Innovative Solutions for De-risking Species Detections in Tidal Energy EEM Programs

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INNOVATIVE SOLUTIONS FOR DE-RISKING SPECIES DETECTIONS IN TIDAL ENERGY EEM PROGRAMS

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Executive Summary

Environmental effects monitoring investigations in marine ecosystems are challenging, particularly in dynamic ecosystems like the Bay of Fundy. We applied new environmental deoxyribonucleic acid (eDNA) tools in an effort to augment the conventional scientific approaches used in monitoring programs gained from monitoring programs specific to tidal turbine projects. Our ultimate goal is to address regional regulatory concerns and increase stakeholder confidence related to monitoring efforts conducted to-date. eDNA is defined as short fragments of genetic material that have detached from an organism into non-living components of an ecosystem (e.g., air, water, sediment) and provides a useful tool for determining species presence in challenging places to access and sample, such as macrotidal environments in the Bay of Fundy. 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 the marine environments.

Our project objectives were to develop and refine species-specific primers for eDNA detection of striped bass, derive estimates of eDNA signal persistence in saline water, and assess whether relationships exist between striped bass densities and eDNA concentration. To accomplish this, we assessed the accuracy and precision of a hand-held point-of-need (PoN) tool which can analyze eDNA *in-situ* to confirm species identification in real-time versus conventional laboratory-based eDNA techniques. These objectives were achieved through a series of manipulative laboratory experiments conducted at Dalhousie University's Aquatron facility.

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-hour period of exposure to striped bass was reliably and consistently detectable with either platform. Our final experiment found that the linear 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 towards complementing previous and ongoing marine tidal energy monitoring efforts and demonstrate the potential for eDNA tools to quantify and identify the spatial and temporal distribution of fish species-at-risk in an open ocean environment. Future research priorities are discussed related to further laboratory-based validation, field study trials, and augmentation with existing marine tidal monitoring data to leverage regulatory and stakeholder confidence in effects monitoring results.

1.0 INTRODUCTION AND OBJECTIVES

Conducting environmental effects monitoring (EEM) investigations of tidal in-stream energy conversion (TISEC) devices in the marine ecosystem is challenging in a naturally variable and high flow tidal environment, both in space and time. This project applied a new technology for rapid species identification in high flow marine conditions using environmental deoxyribonucleic acid (eDNA) tools and to build upon reports/data and experience gained from EEM programs for national and international tidal turbine projects.

1.1 PREVIOUS STUDIES

Previous trawling- and hydroacoustic-based EEM programs (FORCE 2011; Melvin and Cochrane 2014; 2015) for assessing potential effects of TISEC devices on marine fish present or migrating throughout planned project areas have been deemed by Fisheries and Oceans Canada (DFO 2012; 2016) to have not satisfactorily addressed the Environmental Assessment predictions (AECOM 2009) pertaining to potential effects on fish.

The hydroacoustic technique employed in these EEM programs was limited in its ability to: (a) differentiate species; and (b) detect fish near boundaries such as the surface, sea floor, or in the immediate near-field (< 10 m) of a TISEC device (SLR 2015). Melvin and Cochrane (2014; 2015) stated that any reference to species is purely speculative with their hydroacoustic surveys and also reported that entrained air within the upper water column, particularly during high wind events, restricted the use of hydroacoustic technologies at certain times and locations.

The Fundy Ocean Research Center for Energy (FORCE) Environmental Monitoring Advisory Committee (EMAC) has identified:

> "(1) that the highest priority for monitoring, from regulatory and public perspectives, continues to be avoidance behaviour of fish and their potential interaction with operating turbines; and (2) that this has proven to be the most challenging undertaking from an operational perspective because of the high currents and turbulence in the Minas Passage (EMAC 2016)."

EMAC further expressed some reservations regarding the application of vessel-mounted, downward-looking hydroacoustics to monitor fish (EMAC 2016). In 2016, Fisheries and Oceans Canada (DFO) indicated data were lacking for Atlantic sturgeon (*Acipenser oxyrinchus),* striped bass (*Morone saxatilis*), adult American eel (*Anguilla rostrata*), and inner Bay of Fundy Atlantic salmon smolts (*Salmo salar*). In particular, sufficient replication data and information from winter months were lacking for striped bass that exhibited an extended temporal and spatial presence within Minas Passage in (DFO 2016).

FORCE has deployed the latest iteration of the Fundy Advanced Sensor Technology (FAST) platform which includes an upward-looking acoustic zooplankton fish profiler (AZFP). While these acoustic-based methodologies (bottom-deployed or vessel-mounted) can generate large volumes of data on the presence of fish or zooplankton in the water column, the acoustic data are still unable to reliably discern

which species (e.g., striped bass, inner Bay of Fundy Atlantic salmon, or Atlantic sturgeon) are actually present due to limitations in the interpretation of acoustic target strengths and presence of multiple species fish assemblages.

1.2 PROJECT RATIONALE

To overcome these obstacles, eDNA offers a new technology for rapid species detection. eDNA is defined as short fragments of genetic material that have detached from an organism into non-living components of an ecosystem such as air, water, or sediment (Díaz-Ferguson and Moyer 2014; Pilliod et al. 2013). The accumulation of eDNA in the environment occurs either indirectly via shedding or through discharge of mucus, skin, urine, feces, and gametes, or directly via the process of cell death and release of DNA (Díaz-Ferguson and Moyer 2014; Pedersen et al. 2015). Because eDNA is sampled from non-living ecosystem components, this new technology 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., commercial and cultural importance). eDNA methods are also proving to be a safer sampling method, with lower effort and cost, and at a considerably lower sampling effort and cost. Furthermore, eDNA provides a useful tool for evaluating biodiversity in remote or challenging regions to access and sample, such as macrotidal and open environments where TISEC devices are typically placed.

Analysis of eDNA involves taking a sample of the environment (e.g., filtration of water) in which the target species may be present, extracting the DNA, and then using species-specific DNA primers to objectively and unambiguously determine if the DNA of the target species is present in the sample. The test will determine if DNA for the target species is present, regardless of life stage or whether specimens are complete or in fragments.

Over the past decade, eDNA has been employed by Stantec scientists, our collaborators, and the wider scientific community for a variety of applications including: positive identification of rare and endangered species and invasive species (based on genetic markers); inventories of freshwater and marine benthic and fish communities; identification of species from unknown tissues (e.g., blood on wind turbines or aircrafts, confirmation of rare and endangered plants during fall and winter); sampling of potable water quality (using pathogens as markers); and tracing of parasite and disease vectors (e.g., identification of host animals and pathways). 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 sample collection methods in the field (e.g., nets, traps, tissue swabs, sediment grabs) 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 hand-held point-of-need (PoN) tools that analyze eDNA *in-situ* to confirm species identification in real-time. Real-time analyses of eDNA in the field offers substantial costs 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 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.

1.3 PROJECT OBJECTIVES

Scientific literature indicates 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 been tested in remote or challenging regions to access and sample, such as macrotidal environments like the Bay of Fundy (BoF). This 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. Our project objectives were to:

- Develop and refine species-specific primers to target eDNA detection of BoF striped bass.
- Test the PoN tool and species-specific primers against varying known densities of striped bass at Dalhousie University's Aquatron facility to demonstrate the ability to detect and develop empirical relationships to the relative abundance of this species from water sampling to: (a) assess eDNA detection efficiency and signal persistence; and (b) quantify striped bass density based on the eDNA prevalence.
- Assess the accuracy and precision of the PoN tool relative to conventional laboratory-based quantitative polymerase chain reaction (qPCR).

Our ultimate goal was to validate and advance this eDNA approach towards complementing previous and ongoing marine tidal energy monitoring efforts and demonstrate the potential for eDNA tools to quantify and identify the spatial and temporal distribution of fish species-at-risk in marine environments.

2.0 METHODOLOGY

2.1 AQUATRON FACILITY

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 towards the tank drain. The main deck of the tanks allows access to the ends of each tank and the outer sides of the end tanks. The tanks were designed to be rectangular in shape, which allows their volumes to be maximized. The corners of each tank are chamfered to help reduce water flow dead spots in the corners. To aid in circulation, each tank is equipped with two high-tech, carbon fiber mixers. These mixers are located in opposing corners of the tank and can be adjusted to create water flow in any part of the tank. The mixers are controlled with electronic micro controllers, which allow the mixers to provide a number of different flow patterns.

These world-class facilities are backed by a mechanical system, which can provide 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 filling and draining of the tanks is all done with the help of computer software, which can also monitor tank water depth through the use of an electronic level sensor and a submerged pressure sensor. The three tanks are individually supplied by the seawater system and isolated so that each are independent replicates.

Figure 1 Photograph of Pool 1 Empty During Cleaning and Sterilization Protocol Prior to Initiating Experiments

As the largest university aquatic research facility in Canada, this venue was one of the world's most realistic laboratory-based mesocosms in which to conduct genomic experiments on large marine fish under controlled laboratory conditions.

2.2 EXPERIMENTAL DESIGNS

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[1] seawater (Pools 1 to 3) where eDNA was shed and quantified using the eDNA tools outlined below. 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 - October 7, 2018 the water temperature in the tanks ranged from 16.8 – 18.0 ºC. Prior to initiating work in Pools 1 and 2 they were cleaned[2] and free of fish. 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 testing was conducted to verify the process successfully eliminated striped bass eDNA.

The goals of the three proposed experiments were:

- Experiment 1 – Detection – Research question: Does the tool detect striped bass eDNA *in-situ*? Goal: Establish whether PoN 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 hours; n = 3 water samples per time).

- Experiment 3 - Quantification – Research question: Can the tool 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 work spaces were wiped with 10% bleach between all samples and daily before initiating any work. All sample bottles and sampling implements (hoses, glassware, lab materials) were soaked with 10% bleach bath for at least 10 minutes followed by a rinse in

[1] Recirculated seawater was used to reduce risk of contamination with exogenous striped bass eDNA from the Halifax Harbour source water where this species has the potential to occur.
[2] 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.

municipal water in two successive and separate tubs. Water samples collection methods for each of the experiments are outlined below.

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).

The main components of the filtration system are a backpack pump system 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, pre-loaded 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[3] (Maxxam Analytics, Rapid Chemical Analysis package).

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 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 above.

Experiment 3 - Quantification – For each Pool x Time combination, 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. DNA extraction consisted of four reagent steps. Initially, the filter was removed from the ANDe filter housing using sterile disposable forceps and then submerged in a lysis buffer and shaken. A filter-embedded syringe then captured all the liberated cellular material. Subsequent washes liberated proteins, polysaccharides and reagents from the filter, leaving only DNA attached. DNA was then collected by washing in an elution buffer. These extracted DNA elutions 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 ºC. All PCR reactions for each of

[3] Molecules (including naturally occurring compounds from plants and soils) which can interact with target DNA and/or PCR polymerases to prevent or impair PCR amplification, even when sufficient target DNA may be present, leading to conclusions of false negatives or biased abundance estimates (Goldberg et al., 2016).

the lab-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 multi-plex, real-time detection of up to 27 targets from 1 sample or test 9 samples for up to 3 targets each. The Biomeme three9™ has three color channels that detect: FAM / SYBR; TexasRedX; 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 by Biodiversity Institute of Ontario (University of Guelph), is a duplex real-time qPCR TaqMan assay using mitochondrial DNA markers. 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, assay 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.

Tests conducted on the laboratory-based MIC thermocycler used wet reagents freshly prepared in the laboratory prior to use. To maintain common test conditions, qPCR assays using both the Biomeme three9™ and MIC thermocyclers were conducted by Dr. Marc Skinner at a research laboratory at the Centre for Biodiversity Genomics, Department of Integrative Biology at the University of Guelph. DNA extractions (detailed below) were not conducted in the PCR laboratory room to prevent cross-contamination. 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 reactions 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.

For all samples processed with the MIC, three technical replicates[4] 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: (a) testing for the presence of PCR inhibition, which, if not identified, can lead to false negatives; and (b) determining that any negative signal was not caused by reagent failure. No-template controls (NTC[5]) 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.

[4] Replicates used to perform the same test multiple times on a single eDNA extract from a single water sample.
[5] Genetic-grade pure distilled water omits any DNA template from a reaction and serves as a control for extraneous nucleic acid contamination. No PCR amplification should occur for these samples.

Sample processing for the PoN included the addition of an internal positive control (IPC). The IPC is set up such that a delay in the mean quantification cycle (Cq) value of 1 cycle or more for a reaction containing eDNA extract (relative to reactions containing pure water) is indicative of PCR inhibition.

The assay was successfully lab-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 reactions versus no amplification detected when DNA samples from five related non-target species/hybrid combinations were used:

- white bass (*Morone chrysops*);
- white perch (*Morone americana*);
- Morone Hybrid;
- *Morone saxatilis* x *M. chrysops Hybrid*; or
- smallmouth bass (*Micropterus dolomieu*).

Sensitivity and efficiency were established by means of standard curves based on replicate sample serial dilutions (1:10K to 1:100B) of sequence-verified, double-stranded DNA fragments of the target species. 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 each of the PoN and MIC. 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 reaction (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).

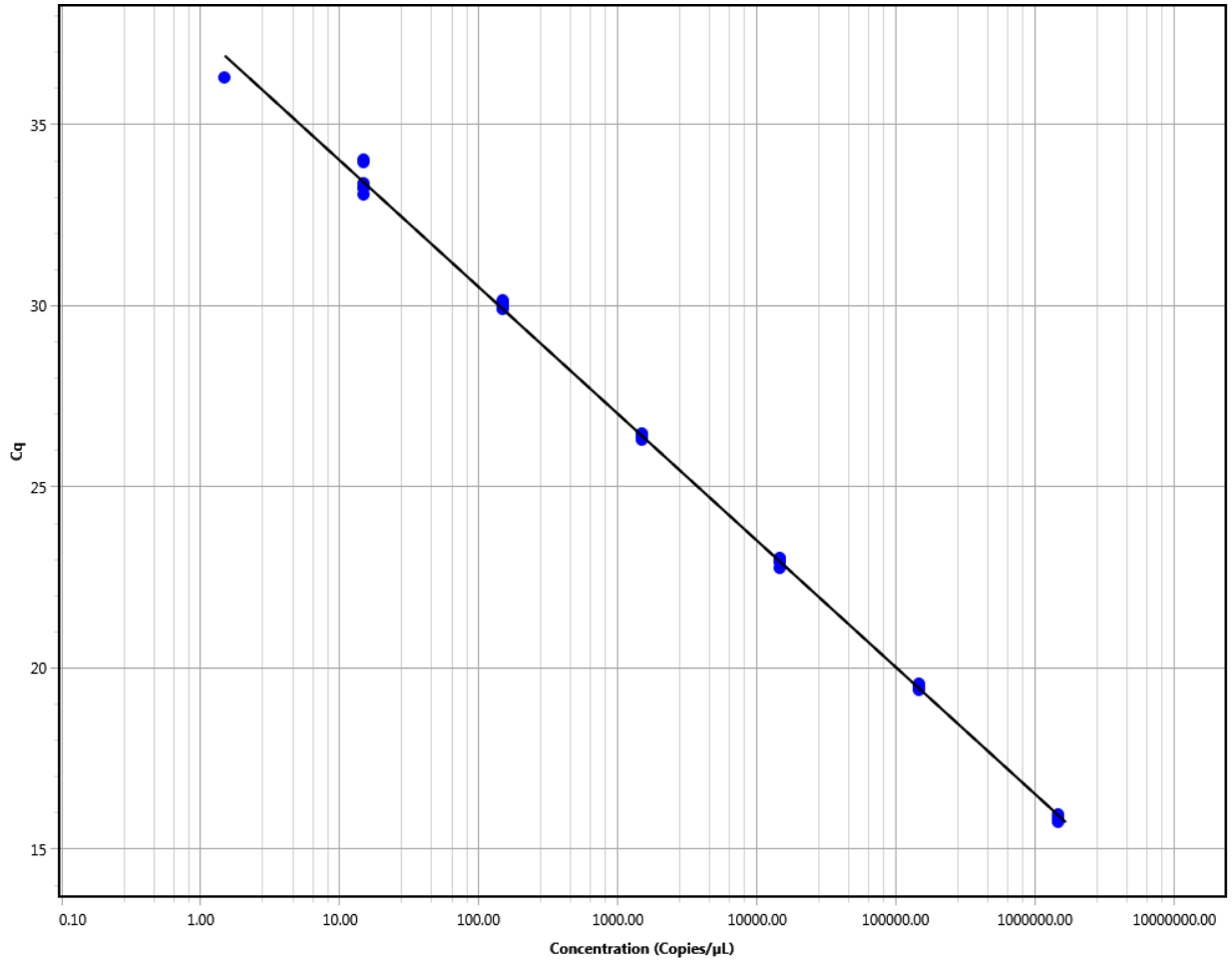

Figure 2 Standard curve of eDNA concentration versus number of DNA amplification cycles generated using the MIC thermal cycler with 6 replicates per dilution.

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 visual inspection of residual plots and boxplots, respectively. When necessary, data were transformed to satisfy these assumptions (Quinn and Keogh, 2002). 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 In Experiment 3, linear regressions were performed on $\log_{10}+2$ transformed eDNA concentration data versus log2 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).

3.0 RESULTS

Analyses of general chemistry and metals scan provided no direct evidence of inhibitory substances/conditions in Aquatron Pool 3 water (Maxxam Analytics, Rapid Chemical Analysis package; Appendix A). That said, the occurrence of false negative results and IPC curve data during initial sampling indicated PCR inhibition was occurring, despite this pool containing 223 striped bass. 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 runs functioned properly.

For all experiments, no striped bass DNA was detected in any negative controls during filtration or from NTC during PCR, indicating that there was no evidence of contamination. 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

Pool 3 contained all experimental animals and no technical replicates from MIC PCR produced false negatives. Across all samples, eDNA concentrations ranged from 4.36 – 399 DNA copies$\cdot\mu L^{-1}$ with a mean of 102.5 DNA copies$\cdot\mu L^{-1}$ (± 114.5 SD) while Cq ranged from 28.4 – 35.3 cycles with a mean of 31.6 cycles (± 2.1 SD). No significant differences in eDNA concentrations were observed among depths sampled ($F_{2,6}$ = 0.946, p = 0.439; Figure 3).

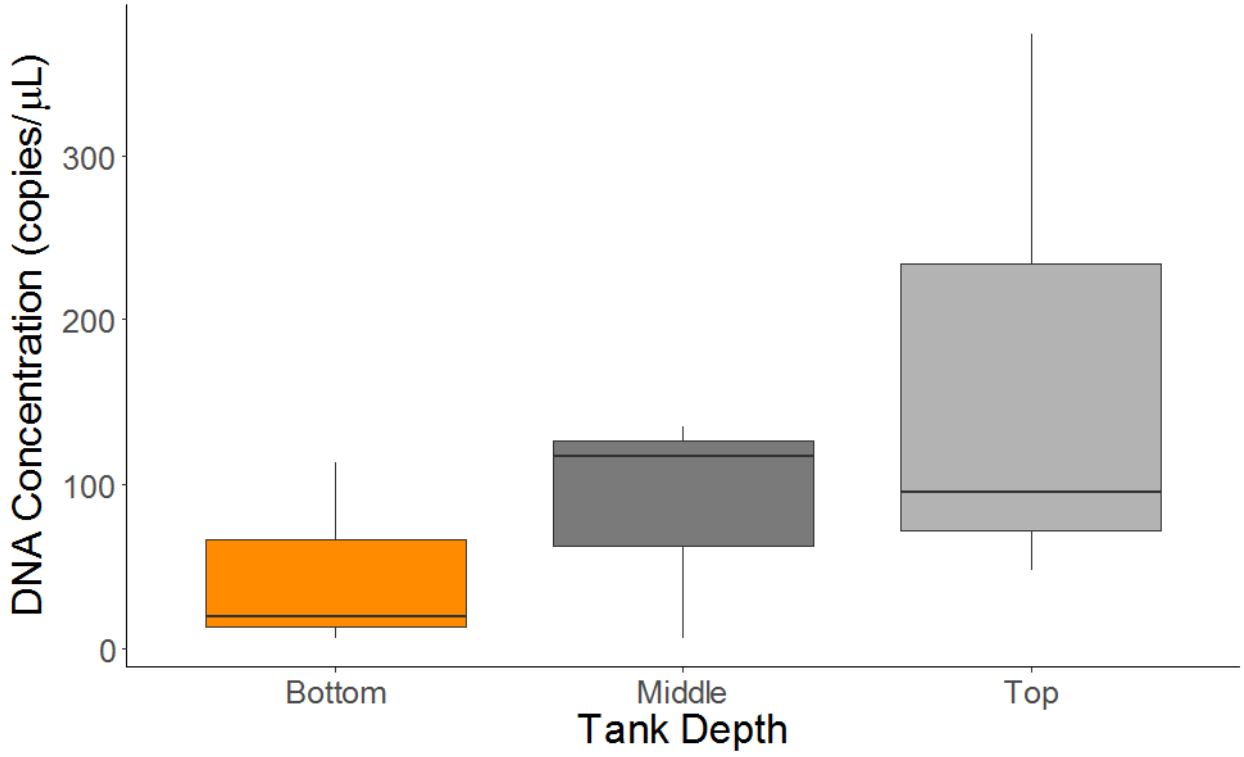

Figure 3 Boxplot of eDNA Concentrations by Tank Depths - MIC Analyses

Notes: n = 3 for each tank depth. The centre line is the median. Ends of the box indicate the lower and upper quartiles. Ends of the whiskers indicate the quartile ± 1.5 x interquartile spread. Asterisks, if present, would indicate values falling within the quartile ± 3 x interquartile spread. Open circles, if present, would indicate values falling outside the quartile ± 3 x interquartile spread.

Using the PoN, three false negatives were detected – one from each of top, middle, and bottom samples. Across all samples, eDNA concentrations with the PoN ranged from 0 – 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 – 35.9 cycles with a mean of 32.4 cycles (± 2.1 SD). No significant differences in eDNA concentrations were observed among depths sampled ($F_{2,6}$ = 0.527, p = 0.616; Figure 4).

Absolute eDNA concentration estimates for MIC samples (Figure 3) were approximately an order of magnitude greater than those observed with the PoN (Figure 4). However, Cq values were also conserved across both systems, giving some indication of comparable performance. Despite this differential in eDNA concentration estimates, both systems provided similar observed relative trends among sample depths as well as the same statistical conclusions.

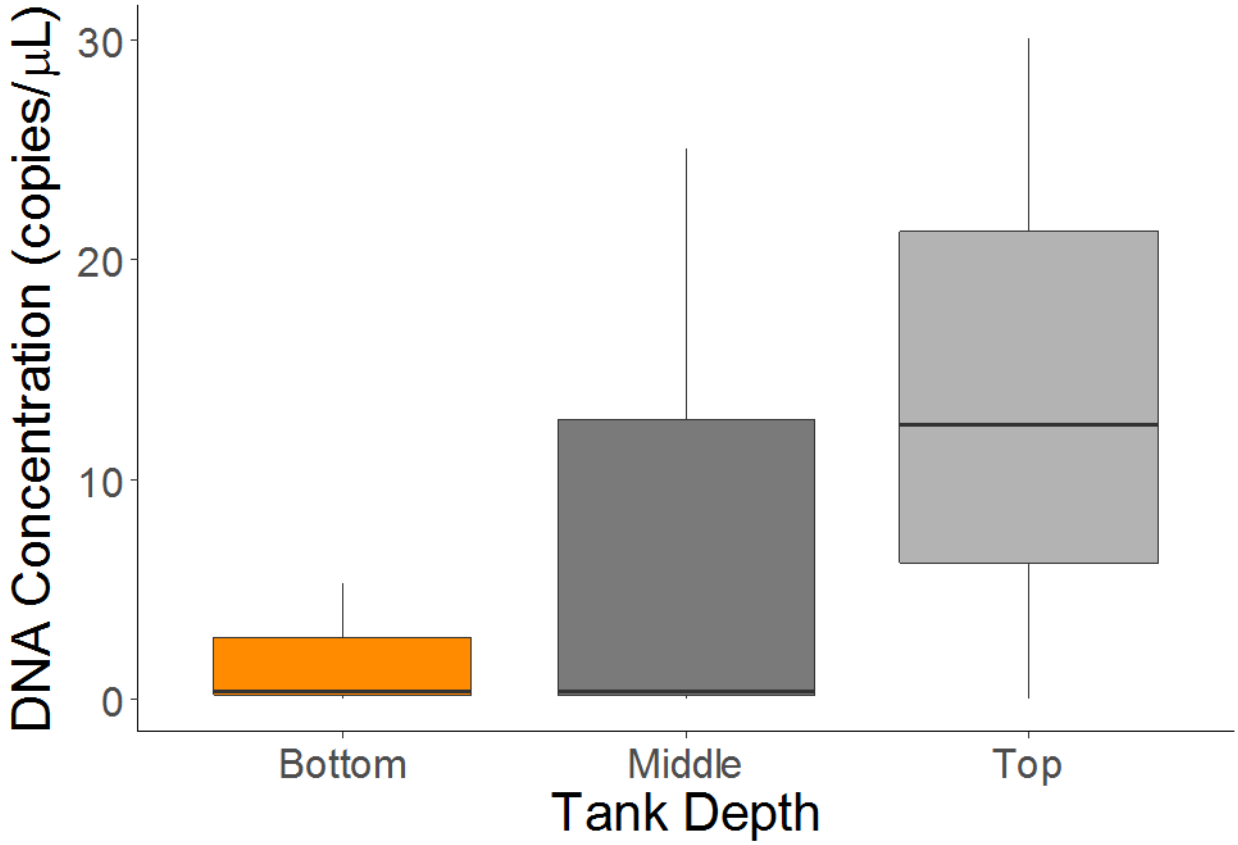

Figure 4 Boxplot of eDNA Concentrations by Tank Depths - PoN Analyses

Notes: n = 3 for each tank depth. The centre line is the median. Ends of the box indicate the lower and upper quartiles. Ends of the whiskers indicate the quartile ± 1.5 x interquartile spread. Asterisks, if present, would indicate values falling within the quartile ± 3 x interquartile spread. Open circles, if present, would indicate values falling outside the quartile ± 3 x interquartile spread.

3.2 EXPERIMENT 2 – PERSISTENCE

Temporal trends in MIC-derived eDNA concentrations showed a consistently detectable signal for the initial 24-hour examination period after which no eDNA was detected in any subsequent samples from 48 – 120 hours (Figure 5). Of note, at least one technical replicate for each sample within each time period from 0 – 24 hours resulted in non-detectable eDNA.

The first evidence of complete signal loss (with all three technical replicates from a sample non-detectable) occurred at 24 hours (Table 1). Within the 0 – 12-hour 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-hour samples as compared to initial samples at 0 hours (Figure 5), though this result was likely a function of the low eDNA concentration (0.68 copies·µL^{-1}) in one of the 0-hour samples biasing the median value (based on three samples) for that group lower.

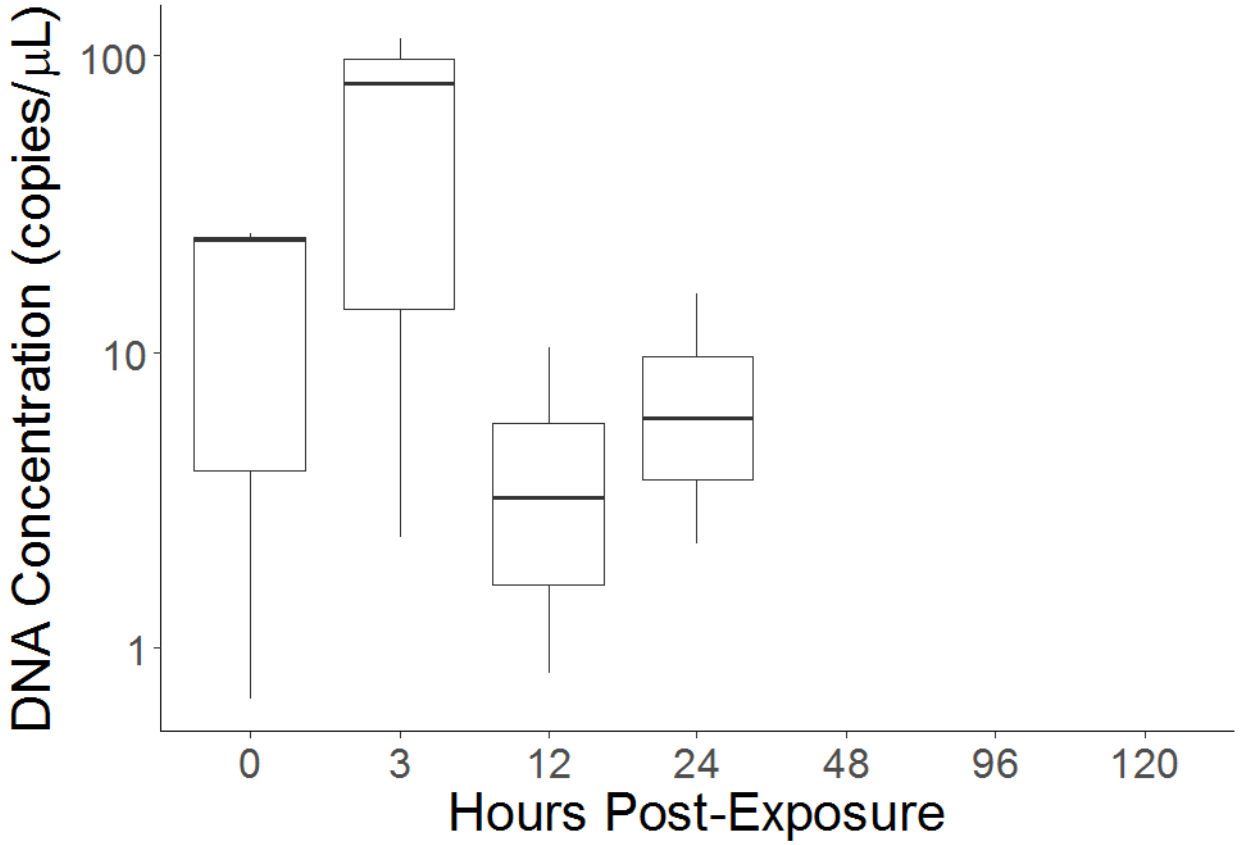

Figure 5 Boxplot of Temporal Trends in eDNA Concentrations - MIC Analyses

Notes: Y-axis presented in $\log_{10}$ scale. n = 3 for each time. The centre line is the median. Ends of the box indicate the lower and upper quartiles. Ends of the whiskers indicate the quartile ± 1.5 x interquartile spread. Asterisks, if present, would indicate values falling within the quartile ± 3 x interquartile spread. Open circles, if present, would indicate values falling outside the quartile ± 3 x interquartile spread.

Table 1 eDNA Concentrations Time Series Data (MIC and PoN)

Time (hours)	eDNA Concentrations (copies·µL^{-1})	
	MIC	PoN
0	25.34	39.03
0	23.88	64.29
0	0.68	nd
3	81.86	33.95
3	2.38	0.55
3	115.63	53.83
12	3.24	nd
12	0.83	0.09
12	10.37	0.93

Table 1 eDNA Concentrations Time Series Data (MIC and PoN)

Time (hours)	eDNA Concentrations (copies·μL^{-1})	
	MIC	PoN
24	nd	nd
24	15.81	1.21
24	2.29	0.01
48	nd	nd
48	nd	nd
48	nd	nd
96	nd	nd
96	nd	nd
96	nd	nd
120	nd	nd
120	nd	nd
120	nd	nd

Notes: Grey 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.

A similar trend was observed in PoN-derived data (Figure 6) which also showed a detectable signal for the initial 24-hour examination period after which no eDNA was detected in any subsequent samples from 48 – 120 hours, with progressively declines in eDNA concentration from 0 – 24 hours (Figure 6). In contrast to MIC-derived data, at least one of three samples within each sampling event from 0 – 24 hours 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. As previously stated, these varying eDNA concentration estimates were most likely a function of the variability between the development of their standard curves. The main finding of Experiment 2 was that regardless of the eDNA platform used, the decay signal indicated that water samples collected within a 24-hour period of exposure to striped bass reliably detected eDNA, with this signal diminishing substantially after a 48-hour period, after which there was no detectable eDNA signal using either method.

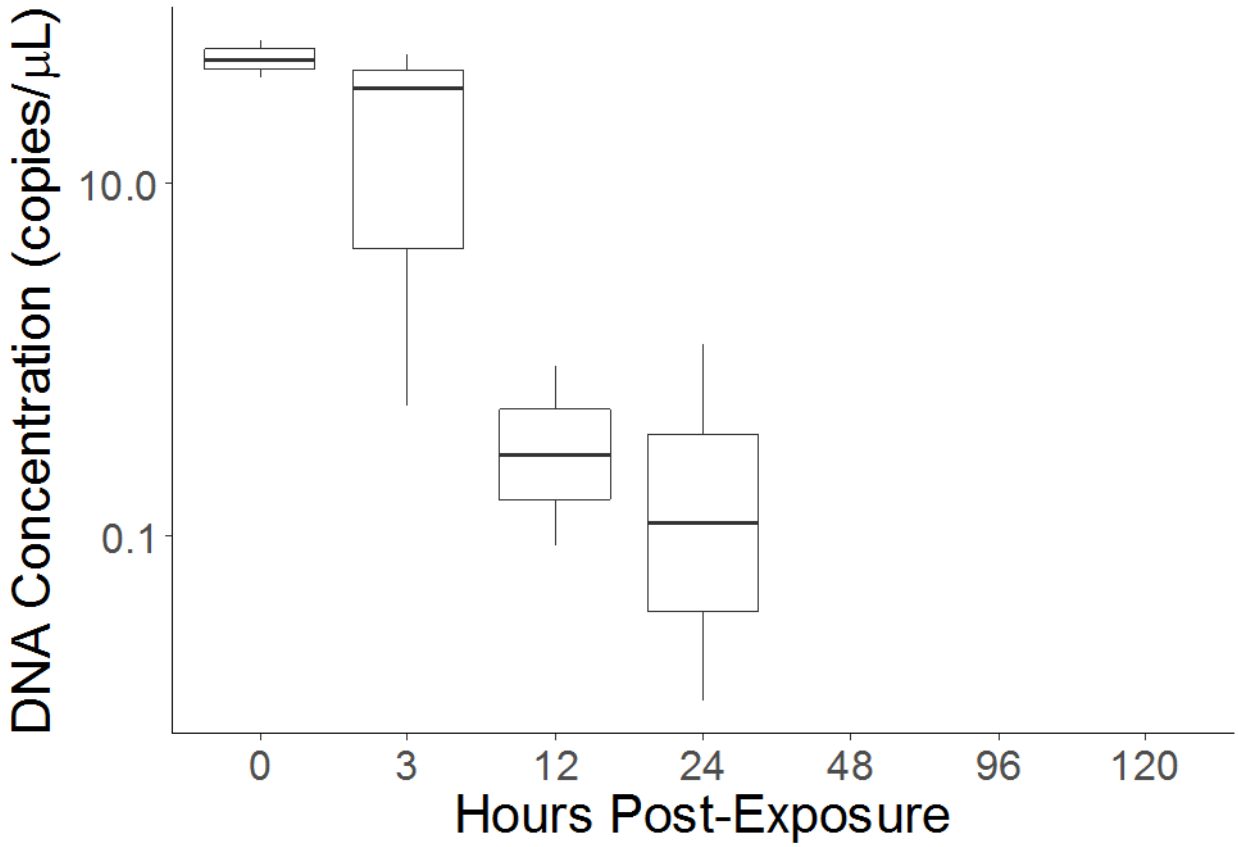

Figure 6 Boxplot of Temporal Trends in eDNA Concentrations (PoN Analyses)

Notes: Y-axis presented in $\log_{10}$ scale. n = 3 for each time. The centre line is the median. Ends of the box indicate the lower and upper quartiles. Ends of the whiskers indicate the quartile ± 1.5 x interquartile spread. Asterisks, if present, would indicate values falling within the quartile ± 3 x interquartile spread. Open circles, if present, would indicate values falling outside the quartile ± 3 x interquartile spread.

3.3 EXPERIMENT 3 – QUANTIFICATION

The linear relationship between eDNA concentrations and manipulated striped bass stocking densities was highly significant and positive ($r^2_{adjusted}$ = 0.225; $F_{1,105}$ = 31.73, p < 0.001; Figure 7) for MIC samples. No significant differences were found in the intercepts through time (p > 0.05) and no significant differences were found between linear models with or without the temporal covariate ($F_{4,85}$ = 1.395, p = 0.409). Trends in the data indicated maximum eDNA concentrations were reached at a density of 26 individuals and plateaued regardless of subsequent increases in stocking density. Interestingly, eDNA concentrations for intermediate stocking densities (5, 26, or 58 individuals) were more variable than either of the lowest (1 or 2 individuals) or highest stocking densities (139 or 223 individuals; Figure 7). False negative results were noted in 10% of samples; with 80% of these occurring in the three lowest density treatments (1, 2, 5 individuals).

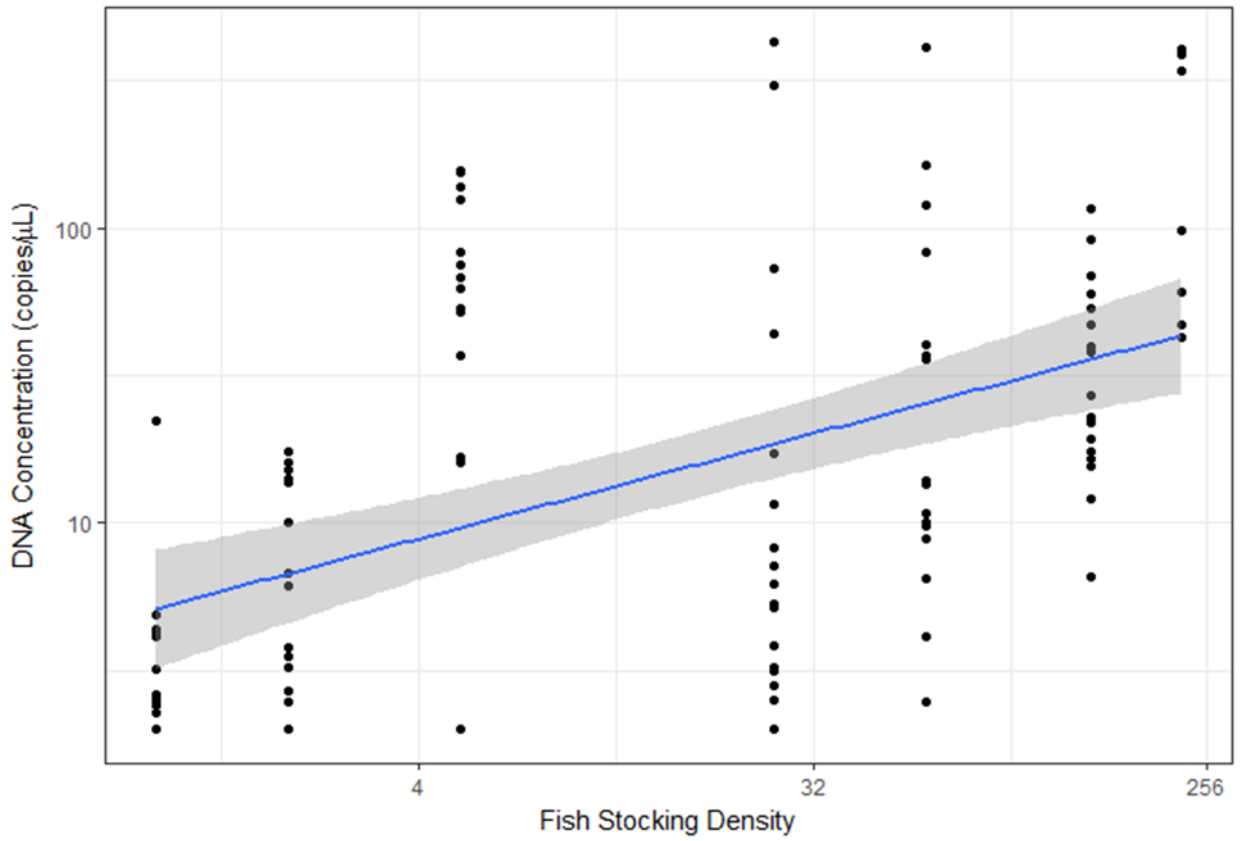

Figure 7 Relationship between eDNA Concentrations and Striped Bass Stocking Density - MIC Analyses

Notes: Y-axis presented in $\log_{10}$ scale, x-axis presented in $\log_2$ scale. Blue line equals regression line (± 95% confidence intervals). eDNA concentrations from Experiment 1 (stocking density = 223 fish) added to dataset.

Similar to the MIC results, the linear relationship between eDNA concentrations and manipulated striped bass stocking densities for the PoN was significant and weakly positive ($r^2_{adjusted}$ = 0.03; $F_{1,103}$ = 4.67, p = 0.033; Figure 8). Trends in these data also indicated maximum eDNA concentrations were reached at a density of 26 individuals and plateaued regardless of subsequent increases in stocking density up to 139 individuals (Figure 8). The addition of data from Experiment 1 for the 223 individual stocking density served to mute the observed linear relationship (Figure 8), in contrast to observed trends for the MIC data. Regardless, these data were still retained in the PoN analyses for the sake of comparison to the MIC results. It is also important to note the differential in eDNA concentration estimates between the MIC and PoN observed in Experiment 1 appears to be repeated for the higher stocking density of 139 individuals (Figure 8).

As was noted for the MIC, 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 8). However, no reduction in variability was noted for the 139 individuals stocking densities (Figure 8), as had been observed for the MIC data (Figure 7).

The main finding of Experiment 3 was that 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).

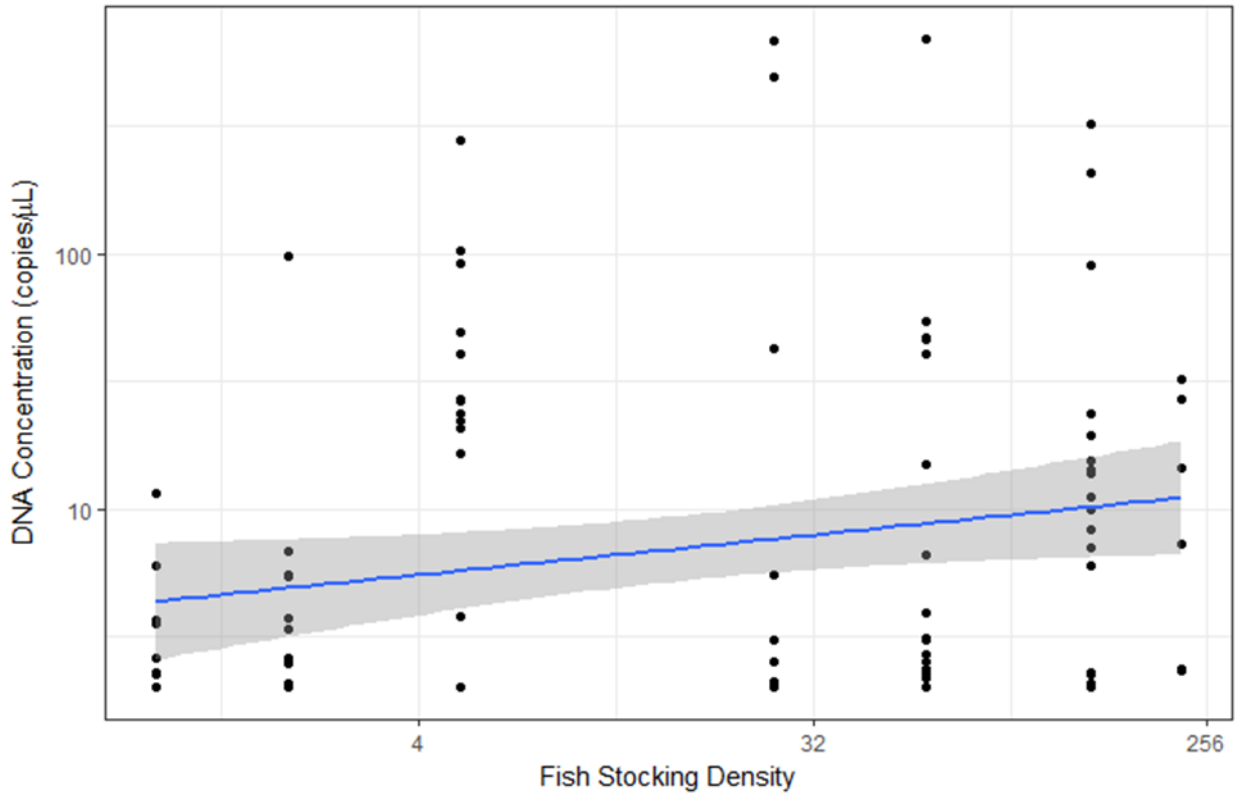

Figure 8 Relationship between eDNA Concentrations and Striped Bass Stocking Density (MIC Analyses)

Notes: Y-axis presented in $\log_{10}$ scale, x-axis presented in $\log_2$ scale. Blue line equals regression line (± 95% confidence intervals). eDNA concentrations from Experiment 1 (stocking density = 223 fish) added to dataset.

4.0 CONCLUSIONS

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 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 of freshwater systems; and by inhibition of molecular procedures by high salinity environments (Díaz-Ferguson & Moyer, 2014). Despite some of these challenges, however, eDNA tools have been successfully used in the marine environment for 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, species-specific primers to target eDNA detection of striped bass were effective and performed with expected specificity and sensitivity. Additionally, these 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 common 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 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%) vs. the MIC (4.42%), these results could indicate lower performance by the PoN. 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 300m^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). These observations of eDNA concentration estimates aside, 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.

The main finding of Experiment 2 was that regardless of the eDNA platform used, the decay signal indicated that water samples collected within a 24-hour period of exposure to striped bass eDNA was reliably detectable and that this signal diminished substantially after a 48-hour period, after which there was no detectable eDNA signal using either method. 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 environments like the Bay of Fundy, particularly the Minas Passage. While our experimental results provide promise of the field utility for eDNA surveys in the BoF, more research is required for tool validation before deployment in such macrotidal, sediment-laden environments.

As hypothesized, significant and positive linear relationships between eDNA concentrations and manipulated striped bass stocking densities were observed for both the laboratory-based and PoN platforms. These 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 and 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 reactions 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.

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 towards complementing previous and ongoing marine tidal energy monitoring efforts while simultaneously increasing the rigour 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 eDNA tools to address monitoring and regulatory challenges faced by marine tidal energy proponents in Atlantic Canada. Other marine industrial proponents, particularly in the oil and gas sector, are also recognizing the potential of eDNA to address environmental management needs and many industry-led research programs are already underway to validate these tools relative to more traditional methods.

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.

5.0 RECOMMENDATIONS

Based on the results of the current study, we provide the following recommendations:

- Our results provide a demonstrable positive relationship between eDNA capture/quantification and fish density. These relationships have not been tested in remote or challenging regions to access and sample, 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 setting such as the Aquatron to confirm the ability to detect and develop empirical relationships for relative abundance of striped bass.
- If the above tests are successfully completed, the efficacy of the striped bass primers 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 more traditional methods. Annual estimations of striped bass densities using traditional fish trapping and acoustic tracking are conducted by DFO in areas of intensive striped bass spawning, such as in the Stewiacke River.
- Given the high-energy environment of the Bay of Fundy, estimates of the transport potential of eDNA particles in the water column are required and this may be possible using existing data collected by marine tidal industry proponents. Additionally, there is high potential to build upon the species-specific movement and behavioural acoustic tracking research conducted by Acadia University. Such results would permit estimation of the provenance of eDNA detected in a given study area to help with interpretation of data and gain confidence regarding potential species distribution in the field.

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APPENDIX A

Maxxam Analytics Water Sample Report

Maxxam
A Bureau Veritas Group Company

Your Project #: 121415636
Site Location: DAL
Your C.O.C. #: D39163

Attention: Marc Skinner

Stantec Consulting Ltd
40 Highfield Park Drive
Suite 102
Dartmouth, NS
CANADA B3A 0A3

Report Date: 2018/09/07
Report #: R5389974
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B8M9161
Received: 2018/09/05, 10:34

Sample Matrix: Water
Samples Received: 3

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Reference
Carbonate, Bicarbonate and Hydroxide	3	N/A	2018/09/06	N/A	SM 22 4500-CO2 D
Alkalinity	3	N/A	2018/09/05	ATL SOP 00013	EPA 310.2 R1974 m
Chloride	3	N/A	2018/09/05	ATL SOP 00014	SM 23 4500-Cl- E m
Colour	3	N/A	2018/09/06	ATL SOP 00020	SM 23 2120C m
Conductance - water	3	N/A	2018/09/06	ATL SOP 00004	SM 23 2510B m
Hardness (calculated as CaCO3)	3	N/A	2018/09/07	ATL SOP 00048	Auto Calc
Metals Water Total MS	3	2018/09/06	2018/09/07	ATL SOP 00058	EPA 6020A R1 m
Ion Balance (% Difference)	3	N/A	2018/09/07	N/A	Auto Calc.
Anion and Cation Sum	3	N/A	2018/09/07	N/A	Auto Calc.
Nitrogen Ammonia - water	3	N/A	2018/09/05	ATL SOP 00015	EPA 350.1 R2 m
Nitrogen - Nitrate + Nitrite	3	N/A	2018/09/06	ATL SOP 00016	USGS I-2547-11m
Nitrogen - Nitrite	3	N/A	2018/09/06	ATL SOP 00017	SM 23 4500-NO2- B m
Nitrogen - Nitrate (as N)	3	N/A	2018/09/07	ATL SOP 00018	ASTM D3867-16
pH (1)	3	N/A	2018/09/06	ATL SOP 00003	SM 23 4500-H+ B m
Phosphorus - ortho	3	N/A	2018/09/06	ATL SOP 00021	SM 23 4500-P E m
Sat. pH and Langelier Index (@ 20C)	3	N/A	2018/09/07	ATL SOP 00049	Auto Calc.
Sat. pH and Langelier Index (@ 4C)	3	N/A	2018/09/07	ATL SOP 00049	Auto Calc.
Reactive Silica	3	N/A	2018/09/05	ATL SOP 00022	EPA 366.0 m
Sulphate	3	N/A	2018/09/06	ATL SOP 00023	ASTM D516-16 m
Total Dissolved Solids (TDS calc)	3	N/A	2018/09/07	N/A	Auto Calc.
Organic carbon - Total (TOC) (2)	3	N/A	2018/09/06	ATL SOP 00203	SM 23 5310B m
Turbidity	3	N/A	2018/09/06	ATL SOP 00011	EPA 180.1 R2 m

Remarks:

Maxxam Analytics' laboratories are accredited to ISO/IEC 17025:2005 for specific parameters on scopes of accreditation. Unless otherwise noted, procedures used by Maxxam are based upon recognized Provincial, Federal or US method compendia such as CCME, MDDELCC, EPA, APHA.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by professionals in Maxxam's profession using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Maxxam in writing). All data is in statistical control and has met quality control and method performance criteria unless otherwise noted. All method blanks are reported; unless indicated otherwise, associated sample data are not blank corrected. Where applicable, unless otherwise noted, Measurement Uncertainty has not been

Your Project #: 121415636
Site Location: DAL
Your C.O.C. #: D39163

Attention: Marc Skinner

Stantec Consulting Ltd
40 Highfield Park Drive
Suite 102
Dartmouth, NS
CANADA B3A 0A3

Report Date: 2018/09/07
Report #: R5389974
Version: 1 - Final

CERTIFICATE OF ANALYSIS

MAXXAM JOB #: B8M9161
Received: 2018/09/05, 10:34

accounted for when stating conformity to the referenced standard.

Maxxam Analytics' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Maxxam has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Maxxam, unless otherwise agreed in writing. Maxxam is not responsible for the accuracy or any data impacts, that result from the information provided by the customer or their agent.

Solid sample results, except biota, are based on dry weight unless otherwise indicated. Organic analyses are not recovery corrected except for isotope dilution methods.
Results relate to samples tested. When sampling is not conducted by Maxxam, results relate to the supplied samples tested.
This Certificate shall not be reproduced except in full, without the written approval of the laboratory.

Reference Method suffix “m” indicates test methods incorporate validated modifications from specific reference methods to improve performance.

* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

(1) The APHA Standard Method require pH to be analyzed within 15 minutes of sampling and therefore field analysis is required for compliance. All Laboratory pH analyses in this report are reported past the APHA Standard Method holding time.
(2) TOC / DOC present in the sample should be considered as non-purgeable TOC / DOC.

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.
Marie Muise, Key Account Specialist
Email: MMuise@maxxam.ca
Phone# (902)420-0203 Ext:253

==========

This report has been generated and distributed using a secure automated process.
Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

Maxxam Job #: B8M9161
Report Date: 2018/09/07

Stantec Consulting Ltd
Client Project #: 121415636
Site Location: DAL
Sampler Initials: MS

ATLANTIC RCAP-MS TOTAL METALS IN WATER (WATER)

Maxxam ID		HQR349			HQR350		HQR351		
Sampling Date		2018/09/05 09:00			2018/09/05 09:10		2018/09/05 09:15		
COC Number		D39163			D39163		D39163		
	UNITS	**1 TOP**	**RDL**	**QC Batch**	**1 MID**	**QC Batch**	**1 BOT**	**RDL**	**QC Batch**
Calculated Parameters									
Anion Sum	me/L	503	N/A	5714273	497	5714273	508	N/A	5714273
Bicarb. Alkalinity (calc. as CaCO3)	mg/L	94	1.0	5714267	95	5714267	95	1.0	5714267
Calculated TDS	mg/L	30000	1.0	5714284	29000	5714284	30000	1.0	5714284
Carb. Alkalinity (calc. as CaCO3)	mg/L	<1.0	1.0	5714267	<1.0	5714267	<1.0	1.0	5714267
Cation Sum	me/L	522	N/A	5714273	530	5714273	531	N/A	5714273
Hardness (CaCO3)	mg/L	5500	1.0	5714269	5600	5714269	5600	1.0	5714269
Ion Balance (% Difference)	%	1.85	N/A	5714271	3.21	5714271	2.18	N/A	5714271
Langelier Index (@ 20C)	N/A	0.0840	N/A	5714281	0.156	5714281	0.134	N/A	5714281
Langelier Index (@ 4C)	N/A	-0.155	N/A	5714282	-0.0830	5714282	-0.105	N/A	5714282
Nitrate (N)	mg/L	0.39	0.050	5714275	0.36	5714275	0.36	0.050	5714275
Saturation pH (@ 20C)	N/A	7.30	N/A	5714281	7.28	5714281	7.29	N/A	5714281
Saturation pH (@ 4C)	N/A	7.54	N/A	5714282	7.52	5714282	7.53	N/A	5714282
Inorganics									
Total Alkalinity (Total as CaCO3)	mg/L	94	5.0	5712531	96	5712531	95	5.0	5712531
Dissolved Chloride (Cl-)	mg/L	16000	120	5712537	16000	5712537	16000	120	5712537
Colour	TCU	<5.0	5.0	5712558	<5.0	5712558	<5.0	5.0	5712558
Nitrate + Nitrite (N)	mg/L	0.43	0.050	5715201	0.40	5715201	0.40	0.050	5715201
Nitrite (N)	mg/L	0.033	0.010	5712565	0.035	5712565	0.034	0.010	5712565
Nitrogen (Ammonia Nitrogen)	mg/L	0.23	0.050	5712645	0.22	5712652	0.34	0.050	5712632
Total Organic Carbon (C)	mg/L	1.4	0.50	5716594	1.4	5716594	1.4	0.50	5716594
Orthophosphate (P)	mg/L	0.10	0.010	5715194	0.10	5715194	0.10	0.010	5715194
pH	pH	7.38	N/A	5716505	7.44	5716505	7.42	N/A	5716505
Reactive Silica (SiO2)	mg/L	0.51	0.50	5712549	0.51	5712549	0.50	0.50	5712549
Dissolved Sulphate (SO4)	mg/L	2300	60	5715190	2300	5715190	2300	60	5715190
Turbidity	NTU	0.44	0.10	5716573	0.24	5716573	0.44	0.10	5716573
Conductivity	uS/cm	46000	1.0	5716506	46000	5716506	46000	1.0	5716506
Metals									
Total Aluminum (Al)	ug/L	<50	50	5716727	<50	5716727	<50	50	5716727
Total Antimony (Sb)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727
Total Arsenic (As)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727
Total Barium (Ba)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727
Total Beryllium (Be)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
N/A = Not Applicable

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Maxxam Job #: B8M9161
Report Date: 2018/09/07

Stantec Consulting Ltd
Client Project #: 121415636
Site Location: DAL
Sampler Initials: MS

ATLANTIC RCAP-MS TOTAL METALS IN WATER (WATER)

Maxxam ID		HQR349			HQR350		HQR351		
Sampling Date		2018/09/05 09:00			2018/09/05 09:10		2018/09/05 09:15		
COC Number		D39163			D39163		D39163		
	UNITS	**1 TOP**	**RDL**	**QC Batch**	**1 MID**	**QC Batch**	**1 BOT**	**RDL**	**QC Batch**
Total Bismuth (Bi)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Boron (B)	ug/L	3900	500	5716727	4000	5716727	3900	500	5716727
Total Cadmium (Cd)	ug/L	0.11	0.10	5716727	<0.10	5716727	<0.10	0.10	5716727
Total Calcium (Ca)	ug/L	370000	1000	5716727	370000	5716727	370000	1000	5716727
Total Chromium (Cr)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727
Total Cobalt (Co)	ug/L	<4.0	4.0	5716727	<4.0	5716727	<4.0	4.0	5716727
Total Copper (Cu)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Iron (Fe)	ug/L	<500	500	5716727	<500	5716727	<500	500	5716727
Total Lead (Pb)	ug/L	<5.0	5.0	5716727	<5.0	5716727	<5.0	5.0	5716727
Total Magnesium (Mg)	ug/L	1100000	1000	5716727	1100000	5716727	1100000	10000	5716727
Total Manganese (Mn)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Molybdenum (Mo)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Nickel (Ni)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Phosphorus (P)	ug/L	<1000	1000	5716727	<1000	5716727	<1000	1000	5716727
Total Potassium (K)	ug/L	340000	1000	5716727	350000	5716727	340000	1000	5716727
Total Selenium (Se)	ug/L	<10	10	5716727	<10	5716727	<10	10	5716727
Total Silver (Ag)	ug/L	<1.0	1.0	5716727	<1.0	5716727	<1.0	1.0	5716727
Total Sodium (Na)	ug/L	9300000	1000	5716727	9400000	5716727	9400000	1000	5716727
Total Strontium (Sr)	ug/L	7200	20	5716727	7200	5716727	7100	20	5716727
Total Thallium (Tl)	ug/L	<1.0	1.0	5716727	<1.0	5716727	<1.0	1.0	5716727
Total Tin (Sn)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Titanium (Ti)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Uranium (U)	ug/L	2.6	1.0	5716727	2.6	5716727	2.6	1.0	5716727
Total Vanadium (V)	ug/L	<20	20	5716727	<20	5716727	<20	20	5716727
Total Zinc (Zn)	ug/L	<50	50	5716727	<50	5716727	<50	50	5716727

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	12.3°C

Sample HQR349 [1 TOP] : Elevated reporting limits for trace metals due to sample matrix.

Sample HQR350 [1 MID] : Elevated reporting limits for trace metals due to sample matrix.

Sample HQR351 [1 BOT] : Elevated reporting limits for trace metals due to sample matrix.

Results relate only to the items tested.

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Report Date: 2018/09/07

QUALITY ASSURANCE REPORT

Stantec Consulting Ltd
Client Project #: 121415636
Site Location: DAL
Sampler Initials: MS

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
5712531	Total Alkalinity (Total as CaCO3)	2018/09/05	NC	80 - 120	107	80 - 120	<5.0	mg/L	1.4 (1)	25		
5712537	Dissolved Chloride (Cl-)	2018/09/05	91	80 - 120	100	80 - 120	<1.0	mg/L	3.3	25	108	80 - 120
5712549	Reactive Silica (SiO2)	2018/09/06	NC	80 - 120	94	80 - 120	<0.50	mg/L	7.4	25		
5712558	Colour	2018/09/06			108	80 - 120	<5.0	TCU	NC	20		
5712565	Nitrite (N)	2018/09/06	82	80 - 120	98	80 - 120	<0.010	mg/L	NC	20		
5712632	Nitrogen (Ammonia Nitrogen)	2018/09/05	NC	80 - 120	97	80 - 120	<0.050	mg/L	1.3	20		
5712645	Nitrogen (Ammonia Nitrogen)	2018/09/05	97	80 - 120	97	80 - 120	<0.050	mg/L	4.5	20		
5712652	Nitrogen (Ammonia Nitrogen)	2018/09/05	96	80 - 120	101	80 - 120	<0.050	mg/L	NC	20		
5715190	Dissolved Sulphate (SO4)	2018/09/06	97	80 - 120	93	80 - 120	<2.0	mg/L	1.4	25		
5715194	Orthophosphate (P)	2018/09/06	95	80 - 120	98	80 - 120	<0.010	mg/L	NC	25		
5715201	Nitrate + Nitrite (N)	2018/09/06	98	80 - 120	97	80 - 120	<0.050	mg/L	0.11	25		
5716505	pH	2018/09/06							0.44	N/A	100	97 - 103
5716506	Conductivity	2018/09/06			100	80 - 120	<1.0	uS/cm	1.2	25		
5716573	Turbidity	2018/09/06			99	80 - 120	<0.10	NTU	NC	20	106	80 - 120
5716594	Total Organic Carbon (C)	2018/09/06	106	85 - 115	100	80 - 120	<0.50	mg/L	NC	15		
5716727	Total Aluminum (Al)	2018/09/07	98	80 - 120	99	80 - 120	<5.0	ug/L	NC	20		
5716727	Total Antimony (Sb)	2018/09/07	104	80 - 120	104	80 - 120	<1.0	ug/L				
5716727	Total Arsenic (As)	2018/09/07	98	80 - 120	97	80 - 120	<1.0	ug/L				
5716727	Total Barium (Ba)	2018/09/07	97	80 - 120	97	80 - 120	<1.0	ug/L				
5716727	Total Beryllium (Be)	2018/09/07	99	80 - 120	99	80 - 120	<1.0	ug/L				
5716727	Total Bismuth (Bi)	2018/09/07	104	80 - 120	104	80 - 120	<2.0	ug/L				
5716727	Total Boron (B)	2018/09/07	101	80 - 120	99	80 - 120	<50	ug/L				
5716727	Total Cadmium (Cd)	2018/09/07	100	80 - 120	99	80 - 120	<0.010	ug/L				
5716727	Total Calcium (Ca)	2018/09/07	104	80 - 120	103	80 - 120	<100	ug/L				
5716727	Total Chromium (Cr)	2018/09/07	98	80 - 120	97	80 - 120	<1.0	ug/L				
5716727	Total Cobalt (Co)	2018/09/07	98	80 - 120	97	80 - 120	<0.40	ug/L				
5716727	Total Copper (Cu)	2018/09/07	95	80 - 120	97	80 - 120	<2.0	ug/L				
5716727	Total Iron (Fe)	2018/09/07	100	80 - 120	100	80 - 120	<50	ug/L				
5716727	Total Lead (Pb)	2018/09/07	99	80 - 120	99	80 - 120	<0.50	ug/L				
5716727	Total Magnesium (Mg)	2018/09/07	101	80 - 120	101	80 - 120	<100	ug/L				
5716727	Total Manganese (Mn)	2018/09/07	99	80 - 120	100	80 - 120	<2.0	ug/L				

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QUALITY ASSURANCE REPORT(CONT'D)

Stantec Consulting Ltd
Client Project #: 121415636
Site Location: DAL
Sampler Initials: MS

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
5716727	Total Molybdenum (Mo)	2018/09/07	106	80 - 120	104	80 - 120	<2.0	ug/L				
5716727	Total Nickel (Ni)	2018/09/07	98	80 - 120	97	80 - 120	<2.0	ug/L				
5716727	Total Phosphorus (P)	2018/09/07	103	80 - 120	102	80 - 120	<100	ug/L				
5716727	Total Potassium (K)	2018/09/07	104	80 - 120	102	80 - 120	<100	ug/L				
5716727	Total Selenium (Se)	2018/09/07	98	80 - 120	97	80 - 120	<1.0	ug/L				
5716727	Total Silver (Ag)	2018/09/07	98	80 - 120	97	80 - 120	<0.10	ug/L				
5716727	Total Sodium (Na)	2018/09/07	97	80 - 120	97	80 - 120	<100	ug/L				
5716727	Total Strontium (Sr)	2018/09/07	104	80 - 120	104	80 - 120	<2.0	ug/L				
5716727	Total Thallium (Tl)	2018/09/07	105	80 - 120	103	80 - 120	<0.10	ug/L				
5716727	Total Tin (Sn)	2018/09/07	105	80 - 120	103	80 - 120	<2.0	ug/L				
5716727	Total Titanium (Ti)	2018/09/07	101	80 - 120	102	80 - 120	<2.0	ug/L				
5716727	Total Uranium (U)	2018/09/07	101	80 - 120	100	80 - 120	<0.10	ug/L				
5716727	Total Vanadium (V)	2018/09/07	99	80 - 120	99	80 - 120	<2.0	ug/L				
5716727	Total Zinc (Zn)	2018/09/07	98	80 - 120	97	80 - 120	<5.0	ug/L				

N/A = Not Applicable

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Matrix Spike: A sample to which a known amount of the analyte of interest has been added. Used to evaluate sample matrix interference.

QC Standard: A sample of known concentration prepared by an external agency under stringent conditions. Used as an independent check of method accuracy.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

NC (Matrix Spike): The recovery in the matrix spike was not calculated. The relative difference between the concentration in the parent sample and the spike amount was too small to permit a reliable recovery calculation (matrix spike concentration was less than the native sample concentration)

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (absolute difference <= 2x RDL).

(1) Elevated reporting limit due to sample matrix.

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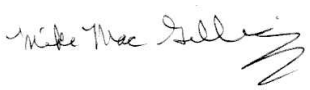
VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).



Mike MacGillivray, Scientific Specialist (Inorganics)

Maxxam has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per section 5.10.2 of ISO/IEC 17025:2005(E), signing the reports. For Service Group specific validation please refer to the Validation Signature Page.

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