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A mesocosm comparison of laboratory-based and on-site eDNA solutions for detection and quantification of striped bass (*Morone saxatilis*) in marine ecosystems

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Abstract

Environmental effects monitoring in marine ecosystems are challenging, particularly in dynamic macrotidal settings like the Bay of Fundy. Environmental DNA provides a useful tool for determining species presence in such challenging places to access and sample. Moreover, recent studies showing a link between eDNA concentration and fish density/biomass reveal the great promise for eDNA tools to improve biodiversity assessments in marine environments. Three mesocosm experiments were conducted to assess the accuracy and precision of a handheld point-of-need (PoN) tool for quantitative polymerase chain reaction (qPCR) assay for eDNA detection of striped bass (*Morone saxatilis*) versus conventional laboratory-based eDNA techniques. The first of these experiments determined that striped bass eDNA was reliably detected using either of the laboratory-based or PoN platforms, with some variation observed in the estimates of eDNA concentrations derived from each. Next, a time series experiment established that eDNA in water samples collected within a 24-hrs period of exposure to striped bass was reliably and consistently detectable with either platform. Our final experiment found that the relationship between eDNA concentrations and manipulated striped bass stocking densities was significant and positive based on results from each of the laboratory-based or PoN platforms. Our results validate and advance eDNA approaches toward environmental monitoring efforts and demonstrate the potential for real-time eDNA tools to quantify and identify the spatial and temporal distribution of species-at-risk in an open ocean environment.

KEYWORDS

environmental DNA, environmental effects monitoring, *Morone saxatilis*, striped bass, tidal energy

1 | INTRODUCTION

Conducting environmental effects monitoring (EEM) investigations in spatially and temporally dynamic marine ecosystems can be particularly challenging in terms of logistics and safety risks, as well as potential low probabilities of encountering target species. For example, previous trawling- and hydroacoustic-based EEM programs for assessing potential effects of instream tidal energy devices on marine fish present or migrating throughout planned project areas (FORCE (Fundy Ocean Research Center for Energy) 2011; Melvin and Cochrane 2014; 2015) have been deemed by Canadian federal regulators (DFO (Fisheries and Oceans Canada) 2012; 2016) to have not satisfactorily addressed the Environmental Assessment predictions (AECOM 2009) pertaining to potential effects on fish.

To overcome such obstacles, environmental DNA (eDNA) presents a novel opportunity to develop new technology for rapid species detection. Because eDNA is sampled from non-living ecosystem components, it provides an entirely non-invasive means of conducting large-scale ecological surveys without physically capturing, handling, or harming organisms (Tréguier et al. 2014), particularly species-at-risk and other species of interest (e.g., species of commercial and/or cultural importance). eDNA methods are also proving to be a safer sampling method, with lower sampling effort and cost (Evans et al. 2017). By analyzing eDNA from a water sample, it is possible to determine whether a species of interest is present, regardless of life stage or whether specimens are complete or fragmented. Therefore, eDNA provides a useful tool for evaluating biodiversity in remote or challenging regions to access and sample, such as macrotidal and open ocean environments.

Over the past decade, eDNA has been employed by scientists for a variety of applications including: positive identification of rare and endangered species and invasive species (Laramie et al. 2015); inventories of freshwater and marine benthic and fish communities (Deiner et al. 2015); identification of species from unknown tissues (e.g., blood on wind turbines or aircrafts; confirmation of rare and endangered plants during fall and winter) (Zielińska et al. 2017); sampling of potable water quality (using pathogens as markers) (Shahraki et al., 2018); and tracing of parasite and disease vectors (e.g., identification of host animals and pathways) (Deiner et al. 2015). eDNA is now accepted as a reliable method for confirming species identifications in these and other applications.

Until recently, typical eDNA sampling involved existing standard ecological sample collection methods in the field (e.g., nets, traps, tissue swabs, sediment grabs, water samples) followed by preservation and submission of samples to largely academic laboratories for analyses, with the delivery timelines for the results on the order of weeks to months. Recent advances in analytical equipment have resulted in handheld point-of-need (PoN) tools that analyze eDNA in situ to confirm species identification in real time (Thomas et al. 2018). Real-time analyses of eDNA in the field offer substantial cost savings over conventional field sampling methods, and efficiency to obtain critical results within hours, as opposed to waiting weeks to months. Specific to ongoing EEM monitoring of marine tidal energy applications, this eDNA advancement has the potential to provide the missing link between hydroacoustic marine survey data and unequivocal species identification using molecular tools, as well as provide a relative eDNA signal strength output that proportionately reflects the density of fish present. Also, eDNA water column sampling would not be susceptible to the weather and seasonal climatic limitations imposed on vessel-based hydroacoustic surveys described above.

FIGURE 1 Photograph of Pool 1 empty during cleaning and sterilization protocol prior to initiating experiments

Evidence is accruing in the scientific literature showing a demonstrable positive relationship between eDNA capture/quantification and fish density and/or biomass (see review by Thomsen and Willerslev, 2015; Sassoubre et al., 2016; Murakami et al., 2019). These relationships have not yet been tested in remote or challenging macrotidal environments like the Bay of Fundy (BoF). A PoN system simplifies the sample filtration, eDNA extraction, and analysis steps as compared to a typical molecular laboratory and allows testing to be conducted on-site with basic operator training. The ultimate goal of this study was to validate the field-based PoN platform versus current laboratory-based eDNA solutions while also exploring the potential for both eDNA tools to quantify fish species-at-risk in marine environments.

2 | MATERIALS AND METHODS

2.1 | Experimental facilities

Our experiments were completed at the Aquatron facility housed within the Department of Oceanography at Dalhousie University. This unique facility offers three, large-volume tanks (300 m^3) each with a width of 7.3 m, length of 9.1 m, and average depth of 4.5 m (Figure 1). The tanks are constructed of reinforced concrete, with a flexible, food-grade liner. Each tank has a sloping bottom, which aids in allowing dirt and detritus to move toward the tank drain. These facilities are backed by a mechanical system, which provides

high-quality, temperature-controlled seawater or freshwater year-round, as well as a dedicated team of both biologists and mechanical operators who were available to run the systems. For our experiments, the tanks were filled with ambient unfiltered seawater from the core Aquatron seawater system, which is sourced directly via an intake pipe located in the Northwest Arm of Halifax Harbour at a depth of approximately 9 m. The three tanks are individually supplied by the seawater system and isolated so that each is independent replicates.

2.2 | Experimental design

The specific objectives of this study were as follows: a) to develop and validate a species-specific TaqMan qPCR assay to target eDNA detection of striped bass; b) to test and assess the accuracy and precision of the PoN tool to confirm species detection in real-time versus conventional laboratory-based qPCR with each platform run in tandem during all three experiments; c) derive estimates of eDNA signal persistence in saline water; and d) assess whether there exists a positive correlation between eDNA concentration and known densities of striped bass. These objectives were achieved through a series of three manipulative laboratory-based mesocosm experiments.

Adult striped bass (n = 223) of comparable total length (mean = 44.7 cm, range 34.3 – 53.3 cm) and weight (mean = 1.43 kg, range = 0.8 – 2.4 kg) originally sourced from Bay of Fundy broodstock were kept in holding tanks of recirculating seawater (Pools 1 to 3) where eDNA was shed and quantified using the eDNA tools outlined below. Recirculated seawater was used to reduce risk of contamination with exogenous striped bass eDNA from the source water where this species has the potential to occur. Animal care during our experiment was managed via Dalhousie University's Committee on Laboratory Animals Protocol Number I18-18. During the experimental period from August 30 to October 7, 2018, the water temperature in the tanks ranged from 16.8 to 18.0 °C. Prior to initiating work in Pools 1 and 2, they were cleaned and free of fish. Tanks were cleaned as follows: completely drained; mechanically power washed with 10% bleach solution: completely filled with seawater diluted to a concentration of 10% bleach and soaked overnight; drained and rinsed with seawater; and finally refilled with new seawater. All experimental fish were held in the same single tank (Pool 3) for approximately six months prior to the initiation of our experiment. Prior to initiating Experiment 3, Pool 3 was also cleaned. Post-cleaning, eDNA samples were analyzed to verify that the process successfully eliminated striped bass eDNA.

For each of three individual experiments, eDNA analyses were conducted with the in situ PoN thermocycler and compared to results using conventional laboratory-based qPCR techniques. The goals of the three experiments were:

- *Experiment 1—Detection*—Research question: Does the assay detect striped bass eDNA in situ? Goal: Establish whether the assay can detect striped bass eDNA stratified in the water column (top, middle, bottom; n = 3 water samples per depth level) in a tank (Pool 3), which held striped bass over previous six months).
- *Experiment 2—Persistence*—Research question: How long will DNA signal be detectable after fish are present? Goal: Establish the temporal persistence/decay rate of eDNA signal using water samples from Experiment 1. Testing for eDNA persistence occurred over a discrete time period (0, 3, 12, 24, 48, 96, and 120 hrs; n = 3 water samples per time).
- *Experiment 3—Quantification*—Research question: Can the assay quantify relative fish density? Goal: Examine the numerical relationship of eDNA concentration with increased fish density. After Time Zero sampling (no fish present) in each of Pools 1 to 3, three levels of fish density (1, 2, and 5 ind.) were established in randomly assigned Pools for a six-day period. After the initial six-day period, fish densities were increased in these three Pools (26, 58, and 139 ind.) for a subsequent six-day period. Water samples (n = 3) were collected daily over each six-day period.

Polyethylene sheeting was hung to a height of ~ 1 m between Pool Tanks to reduce potential for eDNA cross-contamination (splashing). An eDNA extraction working space was established in an adjacent area to avoid eDNA cross-contamination. All work was completed while wearing disposable nitrile gloves and required personal protective equipment for working around water and with chemicals.

2.3 | Water sampling

All sampling equipment and sample analysis workspaces were sterilized with 10% bleach between all samples and daily before initiating any work. All sample bottles and sampling implements (hoses, glassware, and laboratory materials) were also sterilized with 10% bleach bath for at least 10 mins followed by a rinse in fresh tap water in two successive and separate tubs. Water samples collection methods for each of the experiments are outlined below. Experimental blank (site negative) controls were included in all water sampling events.

2.3.1 | Experiment 1—Detection

In Pool 3, which held striped bass over previous six months, Teflon-lined peristaltic pump tubing was run to three water depths: top (0.1 m below the water's surface); mid-water (2.4 m below the water's surface); and bottom (3.5 m below the water's surface/ 0.5 m from the bottom of the tank to avoid residual organic matter on tank bottom). A peristaltic pump (Spectra Field-Pro, Waterra Pumps Limited, Mississauga, ON, Canada) was used to collect water. Prior to collecting a sample, 5 L of water was first pumped and disposed to fully purge and rinse the tube. While pumping, each sample bottle was tripled rinsed with target water to remove residual bleach and/or potential contamination. A total volume of 3.3 L of water for sample processing was then collected in pre-labeled vessels. Replicate

samples (n = 3) were collected from each water depth. Each water sample was then filtered for eDNA using an ANDe Sampling Backpack (Vancouver, WA, USA; Thomas et al. 2018).

The main components of the filtration system are a backpack pump system (ANDe) with a negative pressure inline filtration system with sensor feedback (to control the flow rate and pressure), an extension pole for sampling without entering water, and single-use, preloaded nitrocellulose filter membrane (47 mm diameter, 5-micron pore size). For each sample, a total water volume of 3 L was filtered using a maximum pressure threshold of 12.0 psi and a flow rate of 1.0 L/min.

Prior to starting the experiment, water samples were collected from each of the three tank depths and submitted to a commercial laboratory for analyses of general chemistry and metals scan to confirm the absence of inhibitory substances/conditions (Maxxam Analytics, Rapid Chemical Analysis package).

2.3.2 | Experiment 2—Persistence

Upon conclusion of Experiment 1, triplicate Top water samples were collected from Pool 3 (which held all striped bass over previous six months) using the same peristaltic pump protocol from above. Samples were stored at ambient light and temperature conditions for the discrete time periods described above and then processed using the eDNA filter protocol described in Experiment 1.

2.3.3 | Experiment 3—Quantification

For each Pool x Time combination (0, 3, 12, 24, 48, 96, and 120 hr), water samples were collected using the eDNA filter protocol described above.

Proprietary instrument-free DNA extraction kits (M1 Sample Prep Kit, Biomeme, Philadelphia, PA, USA) were used to extract and isolate eDNA from nitrocellulose filters following the manufacturer protocol. The filter was removed from the ANDe filter housing using sterile disposable forceps and then submerged in a lysis buffer and shaken. These extracted DNA elutions (500 µl) were then equally split into three separate Eppendorf tubes (one for analysis, one for other potential experiments, and one for archive) and frozen at −80°C. All PCR products for each of the laboratory-based and PoN platforms were performed on a common set of samples from this single set of DNA extractions.

2.4 | PCR protocols and assay development

Two separate qPCR platforms were used for this project. The PoN tool consists of a Biomeme three9™ (Philadelphia, PA, USA) coupled with an Android smart device to form a thermocycler for real-time PCR or isothermal analysis. This device enables multiplex, real-time detection of up to 27 targets from 1 sample or test 9 samples for up to 3 unique targets each. The Biomeme three9™ has three fluorescence channels that detect: FAM/SYBR; Texas Red-X; and ATTO647N/CY5. Laboratory-based qPCR was conducted using a benchtop Mic thermal cycler (Biomolecular Systems, Upper Coomera, Australia).

The striped bass qPCR assay (developed and validated at the Biodiversity Institute of Ontario, University of Guelph) is a species-specific real-time qPCR TaqMan assay that uses a 102-bp fragment from the *cytochrome c oxidase subunit I* (COI) mitochondrial marker to detect striped bass. Available COI sequences from striped bass were retrieved from GenBank (www.ncbi.nlm.nih.gov) and BOLD (http://www.boldsystems.org). To ensure assay specificity, available sequences from closely related and sympatric species (non-target species) were also retrieved and analyzed. Sequences from striped bass and non-target species were aligned in MEGA7 (Kumar, Stecher, & Tamura, 2016). All problematic sequences (e.g., extensive gaps, >1% of unknown bases, presence of stop codons, potential mislabel, or misidentification) were eliminated from the alignment (Table S1). Striped bass species-specific primers and probe were designed using AlleleID v.7 (Apte & Singh, 2007) . Primers and probe (Table S1) were tested in silico using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and OligoAnalyzer (www.idtdna.com/calc/analyzer).

In vitro validation and optimization of the striped bass qPCR assay (Table S1) was performed at the Biodiversity Institute of Ontario, University of Guelph, using both the PoN and laboratory-based qPCR platforms. Ethanol-preserved striped bass tissue samples were obtained from fish captive at Dalhousie University's Aquatron facility. Striped bass genomic DNA (gDNA) was isolated using the DNeasy Blood & Tissue Kit (QIAGEN).

Preliminary screening and optimization of the qPCR assay was performed in the Mic thermocycler using 20-µL reactions containing 10 µL of LyoDNA (Biomeme, Philadelphia, PA, USA) master mix, 5 µL of target gDNA template, and testing primers and probe concentrations of 200-900 nM. Thermal cycling conditions were 95°C for 120 s, 40 cycles of 95°C for 15 s, and preliminary annealing of 55–64°C for 45 s. Final assay conditions were as follows: 400nM concentration of each primer and 250 nM of probe with a qPCR cycle of 95°C for 120 s, 40 cycles of 95°C for 15 s, and annealing of 58°C for 45 s. The optimized assay (Table S1) was then tested using the PoN tool.

The assay was successfully validated following the *Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)* guidelines (Bustin et al. 2009). The validation included testing specificity, sensitivity, and efficiency. Specificity verified only striped bass (*Morone saxatilis*) target DNA was successfully amplified in qPCR products versus no amplification detected when DNA samples from five related non-target species/hybrid combinations were used including: White bass (*Morone chrysops*), White perch (*Morone americana*), Smallmouth bass (*Micropterus dolomieu*), Morone Hybrid, and *Morone saxatilis* x *M. chrysops* Hybrid. Sensitivity and efficiency were established by means of standard curves based on replicate sample serial dilutions (1:10K to 1:100B) with known copy number concentrations of sequence-verified, double-stranded DNA fragments of the target species (gBlocks, ® Gene Fragments) (IDT, www.idtdna.com). Standard curves were generated for each qPCR platform, the Mic and the PoN tool. Sensitivity is expressed as the limit of

detection (LOD), which is the minimum DNA concentration that can be detected with 95% of confidence (Bustin et al. 2009). The limit of quantification (LOQ) is the lowest concentration of target that can be accurately quantified with a coefficient of variance below a threshold of ≤ 35% obtained from calculated copy number from replicates in an assay-specific standard curve (Forootan et al. 2017). The qPCR assay had a LOD = 14.9 copies per μL and LOQ = 14.9 copies per μL for the Mic, and a LOD = 14.9 copies per μL and LOQ = 149 copies per μL for the PoN. The coefficients of variation (CV) between technical replicates were 1.2% and 7.2% for the PoN and Mic, respectively.

High qPCR efficiency is indicative of precise and robust qPCR assay performance. Efficiency for the PoN assay was 113% ($y = -3.051x + 34.341$; $R^2 = 0.99$) and 93% for the Mic ($y = -3.498x + 37.51$; $R^2 = 0.99$; Figure 2). These regression equations were used to convert quantification cycle (Cq) data from the qPCR product (i.e., the PCR cycle at which the target is considered positively amplified in a given sample) to the concentration of DNA in a given sample (copies of DNA per unit volume).

Tests conducted on the laboratory-based Mic thermocycler used wet reagents freshly prepared in the laboratory prior to use and supplied from Integrated DNA Technologies (IDT). The TripleLock™ striped bass qPCR assay for eDNA is species-specific and contains proprietary formulation of primers, probes, and master mix to allow for primer binding and DNA amplification. For the PoN tool, the tests were in the form of a dry reagent to be reconstituted with an aqueous nucleic acid sample. A lyophilization (i.e., freeze-drying) process makes the strips stable at ambient temperatures, so they do not require refrigeration for transportation or storage, as intended for use in the field or remote locations (Biomeme, Philadelphia, PA, USA). The reactions were optimized for 5 μL extracted template DNA. For the Biomeme three9™, this template was diluted with 15 μL of genetic-grade pure distilled water for a total 20 μL reaction volume. For the Mic, each total reaction volume of 20 μL consisted of 15 μL of customized master mix and 5 μL of extracted template DNA. All qPCR products were performed according to the following thermal cycling: initial denature phase for 2 min at 95°C, followed by 40 cycles of subsequent denaturing phases for 15 s at 95°C and annealing phases for 45 s at 58°C. Both the lyophilized and wet reagents used LyoDNA mastermix (Biomeme, Philadelphia, PA, USA) with an experimentally optimized concentration of 400 nM for F and R primers as well as 250 nM for the probe.

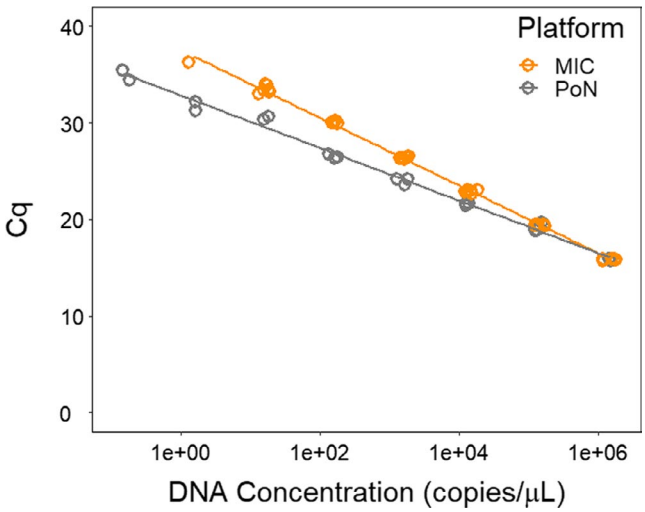

FIGURE 2 Standard curve of eDNA concentration versus number of DNA amplification cycles (Cq) generated using the Mic ($n = 6$ replicates per dilution) and the PoN ($n = 3$ replicates per dilution) platforms

For all samples processed with the Mic, three technical replicates were run. Positive amplification controls (PAC) consisting of reactions containing the target DNA fragment were included in each qPCR run to verify qPCR assay performance by determining that any negative signal was not caused by reagent failure. No-template controls (NTC) were included in each qPCR run to detect the potential presence of sample or reagent contamination during analysis; amplification of target eDNA in the NTC would signal contamination.

Sample processing for the PoN included the addition of an internal positive control (IPC). The IPC (Biomeme, Philadelphia, PA, USA) is set up such that a delay in the mean quantification cycle (Cq) value of 3 cycles or more for a reaction containing eDNA extract (relative to reactions containing pure water) is indicative of PCR inhibition (Goldberg et al. 2016). No technical replicates were used for PoN samples.

2.5 | Data analyses

All non-detect data were set to a Cq value to equal zero (Goldberg et al., 2016). For statistical interpretations, all technical replicates from Mic-based sampling were averaged. All data were assumed to be independent, while normality and homogeneity of variances assumptions were verified by Shapiro-Wilk and Levene's tests, respectively. When necessary, data were transformed to satisfy these assumptions (Quinn & Keough 2002), as described below. For Experiment 1, one-way analysis of variance (ANOVA) was conducted on $\log_{10} + 1$ transformed eDNA concentration data to test the effects of depth levels and for differences between the qPCR platforms. Due to non-normality of data, differences in Cq values between the qPCR platforms were assessed for Experiments 1 and 2 using Kruskal–Wallis rank-sum test. In Experiment 3, polynomial model regressions were performed on $\log_{10} + 2$ transformed eDNA concentration data versus $\log_2$ transformed fish stocking densities with inclusion of time (duration of stocking; 1–6 days) as a categorical covariate. Outliers with studentized residual values greater than 4.0 were assessed for biological or ecological significance for potential removal from analyses. All statistical analyses were performed with R statistical software version 3.4.3 (2017–11–30; R Core Team 2017).

3 | RESULTS

Analyses of general chemistry and metals scan provided no direct evidence of inhibitory substances/conditions in Aquatron Pool 3 water.

That said, the occurrence of false-negative results (Figure 3b) and examination of IPC curve data during initial sampling indicated PCR inhibition was occurring, despite this pool containing 223 striped bass. This PCR inhibition was likely due to the PCR being overloaded with template DNA due to the high density of fish present. Extracted DNA elutions were subsequently diluted (Goldberg et al. 2016) with pure distilled water to a factor of 3:1 to reduce the influence of inhibitory substances relative to the concentration of DNA, and thereafter, the PCR products functioned properly. For all experiments, no striped bass DNA was detected in any negative experimental blank controls during filtration or from NTC during PCR, indicating that there was no evidence of aerosolized cross-contamination of samples during the experimental period or contamination of reagents during PCR preparation. Examination of all PAC and IPC curves for Mic and PoN results, respectively, also indicated successful functioning of the PCR processes.

3.1 | Experiment 1—Detection

No technical replicates from Mic PCR produced false negatives (Figure 3a). Across all samples, eDNA concentrations ranged from 4.36 to 399 DNA copies μL^{-1} with a mean of 102.5 DNA copies μL^{-1} (± 114.5 *SD*; Figure 3a), while Cq ranged from 28.4 to 35.3 cycles with a mean of 31.6 cycles (± 2.1 *SD*; Figure 3b). No significant differences in eDNA concentrations were observed among depths sampled ($F_{2,6}$ = 0.946, p = .439; Figure 3a).

Using the PoN, three false negatives were detected—one from each of the top, middle, and bottom samples (Figure 3b). Across all samples, eDNA concentrations with the PoN ranged from 0 to 30.1 copies μL^{-1} with a mean of 8.17 copies μL^{-1} (± 11.8 *SD*) while Cq for successful reactions ranged from 29.8 to 35.9 cycles with a mean of 32.4 cycles (± 2.1 *SD*; Figure 3b). No significant differences in eDNA concentrations were observed among depths sampled ($F_{2,6}$ = 0.527, p = .616; Figure 3b).

Absolute eDNA concentration estimates for Mic samples (Figure 3a) were approximately an order of magnitude greater than those observed with the PoN ($F_{1,16}$ = 19.4, p < .001). However, Cq values were conserved across both systems (χ^2 = 0.566, df = 1, p = .452; Figure 3b), indicating comparable performance. Despite this differential in calculated eDNA concentration estimates, both systems provided similar observed relative trends among samples with positive detections (Figure 3).

3.2 | Experiment 2—Persistence

Temporal trends in Mic-derived eDNA concentrations showed a consistently detectable signal for the initial 24-hrs examination period after which no eDNA was detected in any subsequent samples

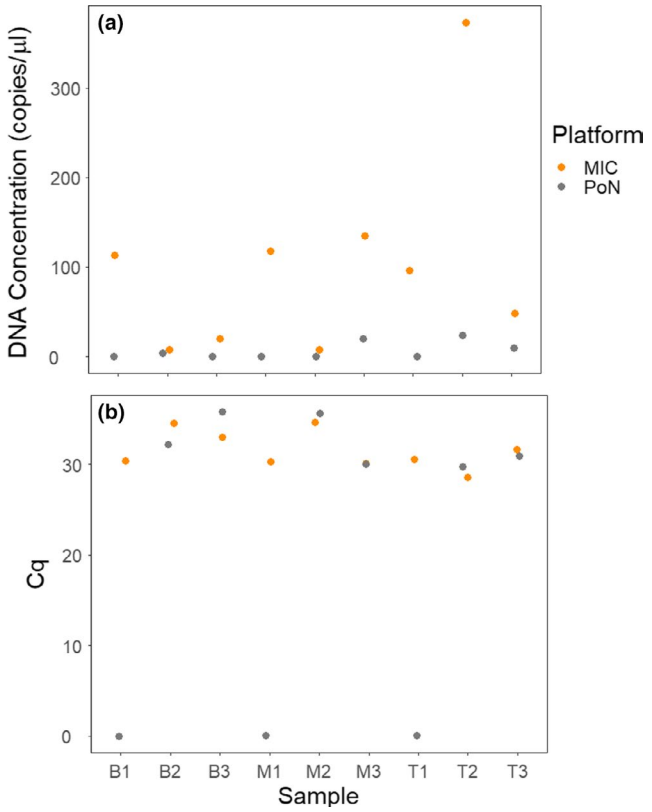

FIGURE 3 Scatterplot of (a) eDNA concentrations and (b) Cq values per sample for Mic analyses and PoN analyses. X-axis letter labels denote sample depth (B = bottom, *M* = middle, T = top), while numbers represent replicate order

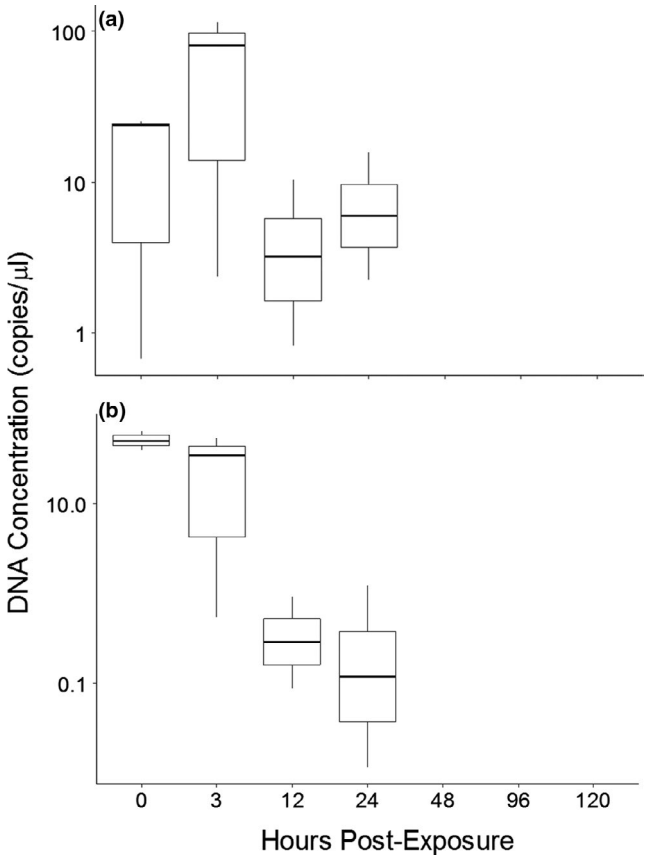

FIGURE 4 Boxplot of temporal trends in eDNA concentrations for (a) Mic analyses and (b) PoN analyses

from 48 to 120 hrs (Figure 4a, Table 1). Of note, at least one technical replicate for each sample within each time period from 0 to 24 hr resulted in non-detectable eDNA.

The first evidence of complete signal loss (no detection in any of the three technical replicates from a sample) using the Mic occurred at 24 hrs (Table 1). Within the 0- to 12-hrs time period, heterogeneity in eDNA concentration was noted at each time point with at least one sample having estimated eDNA concentrations much lower than the remainder of samples. Interestingly, median eDNA concentrations detected increased in 3-hrs samples as compared to initial samples at 0 hr (Figure 4a), though this result was likely a function of the low eDNA concentration (0.68 copies μL^{-1}) in one of the 0-hr samples biasing the median value (based on three samples) for that group lower.

A similar trend was observed in PoN-derived data (Figure 4b), which also showed a detectable signal for the initial 24-hs examination period after which no eDNA was detected in any subsequent samples from 48 to 120 hrs, with progressively declines in eDNA concentration from 0 to 24 hrs (Figure 4b). In contrast to Mic-derived data, at least one of three samples within each sampling event from 0 to 24 hrs was non-detectable for the PoN samples (Table 1). Similar to the observations in Experiment 1, PoN-derived data estimated eDNA concentrations lower than those from the lab-based Mic platform. Examination of Cq values further indicated the differential performance between each platform, with the greatest divergence in Cq values observed during the periods with the highest and lowest (0 and 24 hrs, respectively) eDNA concentrations (Table 1). Despite this finding, Cq values were conserved across both systems (χ^2 = 0.687, *df* = 1, *p* = .407; Figure 4), indicating comparable performance. Regardless of the eDNA platform used, the decay signal indicated that water samples collected within a 24-hrs period of exposure to striped bass reliably detected eDNA, with this signal diminishing substantially after a 48-hrs period, after which there was no detectable eDNA signal using either method.

TABLE 1 eDNA Concentrations and Cq Values (Mic and PoN) from Time Series Experiment 2

	eDNA Concentrations (copies μL^{-1})		Cq	
Time (hours)	Mic	PoN	Mic	PoN
0	25.34	39.03	32.63	29.49
0	23.88	64.29	32.71	28.82
0	0.68	nd	36.43	nd
3	81.86	33.95	30.84	29.67
3	2.38	0.55	35.70	35.13
3	115.63	53.83	30.30	29.06
12	3.24	nd	35.82	nd
12	0.83	0.09	36.12	37.54
12	10.37	0.93	34.03	34.43
24	nd	nd	nd	nd
24	15.81	1.21	33.32	34.09
24	2.29	0.01	35.73	40.23
48	nd	nd	nd	nd
48	nd	nd	nd	nd
48	nd	nd	nd	nd
96	nd	nd	nd	nd
96	nd	nd	nd	nd
96	nd	nd	nd	nd
120	nd	nd	nd	nd
120	nd	nd	nd	nd
120	nd	nd	nd	nd

Note: Gray cells indicate non-detected (nd) samples with Cq = 0 (Goldberg et al. (2016). Mic data presented in each cell are averages of three technical replicates.

3.3 | Experiment 3—Quantification

The cubic relationship between eDNA concentrations and manipulated striped bass stocking densities was highly significant and positive ($r^2_{adjusted}$ = 0. 2,561; $F_{3,96}$ = 12.36, *p* > .001; Figure 5a) for Mic samples. No significant differences were found in the intercepts through time (*p* > .05), and no significant differences were found between models with or without the temporal covariate ($F_{4,85}$ = 1.395, *p* = .409). These findings indicated there was no evidence of cumulative accumulation of eDNA in the tanks over the 6-days duration of the experiment.

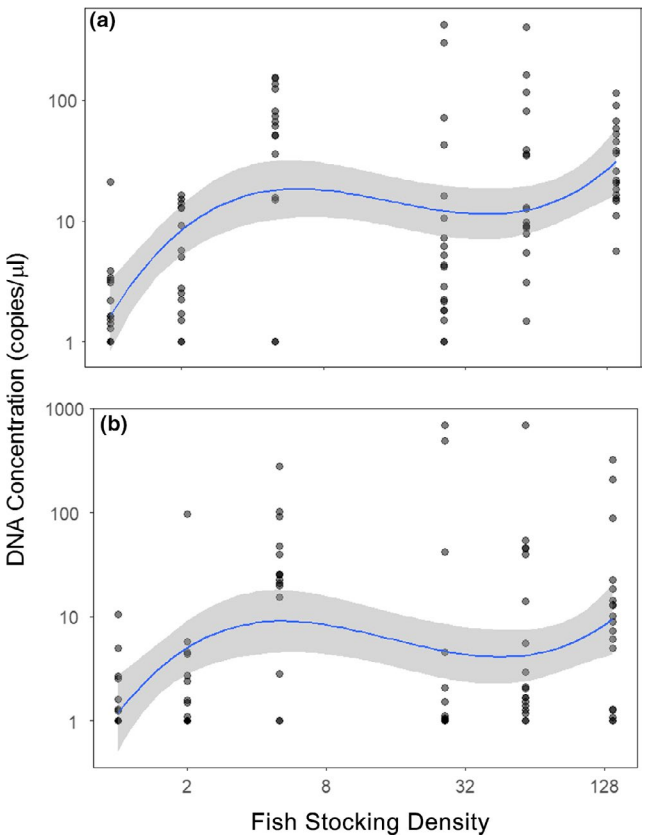

FIGURE 5 Relationship between eDNA concentrations and striped bass stocking density for (a) Mic analyses and (b) PoN analyses. Y-axis presented in $\log_{10}$ + 1 scale; x-axis presented in $\log_2$ scale. Blue line equals regression line (± 95% confidence intervals)

Trends in the data indicated maximum individual eDNA concentrations were reached at a density of 26 individuals, while mean eDNA concentrations plateaued for intermediate stocking densities (5, 26, or 58 individuals) with slope subsequently increasing for the highest stocking density (139 individuals; Figure 5a). Interestingly, eDNA concentrations for these intermediate stocking densities were more variable than either of the lowest (1 or 2 individuals) or highest stocking densities (139 individuals; Figure 5a). False-negative results were noted in 10% of samples, with 80% of these occurring in the three lowest density treatments (1, 2, 5 individuals).

Similar to the Mic results, the relationship between eDNA concentrations and manipulated striped bass stocking densities for the PoN was significant and weakly positive ($r^2_{adjusted}$ = 0.101; $F_{3,95}$ = 4.65, p = .004; Figure 5b). Trends in these data also indicated maximum eDNA concentrations were reached at a density of 26 individuals with a plateau for intermediate stocking densities followed by an increased slope at the highest stocking density (139 individuals; Figure 5b). As was noted from the Mic results, eDNA concentrations from the PoN for intermediate stocking densities (5, 26, or 58 individuals) were more variable than either of the lowest (1 or 2 individuals; Figure 5b). However, no reduction in variability was noted for the 139 individuals stocking densities (Figure 5b), as had been observed for the Mic data (Figure 5a).

Regardless of the eDNA platform used, a quantifiable and significant relationship existed between eDNA concentrations and manipulated striped bass stocking densities. Many more false negatives were noted for the PoN as compared with the Mic. Of the 105 samples processed, 27 (26%) from the PoN were false negatives, of which 16 were found for low-density striped bass (1 and 2 individuals) with another 8 from the intermediate density (26 individuals).

4 | DISCUSSION

Environmental DNA tools are a means to address a broad array of environmental management questions in aquatic systems, including conservation biology, detection of cryptic or rare species, detection of invasive species, population dynamics, indicators of health in aquaculture operations, wildlife forensics, trophic interaction, dietary studies, species historical patterns, ecosystem health, and community assessment (reviewed by Díaz-Ferguson and Moyer 2014). Although eDNA technologies are being widely investigated in freshwater habitats, their application in marine systems has lagged (Díaz-Ferguson and Moyer 2014). This disparity is at least partly attributed to the relative ease of sample collection in freshwater environments; the likely higher ratio of water volume to target eDNA fragments in the marine environment compared to freshwater systems; the much larger scale of hydrographic processes in oceans compared to those in freshwater systems; and by inhibition of molecular procedures in high salinity environments (Díaz-Ferguson & Moyer, 2014). Despite some of these challenges, eDNA tools have been successfully used in the marine environment for the detection of microbial communities, phytoplankton, fish, and marine mammals (Díaz-Ferguson and Moyer 2014; Foote et al. 2012; Doi et al. 2015).

Our results demonstrated that all project objectives were achieved. First, the species-specific assay was successful in detecting eDNA from striped bass and performed with high specificity and sensitivity. Successful and consistent detections were achieved across both the laboratory-based and PoN platforms. We found, however, eDNA concentration estimates for Mic samples were greater than those from samples analyzed with the PoN. Cq values were also conserved across both systems; however, indicating these varying eDNA concentration estimates could be a function of the variability between the development of their standard curves. With the lower precision and accuracy of the PoN relative to the Mic, as evidenced by the greater observed false-negative values as well as higher coefficient of variation values for its standard curve (mean = 13.3%) versus. the Mic (4.42%), these results could indicate lower performance by the PoN. Possible explanations for this lower performance could include less robust optical sensors in the PoN relative to the Mic and/or the lyophilization of the reagents used with the PoN versus the fresh wet reagents used with the Mic. Also, PCR inhibition occurs in samples with very high initial concentrations of DNA template (Opel et al., 2010). Given Experiment 1 was conducted in a 300-m^3 tank with 223 adult striped bass, it is reasonable to infer that PCR inhibition (in the absence of other potential physical/chemical inhibitors) occurred in this particular situation with the less-precise PoN tool (note that no similar PCR inhibition was noted in Experiment 3 at lower stocking densities). Despite the differing eDNA concentration estimates described, these results provide confidence in the potential utility of employing the PoN tool for field-based applications for tracking striped bass, from a detection of species perspective. Our results suggest that the use of the PoN tool for field-based applications has potential for tracking the presence/absence of striped bass. However, the results indicate that the PoN tool provides less precise and accurate abundance estimates compared with the Mic platform.

Findings from Experiment 2 suggest that, regardless of the eDNA platform used, the eDNA signal from water samples collected within a 24-hr period of exposure to striped bass was reliable. In a recent investigation of eDNA dynamics under natural freshwater conditions, Barnes et al. (2014) unexpectedly detected a declining rate of target eDNA degradation as biochemical oxygen demand, chlorophyll, and total eDNA concentration (from any organism) increased. This finding could not be attributed to any specific cause, and the authors concluded environmental differences between various freshwater studies may be sufficient to explain variation in eDNA degradation rates reported in the literature (Barnes et al. 2014). An extreme example of this is the disparity of eDNA persistence between freshwater (median = 30 days) and marine habitats (median = 7 days) (Díaz-Ferguson and Moyer 2014; Foote et al. 2012). Our findings are much shorter in duration than the median values presented by Foote et al. (2012), suggesting that a positive detection of striped bass in marine water reflects recent occupancy of this species. A few assumptions,

however, would be implicit in such a conclusion. First, our results are from a laboratory-based study under very controlled conditions; therefore, our ability to detect eDNA in minute quantities nearing the LOD limits is heightened relative to field studies. Many environmental conditions influence the persistence of eDNA fragment length (and therefore detectability, reviewed by Goldberg et al., 2016) including adsorption of DNA to mineral or organic matter that may favor preservation in bottom sediments or inhibit polymerase activity in the PCR process (Díaz-Ferguson and Moyer 2014; Tréguier et al. 2014). This could be particularly influential in turbid waters with high total suspended solid loading, such as many regions of the Bay of Fundy and its tributaries. While the detection of a species' eDNA provides a reasonable deduction that a live individual was recently present in a given area, this assumption may not hold true in hydrodynamically variable marine environments. While our experimental results provide promise of the field utility for eDNA surveys in the Bay of Fundy, more research is required for tool validation before deployment in such macrotidal, sediment-laden environments. For instance, refinements in assay developments could include the development of an endogenous positive control that utilizes universal primers to assist in overcoming field false negatives.

Significant and positive relationships between eDNA concentrations and manipulated striped bass stocking densities were observed for both the laboratory-based and PoN platforms. Originally, it was hypothesized this relationship would follow a monotonic linear trend. While significant linear relationships were observed upon initial analyses, we found a cubic polynomial best fit the data, driven by the observed plateau for intermediate stocking densities. We are unaware of a potential biological basis as to why eDNA concentrations would plateau at intermediate fish densities; however, this result may be a limitation of the qPCR to discern differences over such a narrow range of fish densities. Lacoursière-Roussel, Rosabal, and Bernatchez (2016) found similar results where exponential models increased the power of predicting fish abundance-/biomass-based on measured eDNA concentrations relative to linear models. Our results lend weight to the growing body of research confirming the ability to derive relationships between eDNA capture/quantification and fish density and/or biomass (Thomsen & Willerslev, 2015; Sassoubre et al., 2016; Murakami et al., 2019). Such findings provide reasonable expectations that, with appropriate validation and study designs, eDNA tools may prove valuable as a tool for determining the relative abundance of marine species.

New methods are promising to refine our ability to determine the concentration of target DNA in a sample. A recently developed "third-generation" DNA detection method, known as digital droplet PCR (ddPCR), can provide absolute quantification of target DNA without a standard curve for reference. This method uses emulsion chemistry to distribute PCR products into thousands of nanodroplets from which PCR amplification can be detected, and statistical analysis of the nanodroplet results can determine the concentration of target DNA from the original sample (Doi et al. 2015). These same authors used a mesocosm stocked with a defined number of fish of a certain size range (juveniles) to compare the accuracy of ddPCR to that of quantitative real-time PCR (qPCR). The authors reported the ddPCR method allowed for quantification of target species eDNA, species abundance, and biomass more accurately than qPCR. Moreover, abundance of target species had a higher regression model estimation accuracy for both ddPCR and qPCR methods than did biomass, indicating that concentration of eDNA is more highly correlated to abundance when target organisms have a similar body mass (Doi et al. 2015). As application of eDNA technology becomes more common, incorporation of suitable statistical methods, such as site occupancy models to design eDNA studies, will provide a high probability of detection if the species is truly present and build regulator and stakeholder confidence in the use of eDNA tools for biodiversity and effects monitoring studies relative to more traditional field sampling methods. While few examples exist in marine eDNA literature, promising results of such an approach of model development and field validation has been demonstrated in estimating species distribution and abundance of salmonids in river networks using eDNA (Carraro et al. 2018) may provide a framework to be adapted for coastal eDNA applications.

Our results provide a demonstrable positive relationship between eDNA capture/quantification and striped bass density. However, these relationships have not been tested in remote or challenging sampling settings, such as macrotidal, sediment-laden environments like the Bay of Fundy. Prior to embarking on field studies, research should test the striped bass primers against varying densities of striped bass and total suspended solids in a laboratory-controlled confirm the ability to detect and develop empirical relationships for relative abundance of striped bass. Subsequently, the efficacy of the striped bass assay should be field-tested at a location of known and high concentrations of target species. For example, striped bass spawning occurs in the Bay of Fundy on the Stewiacke River and the water column at this location would be saturated with striped bass eDNA fragments due to the high densities of fish combined with the release of eggs and milt. Likewise, estimates of density/biomass from eDNA-based field studies could be compared to estimates derived from the same site(s) using conventional assessment methods. Annual estimations of striped bass densities using traditional fish trapping and acoustic tracking are conducted by Fisheries & Oceans Canada in areas of intensive striped bass spawning, such as in the Stewiacke River.

eDNA tools show great promise to improve our ability to assess biodiversity and monitor for environmental effects in the marine environment. Our ultimate goal was to validate and advance this eDNA approach toward complementing previous and ongoing marine monitoring efforts while simultaneously increasing the rigor in the quantification and identification of the spatial and temporal distribution of fish species-at-risk. More work is required to evaluate the beneficial application of eDNA tools to address monitoring and regulatory challenges faced by marine proponents. Ultimately, the success and recognition of potential benefits of this new technology will depend

on its effectiveness through testing and confidence gained by regulators and stakeholders.

DATA ARCHIVING STATEMENT

Data for this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.qz612jm9h.

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AUTHOR CONTRIBUTIONS

MAS conceived and designed the framework for the study with input from all co-authors. MAS compiled and analyzed the data and drafted the original manuscript. TLQ and SC provided extensive laboratory assistance to MAS. MM, TLQ, SC, and RHH contributed substantially to manuscript writing and review.

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SUPPORTING INFORMATION

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

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