection, two lakes were omitted from our analysis because their walleye populations were uncharacteristic of the walleye lakes in this region (Figure S2), and because their walleye abundance relationships to their eDNA values represented extreme outliers compared to the other lakes in our study. Bearskin Lake was omitted because its walleye density (52.4 individuals/ha) was nearly 7 times higher than the mean value of regional walleye populations (Figure S2a). In a linear regression of walleye density and eDNA, Bearskin Lake's Cook's Distance value (18.3) was over 55 times the next highest value among our study lakes. Long Lake was also omitted from our analysis because its walleye population size structure was dominated by large individuals, leading to walleye biomass (kg/

ha) and mean walleye size (kg) estimates uncharacteristic of regional walleye lakes. Long Lake's walleye biomass (46.8 kg/ha) and mean walleye size (4.0 kg) were 7 and 4 times higher than the mean values of regional walleye populations, respectively (Figure S2b,c). In a linear regression of walleye biomass (kg/ha) and eDNA concentration, Long Lake's Cook's Distance value (29.6) was over 90 times the next highest value among our study lakes. Results that do not omit these lakes can be found in Figure S1.

We analyzed the relationship between walleye abundance and eDNA concentration by linear regression. Stepwise (bidirectional; AIC) and all subsets (BIC) model selection was performed to explain eDNA concentration from either walleye density (individuals/ha) or walleye biomass (kg/ha) combined with a suite of other relevant parameters likely to influence the production, persistence, or capture of eDNA. These parameters were mean walleye size (kg), age-0 walleye catch per unit effort (CPUE; individuals/km of shoreline collected in the fall of each year by WDNR and GLIFWC; Cichosz, 2016), water clarity (Secchi depth; m), lake pH, $\log_{10}$ of lake surface area (ha), and maximum lake depth (m) (Papes & Vander Zanden, 2010).

To accommodate for heterogeneity in the variance of the mean eDNA concentration values, we applied a weighted least squares approach: We weighted observations by the inverse proportion of the variance in eDNA concentration among the nine sites within each lake. This approach reduces the influence of lakes with high within-lake eDNA variance (Gelman & Hill, 2007). We applied the weighted least squares approach only for the best performing models (Table 1).

Because the mark–recapture and eDNA surveys were spread over two years, and because the mark–recapture surveys were performed by two different management agencies with differing sampling approaches, we performed ANCOVAs (Type I SS) to test for the significance of year and agency as interaction terms in the best performing models. We used an $\alpha = 0.05$ for all statistical analyses. All analyses and figures were produced in R version 3.4.3 (R Development Core Team, 2017).

TABLE 1 Best performing models for predicting eDNA concentration (copies/L) from walleye (*Sander vitreus*) density (individuals/ha) and walleye biomass (kg/ha) from Ceded Territory of Wisconsin lakes during 2017 and 2018, with other walleye population and lake characteristic parameters (mean walleye size (kg), age-0 walleye catch per unit effort (CPUE; individuals/km of shoreline), water clarity (Secchi depth; m), lake pH, $\log_{10}$ of lake surface area (ha), and maximum lake depth (m))

Model parameters	R^2_{adj}	Residual SE	AIC	BIC
walleye density	.62	0.38	23.15	26.42
walleye density * mean walleye size (kg)	.64	0.36	22.77	28.23
walleye density * mean walleye size (kg) (*weighted*)	.81	0.28	13.99	19.45
walleye biomass	.42	0.45	31.28	34.55
walleye biomass + mean walleye size (kg)	.64	0.36	21.68	26.05
walleye biomass + mean walleye size (kg) (*weighted*)	.82	0.27	12.55	16.91

3 | RESULTS

3.1 | Model performance

We found a significant positive relationship between eDNA concentration and walleye density ($n = 22$, $R^2_{adj} = .62$, $p < .001$; Figure 1a). After applying the weighted least squares approach, and after model selection added mean walleye size (kg) and its interaction to the linear model, the relationship between eDNA concentration and walleye density improved ($R^2_{adj} = .81$, $p < .001$; Table 1). Models predicting eDNA concentration from walleye biomass instead of walleye density exhibited similar performance (Figure 1b). Generally, the best performing models did not include age-0 walleye or abiotic lake characteristics but were some combination of walleye abundance estimates (density or biomass) with walleye size and the weighted least squares approach (Table 1).

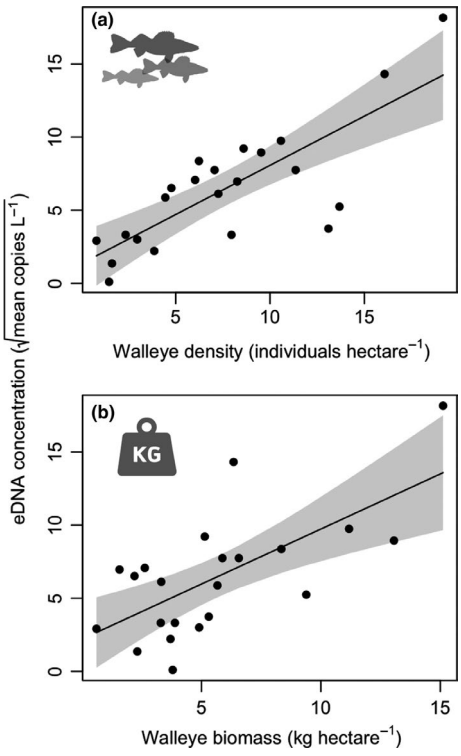

FIGURE 1 Simple linear regressions (black lines) of (a) eDNA concentration versus walleye (*Sander vitreus*) density (individuals/ha) and (b) walleye biomass (kg/ha) as determined by traditional mark–recapture methods in Ceded Territory of Wisconsin lakes during 2017 and 2018. Both demonstrate significant, positive relationships. Gray polygons represent 95% confidence intervals

3.2 | Year and Agency as Potential Factors

ANCOVA revealed that sampling year (2017 vs. 2018) was not a significant term in the best performing (those including mean walleye size and weighted least squares) walleye density ($p > .05$) or walleye biomass ($p > .05$) models. Similarly, mark–recapture agency (WDNR or GLIFWC) was not a significant term in those models (both $p > .05$). For the simple model of walleye density versus eDNA concentration (no additional terms or weighting), the agency interaction term had a p value of 0.041. With such a small sample size of GLIFWC lakes ($n = 7$), it is difficult to make strong inferences about differences among the two agencies.

4 | DISCUSSION

Our results build on a growing body of evidence that eDNA quantity correlates with organism abundance and suggest that quantifying eDNA could be a useful population monitoring tool. We demonstrate that walleye density explains a majority of the variance in walleye eDNA recovered from lake surface water ($R^2 = .62$) across natural systems ($n = 22$) in a fishery of critical management concern. Incorporating mean walleye size and accounting for variability in eDNA concentration among samples within a lake improves that relationship ($R^2 = .81$). Our significant, positive, and linear relationship is stronger than the average relationship found in other quantitative eDNA studies in natural systems ($R^2 = .50$; Yates et al., 2019) and approaches the average performance of quantitative eDNA in mesocosm studies ($R^2 = .82$; Yates et al., 2019).

4.1 | Density versus biomass

Walleye density (individuals/ha) alone slightly outperforms walleye biomass (kg/ha) alone in explaining eDNA concentration ($R^2 = .62$ and .42; BIC = 26.42 and 34.55, respectively). How an organism's size affects its eDNA production remains poorly understood, but it likely varies by species and life stage (Lacoursière-Roussel et al., 2016). Yates et al. (2019) found organism density and biomass were similar in explaining eDNA concentration in their metanalysis of quantitative eDNA studies. However, our best models incorporated both walleye density and biomass by including mean walleye size (kg), which suggests walleye eDNA abundance is driven both by the number of individuals and their relative size. In fact, in our (weighted) model predicting eDNA concentration from walleye biomass and mean walleye size, mean walleye size had a negative coefficient, suggesting that a given biomass of a few large fish produces less eDNA than an equivalent biomass of many small fish.

We may gain additional insights on eDNA's relationship with fish density and biomass from the two lakes which we omitted from our analysis for being uncharacteristic of regional walleye populations. These two lakes are included in a regression analysis in Figure S1. Long Lake, having a population of average density but dominated by large individuals (Figure S3), fit well with the overall relationship when using walleye density to predict eDNA concentration (Figure S1a), but its walleye population is overestimated when using walleye biomass (Figure S1b). This further supports that a few large fish produce less eDNA than an equivalent biomass of many small fish. Bearskin Lake, having a population of high density with average-sized individuals (Figure S3), was overestimated by regressions using both walleye density (Figure S1a) and walleye biomass (Figure S1b). This suggests our relationship may not hold for lakes with extreme walleye abundance, though such lakes would be of minimal management concern.

Our mark–recapture walleye density and biomass metrics include only adult fish, whereas eDNA is presumably sourced from all life stages, presenting a complication for accurately assessing whether eDNA is best predicted by number or size of fish. In addition, mark–recapture surveys were performed in early spring, likely before or during walleye spawning, whereas eDNA was collected in mid-July after walleye spawning, introducing a potential disconnect between the two methods. However, the inclusion of age-0 walleye CPUE data, collected in early fall, did not improve our models, suggesting that the reproductive differences among the walleye populations sampled did not explain significant variation in eDNA concentration. Because management of this fishery is based on adult populations, the strong correlation of eDNA with adult walleye density is useful from an applied ecological perspective.

4.2 | Year and agency as potential factors

The significance of mark–recapture agency as an interaction term in the simple model of walleye density versus eDNA concentration (no additional terms or weighting) may be an artifact of the large difference in ranges of the two agency groups' walleye density values. Though the means of the two groups' walleye densities were not significantly different ($p > .5$; two-sample t test), the range of walleye densities from lakes surveyed by GLIFWC is roughly half of that for lakes surveyed by WDNR (Figure S3). In addition, the sample size of the GLIFWC group ($n = 7$) is too small to make strong inferences. Therefore, we present models with no agency or agency interaction terms, but we recognize that additional sampling to expand the range of GLIFWC walleye densities could better determine whether the different mark–recapture methods used by the two agencies affects the relationship between eDNA concentration and walleye abundance.

4.3 | Qualitative analysis for applied management

To apply our results to real-world management scenarios, it may be helpful to analyze this quantitative data more qualitatively. The linear regression models described above treat walleye density as a continuous measure (individuals/ha). However, fisheries managers in Wisconsin bin lakes into "high," "medium," and "low," walleye categories, prescribing management strategies for

each category rather than individual lakes (Gregory Sass, WDNR, Boulder Junction, WI, personal communication, 2019). To test whether the eDNA assay correctly assigns our 22 walleye lakes into their qualitative management categories, we flipped the axes on our model to predict walleye density from eDNA concentration and then calculated Agreement Percentage and Cohen's Kappa coefficient between the predicted and observed walleye density values. We used three walleye density categories derived from WDNR's management thresholds for walleye populations (Gregory Sass, WDNR, Boulder Junction, WI, personal communication, 2019): "nonfishable" (0–3.7 individuals/ha), "fishable" (3.7–7.4 individuals/ha), and an ideal "management goal" (7.4 + individuals/ha).[1] Using these categories, we calculated an Agreement Percentage of 50% and a Kappa coefficient of 0.2, indicating "poor" agreement between eDNA-predicted and field-observed walleye densities (Fleiss, 1981; Figure 2a). We repeated this qualitative analysis using only two categories, "nonfishable" (0–3.7 walleye/ha) and "fishable" (3.7 + walleye/ha), which yielded a greater Agreement Percentage of 82% and a Kappa coefficient of 0.4, indicating "fair to good" agreement (Figure 2b).

This qualitative analysis of the ability of eDNA to monitor walleye populations gives a different perspective on its utility for applied fisheries management. eDNA performed "fair to good" in correctly identifying populations as "fishable" or "nonfishable," demonstrating its potential as a tool to assess population status across an important management strategy threshold. However, its "poor" ability to correctly assign population status using all three management categories demonstrates the significant amount of unexplained variation remaining in the relationship between eDNA and population size. This sensitivity to where we set category thresholds suggests that this qualitative method may not be the best indicator of our model's performance. Nonetheless, its ability to distinguish "fishable" versus "nonfishable" populations suggests that eDNA could be an early-warning indicator of population decline. In lakes currently lacking monitoring, an eDNA survey could serve as a fishery "firewall" to protect against overharvest. If eDNA monitoring indicates a population is "fishable," managers might be comfortable with business-as-usual strategies. But if eDNA returns a "non-fishable" result, it could alert managers to adjust management strategies or to prioritize that lake for a traditional mark–recapture survey.

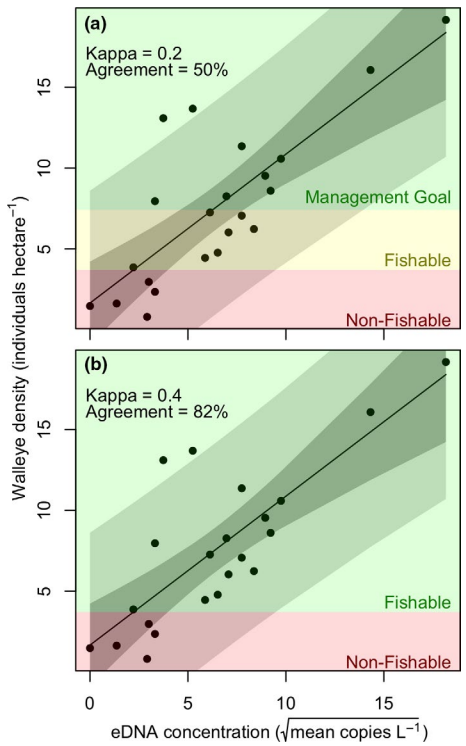

FIGURE 2 Cohen's Kappa coefficients and Agreement Percentages between observed and predicted walleye (*Sander vitreus*) density (individuals/ha) values from our model, broken into (a) three or (b) two categories based on thresholds for walleye population management in the Ceded Territory of Wisconsin during 2017 and 2018

4.4 | Uncertainty

When evaluating the uncertainty of a new monitoring method, it is important to consider that traditional methods contain uncertainty too. Viewing a novel method's uncertainty as a tradeoff for, not an addition to, a traditional method's uncertainty is critical for fair assessment. Traditional mark–recapture surveys are only estimates, possessing their own precision and accuracy like any other method—just precision and accuracy to which managers have become accustomed over time. The uncertainty in eDNA that comes from not having a fish "in hand" may be difficult for some fisheries managers to look past, but when electroshocking debuted as a population estimation method it, too, was initially met with skepticism (Jerde, 2019). Now, we regard electroshocking as "traditional" sampling in comparison with eDNA.

The mean coefficient of variation (CV) of the traditional mark–recapture population estimates in our study was 17% (see supplementary material). However, in our linear regressions, we treated these population estimates as certain. Therefore, our regression metrics (such as R^2) reflect not only the uncertainty of using eDNA to estimate walleye density, but also the 17% CV built into the mark–recapture method's estimation of walleye density. It is difficult to directly compare the uncertainty of our eDNA and mark–recapture methods because our within-lake eDNA values are zero-inflated. Instead, we have tried to build the uncertainty of the eDNA method into the regression using the weighted least squares approach. Measurement error in an independent variable can lead to attenuation bias in linear regression estimates, which is a commonplace violation of regression assumptions because there will "nearly always be error in *X*, particularly in the field sciences" (McArdle, 2003). Based on the variance of our walleye abundance measurement errors as a proportion of the total variance in our walleye abundance observations, we approximate this bias is less than 2% of our regression estimates.

5 | CONCLUSIONS

Walleye eDNA recovered from lake surface water correlates strongly with walleye abundance in Wisconsin lakes. Our quantitative eDNA survey (R^2 = .81; n = 22) performs well above the average for quantitative eDNA studies in natural systems (R^2 = .50). Though eDNA lacks critical demographic information gained from traditional surveys, the cost and personnel requirements of mark–recapture surveys currently limit monitoring to ~5% of walleye lakes in Wisconsin's Ceded Territory. Implementing eDNA surveys as an additional population assessment tool would provide complementary information on many more lakes than are currently monitored. The ability of our eDNA survey to correctly assign lakes to real-world management categories ("fishable" vs. "nonfishable") could provide an early warning for at-risk lakes in need of attention, more widely informing management of this important inland fishery.

The inland fisheries case we present here is contextualized by a single species approach in a limited set of systems, but it demonstrates the powerful potential of quantitative eDNA for monitoring populations. Future research to understand the principles governing the production, persistence, and capture of eDNA, as well as the standardization of eDNA survey protocols, may help translate specific relationships between eDNA concentration and organism abundance into general tools for ecological research, natural resource management, and conservation (Jerde, 2019).

DATA AVAILABILITY STATEMENT

Environmental DNA starting quantities for each sample as well as mark–recapture data and other sampling location information (site coordinates, qPCR performance metrics, abiotic lake information, etc.) are included in the Supporting Information of this article. These data are also archived with the Environmental Data Initiative [https://doi.org/10.6073/pasta/1dd7cbc2c65c8ea7804e3582ee3f4d90].

ENDNOTE

[1] Management thresholds are converted here from 1.5 to 3.0 individuals acre^{-1}.

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SUPPORTING INFORMATION

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

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APPENDIX

BIOMASS CALCULATION

To calculate population biomass for lakes, we adapted methods from Embke et al. (2019), as follows. First, we assigned lengths to all fish that were captured but not individually measured. For lakes with individual fish length information for most fish (2017: n = 4; 2018: n = 9), we randomly sampled with replacement from the available subset of length data for that lake-year combination and then assigned those values as lengths to the unmeasured fish from that same lake-year combination. The remaining lakes (2017: n = 10; 2018: n = 9) did not have any individual fish length measurements. Instead, all fish collected from these lakes were assigned to one of four length bins (0–304, 305–380, 381–508, >508 mm). From this information, we randomly sampled with replacement integers from each bin's range and assigned those integers as lengths to the unmeasured fish in that bin. We capped the final length bin at 880 mm as this was below the maximum length of fish collected in all lakes.

Once we had assigned length measurements to every fish captured, we used available weight data to develop lake-specific length–weight regressions. We used those regressions to assign a weight to each fish and calculate total population biomass (kg/ha) for each lake. For lakes in which some fish received direct length and weight measurements, we calculated log-log length–weight regressions from these fish to assign weights to fish that were not directly weighed. For lakes in which no fish received direct length and weight measurements, we calculated log-log length–weight regressions using data from that lake's most recent mark–recapture survey in which length and weight were directly measured for some fish, again assigning weights to the fish with unknown weights (in this case, all fish).

We determined whether each lake-specific regression was valid according to specific criteria: number of fish >25, R^2 > .85, and 2 < b < 4 (b = slope). Our criteria range for b was adapted from Froese (2006), who empirically demonstrated that, across systems, mean values of b were between 2.5 and 3.5 for over 1,700 fish species. We expanded the acceptable range of b because individual lake values likely exhibit greater variability than Froese's cross-system approach. If a lake-specific regression violated these criteria, we calculated length–weight regressions from directly measured walleye found in regional lakes of the same WDNR "lake classification" (e.g., cool and clear, warm and dark, and riverine/reservoir; Rypel et al., 2019). We then applied the appropriate lake-class length–weight regression to all fish with unknown weights in each remaining lake. Following the assignment of weights to all fish using length–weight regressions, we summed individual weights to calculate total walleye biomass (kg/ha) for each lake.

ATTENUATION BIAS CALCULATION

Despite measurement error in our walleye abundance estimates, we analyzed the relationship between walleye abundance and eDNA

concentration using OLS linear regression. We determined the relative attenuation bias in our regression estimates introduced by this measurement error to be small because the variance of measurement error of walleye abundance is small relative to the total variance in our walleye abundance observations. Using the following calculation adapted from McArdle's (2003) "Practical Considerations" for addressing measurement error in regressions, we approximate the relative attenuation bias to be ~2%.

$$\text{Relative Attenuation Bias} = \frac{p}{(1-p)} \text{ where } p = \frac{\text{var(measurement error in } x)}{\text{total var } (x)} \quad \text{(A1)}$$

where x is walleye density (individuals hectare^{-1}) and measurement error in x is the standard deviation of walleye density derived from the CV of each mark–recapture adult walleye population estimate (see supplementary material).