Evaluating an Autonomous eDNA Sampler for Marine Environmental Monitoring: Short- and Long-Term Applications

Mallory Van Wyngaarden[1], Nicholas W. Jeffery[1*], Edward Horne[1], Rute B.G. Clemente-Carvalho[2], Matt Lemay[2], Iain Grundke[3], Eddy Luy[3], Andre Hendricks[3], Colin Sonnichsen[3], Vincent Sieben[3,4], Julie Laroche[3,5], Robert G. Beiko[3,6], Arnold Furlong[3], Tom Knox[3], Harri Pettitt-Wade[1], Ryan R.E. Stanley[1]

[1] Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

[2] Tula Foundation/Hakai Institute, Campbell River, British Columbia, Canada

[3] Dartmouth Ocean Technologies Inc, Dartmouth, Nova Scotia, Canada

[4] Department of Electrical & Computer Engineering, Dalhousie University, Halifax, Nova Scotia, Canada

[5] Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

[6] Faculty of Computer Science, Dalhousie University, Halifax, Nova Scotia, Canada

[*]Corresponding author: Nick.Jeffery@dfo-mpo.gc.ca

Abstract— **Monitoring biological diversity is essential for monitoring ecosystem health and evaluating conservation efforts. Environmental DNA metabarcoding has emerged as a powerful, scalable, and minimally invasive tool for assessing biodiversity. In this study, we evaluate the performance of an autonomous eDNA sampling platform deployed over a nine-week period and compare it with a traditional filter-at-sample (FAS) sampling protocol. Our results show that the autonomous sampling platform consistently captures and preserves DNA with comparable effectiveness to conventional in-situ filtering and preservation at -80 °C. Using two eDNA markers, we found that fish communities (12S marker) identified by both sampling methods largely overlapped, while invertebrate detections (COI marker) differed between methods, likely due to differences in filter specifications. These findings demonstrate that the autonomous samplers worked effectively in comparison to traditional methods, highlighting their potential to expand the temporal and spatial coverage of eDNA-based biodiversity monitoring. The ability of these samplers to facilitate long-term and continuous sampling in challenging environments shows promise for advancing eDNA applications in diverse and remote settings. Further research is needed to assess their performance in deeper waters and over extended periods, particularly to evaluate eDNA preservation at ambient ocean temperatures.**

Keywords—eDNA, autonomous sampling, oceans, biofouling, metabarcoding

I. Introduction

Biodiversity loss represents a key challenge to natural ecosystems, diminishing their resilience and the vital services they provide [1] [2]. In response, strategies such as Marine Protected Areas (MPAs) have been implemented globally as part of efforts to mitigate biodiversity loss and sustain ecosystem functions amidst evolving environmental dynamics and varied threats. The global expansion of MPAs highlights the increasing commitment to protecting marine life, yet it also underscores the need for effective monitoring technologies that can be deployed effectively and at scale [3]. Effective management and conservation of biodiversity require detailed information on the distribution of species, the threats they face, and how these dynamics are changing over time [4]. Comprehensive baseline ecological data is essential for detecting and attributing changes in ecological conditions to specific management actions [5]. The absence of such baseline data often hinders the proper assessment and management of protected areas, resulting in inefficient monitoring programs and unresolved ecological issues that still demand spatial and temporal management. Understanding the distribution of species within protected areas is crucial for informed decision-making related to zoning, risk assessment, and management effectiveness.

To achieve this, monitoring must be minimally invasive to protect the integrity of sensitive environments, particularly in areas with high biodiversity [6]. The deployment of advanced technologies that can be implemented at scale and provide comprehensive data on species diversity without the need for cumbersome or specialized equipment (e.g., large trawl gear for benthic marine diversity monitoring that requires commensurately large vessels for deployment) is essential. Such non-invasive methods ensure that information is gathered in alignment with the conservation goals of MPAs. For instance, to avoid disruptive techniques like trawling in sensitive areas that are managed to prevent such activities, thereby preserving the habitats aimed to be protected [7].

Environmental DNA (eDNA) has emerged as a promising tool for biodiversity monitoring that has been gaining increased attention in marine research [6] [8]. By sampling small volumes of water (~1 to 10+ liters), millions of eDNA fragments can be filtered and quantified by real-time PCR and sequenced on high-throughput platforms for metabarcoding. This approach relies on DNA barcoding principles, identifying species through unique DNA sequences at specific genetic markers using a reference library. eDNA metabarcoding is effective, efficient (e.g., able to assess both fish and invertebrate diversity, simultaneously), and non-invasive, avoiding disruption to habitats and resident animals. It has demonstrated comparable effectiveness to traditional biodiversity survey methods and can be used by researchers to rapidly conduct biodiversity surveys over relatively large areas [9-11].

Even with the promise of new approaches like eDNA for biodiversity, monitoring still presents a significant challenge, limiting the ability to conduct comprehensive sampling across various environments. In marine settings, this limitation is particularly pronounced, where access can be limited by the availability of specialized vessels to reach remote or deep-water areas. Consequently, surveys are often confined to specific seasons, providing a narrow temporal window for assessing biodiversity and ecological changes. These constraints hinder the ability of monitoring programs to gather temporally (e.g., seasonally) representative data, potentially leading to gaps in the ability to characterize the status of and changes in biodiversity.

Autonomous eDNA samplers offer a promising solution to temporally limited sampling, by enabling regular sampling without the need for frequent and costly trips on research vessels. These samplers can be programmed to collect and preserve multiple eDNA samples at defined intervals, with built-in sterilization between samples to prevent cross-contamination. Their ability to effectively sample and preserve eDNA is key to their utility. In this study, we conducted a test deployment of autonomous eDNA samplers developed by Dartmouth Ocean Technologies (DOT) [12]. We assessed the ability of the units to sample eDNA in an aquatic environment and preserve it under ambient conditions over a three-month period. We also compared eDNA metabarcoding results at two markers with seawater filtered using a conventional filter-at-sample system, commonly employed in marine and freshwater systems. This testing serves as the first step towards deploying these eDNA samplers in remote locations for biodiversity monitoring, addressing areas of conservation interest such as MPAs, where year-round access can be limited.

II. Materials and Methods

To assess the capabilities of autonomous samplers in the coastal ocean, we conducted a paired sampling trial using the DOT eDNA samplers (AUTO) and a conventional filter-at-sample (FAS) approach using a Smith-Root vacuum pump eDNA sampler. We deployed three battery-powered DOT eDNA samplers in the Bedford Basin (Nova Scotia, Canada) at a depth of approximately 5 metres from August to October 2023. Each system filtered a pre-programmed target volume of water (125mL) weekly over a 9-week period in the Bedford Basin (Nova Scotia, Canada, 44.684°N 63.614°W). Water was filtered using polycarbonate filters with 0.22 µm pores in the AUTO system and PES filters with 1.2µm pores for the FAS system following standard protocols [9]. For a more direct comparison, rather than filtering while submerged, we collected weekly seawater samples in a sterilized 5-gallon bucket to provide the same water source for both the AUTO samplers and the FAS samples. This seawater was then filtered by all three AUTO samplers and three FAS samples using the same water source while on board a boat. The samples from DOT samplers were automatically preserved within the unit using RNA*later*, while the FAS filters are self-preserving [13] but were stored at –80°C until extraction. A field control, consisting of one litre of deionized water, was also filtered using the Smith-Root sampler. Photographs of each unit, including the inlet and outlet ports, were taken each week to assess biofouling and potential clogging issues, testing the unit's ability to sample in the nearshore photic environment where biofouling is expected. The autonomous samplers were then redeployed from the same boat until sampling in subsequent weeks.

After nine weeks of sampling, the paired AUTO (n=27) and FAS (n=20) filters were processed at the Hakai Institute, British Columbia. All DNA extractions were carried out in a clean room dedicated to processing eDNA samples. Each filter was cut into small pieces and placed in a 1.5 mL tube containing 720 µL buffer ATL (Qiagen) and 80 µL proteinase K. Between each sample, the workstation and tools were cleaned with 10% bleach. Samples were placed tubes and incubated at 56°C in a centrifuge (~900 rpm) for digestion overnight. After incubation, the lysate was transferred to QIAShredder spin columns (Qiagen) and centrifuged for 2 min at maximum speed. The flowthrough was transferred to a clean 2 mL tube, and further DNA extraction steps followed Qiagen Blood and Tissue kit protocol without modifications. Samples and negative controls (field and laboratory blanks) were processed alongside each batch of samples.

We used universal metabarcoding primers to generate a library of sequences for identifying fish species (12S rRNA gene, MiFish-U) [14] and general invertebrate diversity (COI gene) [15]. The full library preparation protocols can be viewed at protocols.io (12srRNA - dx.doi.org/10.17504/protocols.io.n2bvjnn25gk5/v1, and COI - dx.doi.org/10.17504/protocols.io.261ge5d7yg47/v1). The final libraries were quantified using the NEBNext

Library quant kit for Illumina (NEB) and quality checked on Qiaxcel (Qiagen) using the DNA High-Sensitivity kit. Libraries were sequenced at the Hakai Institute, Quadra Island, BC, using the Illumina MiSeq V3 600 cycle chemistry.

All sequences were demultiplexed and primers trimmed in QIIME2 v. 2023.9 [16]. Low-quality sequences were removed and denoised using the dada2 plugin [17]. The 12S data was truncated to 134bp while the COI data was truncated to 240bp. An ASV table was generated for each marker and exported from QIIME2. Denoised ASVs were assigned taxonomy using BLASTn v.2.14. ASVs with reads representing <1% of the reads within a sample were removed, as well as ASVs with a percent sequence match <98%, and terrestrial and freshwater organisms were also removed. If reads were detected in the lab or field blanks, we removed that same number of reads per ASV for each sample. Our filtered data thus focused on marine and diadromous/brackish fishes and invertebrates.

Our evaluation of the autonomous sampling units focused on four key areas: biofouling, DNA yield, species richness, and species composition. To assess biofouling, we examined the amount of build-up on the intake filters and monitored any changes in sample volume over time, which could indicate clogging or other sampling issues. For DNA yield, we standardized each yield value by dividing it by the maximum observed yield within each replicate and method. We then used a linear model to evaluate whether there was a significant difference in DNA yield between the autonomous units and the conventional FAS method over time. Specifically, we tested whether the slopes of DNA yield versus duration differed between the two methods, with divergent slopes indicating a significant effect of the sampling method on DNA yield.

In addition to yield, we compared species richness by counting the total number of species detected by each sampling method. This comparison aimed to determine whether the autonomous samplers could capture as many species as the conventional method and how that method varied amongst taxonomic groups (invertebrates and fishes) and marker. Species richness analyses were conducted using the *vegan* package in R [18]. We used non-metric multidimensional scaling (nMDS) analyses to explore differences in species composition between the two methods based on Euclidean (presence/absence) and Bray-Curtis dissimilarity for both markers. To evaluate differences in species composition between Autonomous and FAS sampling methods and across sequential weeks, we performed a permutational multivariate analysis of variance (PERMANOVA) using the *vegan* package. These analyses provided insights into the utility of the autonomous samplers for detecting changes in species composition over the study period, particularly in identifying transitions in species presence and abundance.

All analyses were conducted in R v. 4.4.0 with data visualizations generated using the *ggplot2* package [19]. Species detection summary and DNA yield data, code, and associated outputs are available on GitHub [20]. All raw 12S and COI fastq files can be found in the NCBI Sequence Read Archive PRJNA1139183.

III. Results

A. Biofouling

The copper mesh pre-filter on the inlet spigot remained free of any biofouling across the nine-week period (Fig. 1). The samplers did accumulate biofouling from colonial tunicates and bryozoans, primarily on the top of the samplers amongst the filter housings. A number of organisms also were found within the reagent housing area of the units, including rock gunnels (*Pholis* sp.), sea stars (*Asterias* spp.), and invasive European green crab (*Carcinus maenas*). Throughout the study period, all autonomous samplers filtered their programmed volumes of 125 mL and did not experience issues such as a loss of pressure from biofouling.

Fig. 1. Inlet filter of one autonomous eDNA sampler (Dartmouth Ocean Technologies, Canada) after being submerged in the Bedford Basin at weeks 2, 5, and 9 during the experiment. The centre of the inlet contains a copper mesh screen to filter out large particles and prevent biofouling. Over the duration of the experiment the inlet screening did not experience any biofouling, but did develop a patina that could potentially clog the inlet over long periods.

B. DNA Preservation

The linear model analysis suggested a general negative association between duration and standardized DNA yield; however, this trend was not statistically significant ($p > 0.05$, Fig. 2). Similarly, paired differences in DNA yield over time also revealed no significant trend. Importantly, there was no significant difference in the slopes of DNA yield versus duration between the two sampling methods, indicating that both methods exhibited similar trends in DNA yield over time. This lack of significant interaction effect suggests that the

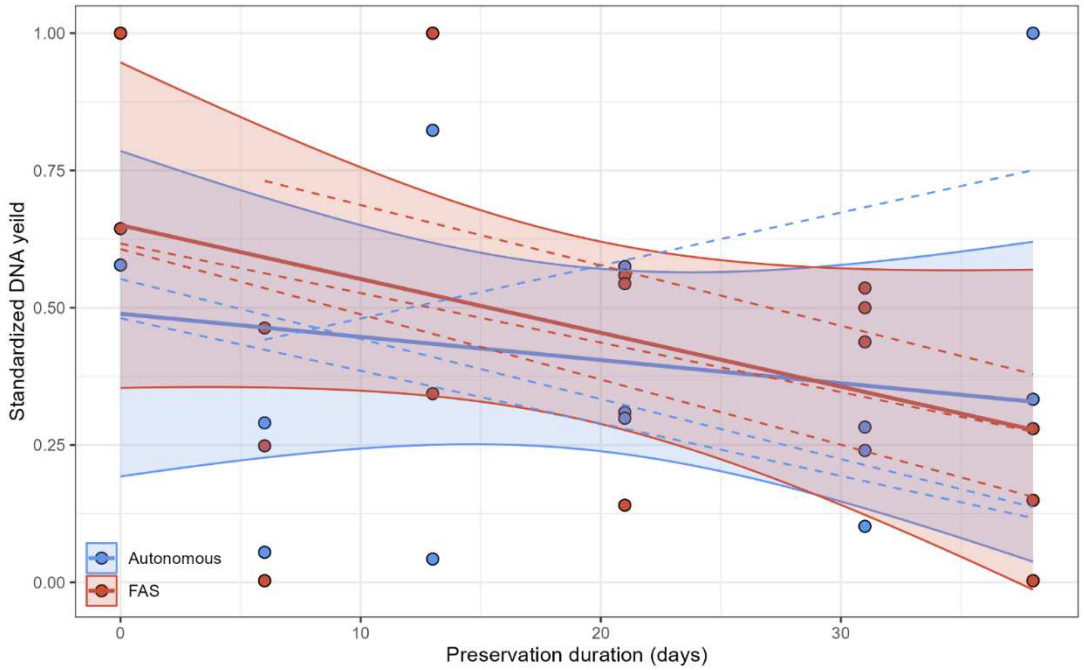

Fig. 2. Trends in DNA extraction yield over time, comparing samples preserved in RNA*later* by the autonomous samplers and those preserved at -80°C after filtering at the time of sample (FAS).

autonomous units provided suitable DNA preservation with RNA*later* in comparison to filtered samples stored at -80°, maintaining the integrity of DNA yield comparable to the conventional method. Despite the observed variability in the slopes among replicates within each method, the overall trends were consistent, reinforcing the reliability of the autonomous samplers in capturing changes in DNA yield over the study period.

C. Species Richness

The eDNA extraction and PCR negatives included low read counts of species not expected in the Atlantic Ocean, and these were removed as suspected contamination. Similar to DNA yield, species richness generally increased throughout the sampling period; however, this trend was not statistically significant and did not differ between sampling methods ($p > 0.05$). Vertebrate species richness, as revealed by the 12S marker, averaged 7.7 species per sample (± 2.55 SD) and did not differ significantly between methods. Although the total number of species detected with the 12S marker was consistent across methods, they shared an average of 41.1% of detected species (± 12.4% SD). Invertebrate species richness associated with the CO1 marker was on average 61% higher in FAS samples (14.4 ± 3.52 species per sample) compared to the autonomous filter (7.67 ± 2.80 species per sample). Similar to the 12S marker, the CO1 marker showed that, on average, 44% of detected species (± 8.6%) were shared among methods (Fig. 3).

During the test period, the total number of species detected was higher with the autonomous sampler using the 12S MiFish marker (29) compared to the FAS method (23). Conversely, the FAS method detected more species with the CO1 marker (35) compared to the autonomous sampler (28). Extrapolated species richness estimates for each sampling method, effort, and period showed that the autonomous sampler was associated with a larger estimated species pool than the FAS method for both markers: 30.72 ± 2.12 versus 26.96 ± 4.66 for the 12S marker, and 43.41 ± 15.9 versus 39.27 ± 4.59 for the CO1 marker (Fig. 4).

D. Species Comparisons

The 12S MiFish marker detected 32 fish species, and several other vertebrates including Mallard ducks (*Anas platyrhynchos*) and red-backed salamander *(Plethodon cinereus*), both commonly found in the area. Most eDNA reads belonged to species common in the northwest Atlantic, including Atlantic herring (*Clupea harengus*), mackerel (*Scomber scombrus*), gaspereau (*Alosa* spp.), sticklebacks, and gunnels (*Pholis* sp.). Several non-native species were also detected, including three species of Pacific salmon (*Onchorhynchus mykiss, O. tshawytscha,* and *O. keta)*, as well as Nile tilapia (*Oreochromis niloticus*). The NMDS showed substantial overlap between filter types at the 12S marker using both Euclidean distance and Bray-Curtis dissimilarity matrices (Fig. 5). The PERMANOVA analyses revealed that neither sampling method nor week had a statistically significant effect on species composition ($p < 0.05$). This suggests that the Autonomous and FAS methods captured similar species assemblages, and there was no clear temporal trend in species composition over the sampling period.

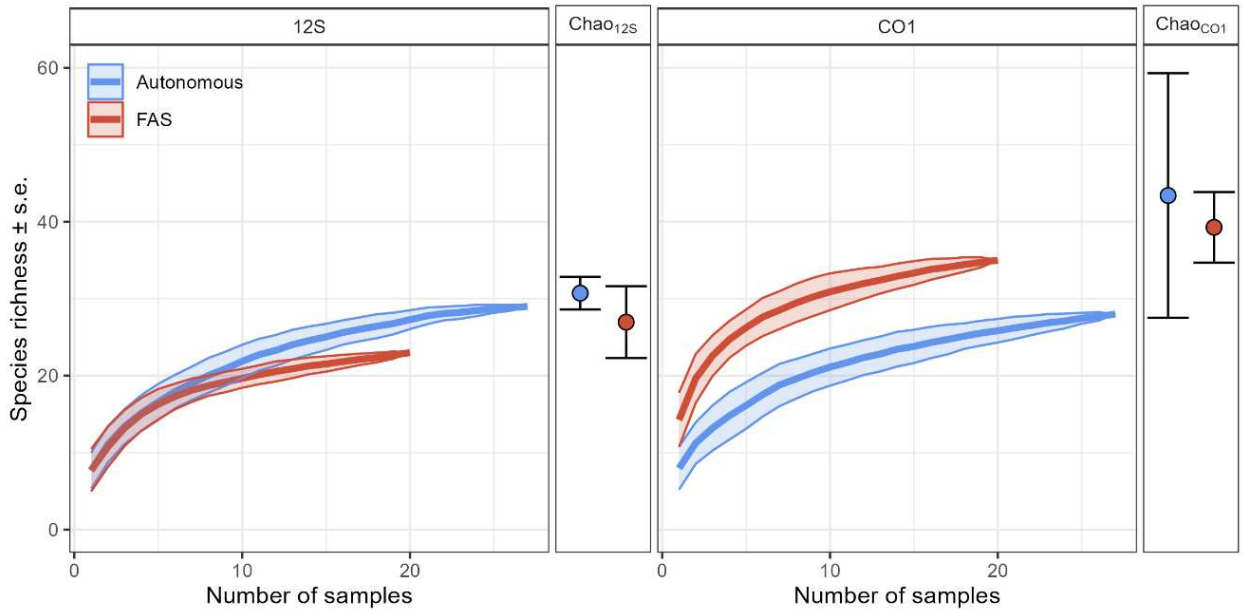

Fig. 3. Comparison of species richness trajectories by sampling method and eDNA marker. Ribbon plots represent species accumulation curves, while points indicate the Chao extrapolated richness in the sampled species pool (± s.e.) for each sampling method over the sampling period.

At the COI marker, invertebrate species detections varied substantially between the autonomous and time-of-sample filtered DNA, corresponding to the richness analysis. This corresponds with the richness analysis, which showed greater species richness in the TAS samples. The TAS samples detected many more copepod species and reads, especially the genera *Acartia*, *Centropages*, *Eurytemora*, *Paracalanus*, and *Oithona*. The TAS samples also detected more molluscs than the autonomous samplers, including *Modiolus modiolus*, *Mytilus edulis*, and *Tritia trivittata*, as well as the invasive bryozoan *Membranipora membranacea*. PERMANOVA analyses found that the two sampling methods detected statistically different assemblages of species. The effect of week was not as pronounced and was only observed to be statistically significant when accounting for community differences using Bray-Curtis dissimilarity. However, both sample types detected *Mytilus trossulus* in most samples. In contrast, the autonomous samples primarily captured marine fungi, diatoms, and other unicellular organisms. The amphipod *Monocorophium insidiosum* and the harpacticoid copepod *Sarsamphiascus kawamurai* were solely detected by the autonomous samplers as well. For both markers and sampling methods, there was considerable variability associated with replicates and sampling period (week), with no clear trends (Fig. 5).

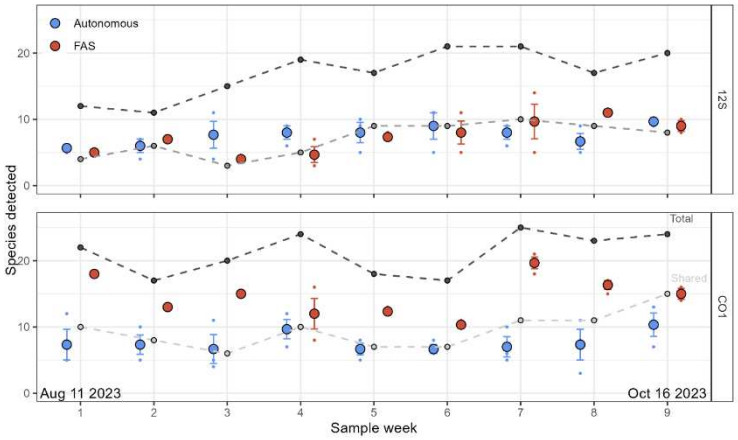

Fig. 4. Comparison of species richness by sampling method and sample time. Dashed lines represent the average number of shared species and the total number of species detected among sampling methods at each time point. Error bars indicate standard error.

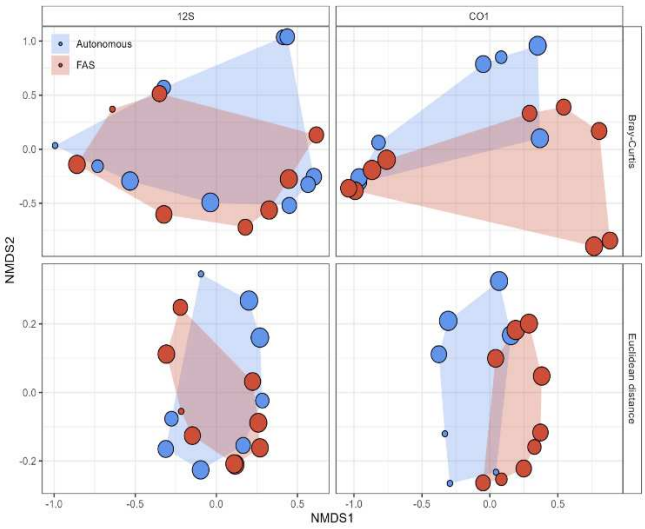

Fig. 5. Plot comparing compositional changes in species richness as a function of sampling period, amplification primer, and community dissimilarity metric using non-metric multidimensional scaling. Size of points corresponds to the sampling period scaling from smallest (week 1) to largest (week 9).

IV. Discussion

Biodiversity monitoring presents a significant challenge for ocean managers, particularly as ecosystems respond to various environmental changes and anthropogenic threats. The imperative for broadscale monitoring of biodiversity changes is increasingly recognized as crucial for managing these challenges effectively. Traditional methods of biodiversity assessment, though valuable, often face limitations in terms of spatial coverage, temporal resolution, and cost. This underscores the growing need for new technologies capable of providing comprehensive and efficient monitoring solutions, especially in remote and sensitive environments where human access is limited or undesirable. Autonomous eDNA samplers represent a promising advancement in this regard. In our study, these devices demonstrated their capability to collect and preserve eDNA samples over an extended period, even in challenging coastal marine conditions. The success of these samplers, which in our test functioned without significant biofouling and maintained sample integrity, highlights their potential to support continuous and wide-ranging biodiversity assessments.

DNA concentrations did not decline significantly over the nine-week period whether preserved in RNA*later* or flash-frozen at -80°C. These results are comparable to other studies comparing RNA*later* and flash-freezing, as well as DNA concentration from higher-volume samples over a minimum of three weeks [21]. As flash-freezing filters is not possible inside an autonomous eDNA sampler at ambient ocean temperatures, our results suggest that RNA*later* will adequately preserve captured eDNA, at least over a period of up to three months. In terms of biofouling, over the nine-week experimental period, the samplers accumulated small amounts of colonial tunicate and bryozoan growth on the tops of the samplers. However, no biofouling was found on the inlets or outlets of the samplers, suggesting the copper screening, a commonly used biocide, was successful in preventing growth [22]. The copper mesh did develop a patina coating after several weeks which did not appear to affect water filtration but could be monitored for any impacts on sampler vacuum pressure and filtering ability. Both the autonomous eDNA samplers and the traditional filter-at-sample methods detected similar fish communities in the Bedford Basin, capturing the most common species such as cunner, alewife, gunnels, mackerel, and herring. However, differences were observed in the invertebrate communities detected by each method. The autonomous samplers predominantly captured diatoms and algae using the COI marker, whereas the FAS filters were more effective at capturing animal eDNA, particularly from copepods, sea stars, and molluscs. Given that all samples were filtered from the same source water each week, these differences are likely due to the different pore sizes used (1.2µm in FAS and 0.22µm in the autonomous samplers). Additionally, the small volume of water sampled weekly (125mL per replicate sampler) and potential preservation effects on certain taxa may also contribute to this variation [23].

The analysis of species richness revealed that both methods had comparable results for vertebrates, with an average of 7.7 species per sample detected using the 12S marker. However, invertebrate species richness was significantly higher in the FAS samples, averaging 14.4 species per sample compared to 7.67 species in the autonomous samplers. Trends in richness overtime were similar among sampling methods, suggesting that the difference in invertebrate detection is likely most impacted by the pore size, particularly with the COI marker in this field test. The difference in invertebrate communities revealed between sample types underscores the need to understand the potential impacts of sampling strategies for eDNA, including filter pore size, material, water volume, and even DNA extraction method [23].

Despite these differences in species detection, the species composition detected by both methods showed substantial overlap, particularly for vertebrates. The nMDS and PERMANOVA analyses revealed that neither the sampling method nor the sampling period (week) had a significant effect on vertebrate species composition. This suggests that both autonomous and FAS methods are effective in capturing the same fish communities, suggesting both methods offer utility for biodiversity monitoring applications. However, the differences observed in invertebrate community composition highlight the importance of considering filter pore size and sampling

volume when designing and/or integrating eDNA into biodiversity monitoring protocols.

Interestingly, both methods detected non-native species, including three Pacific salmon species and Nile tilapia, highlighting the potential of eDNA methods for invasive species monitoring. The presence of unique taxa detected only by the autonomous samplers, such as marine fungi and the copepod *Sarsamphiascus kawamurai*, further underscores their utility in capturing a broad spectrum of biodiversity, including less commonly detected species. These results reinforce that non-native and potentially invasive species can be detected from relatively small water volumes, even if the organisms themselves are not observed [24]. This may be particularly relevant in harbours with high amounts of marine traffic, such as Halifax Harbour.

Design improvements to the eDNA samplers including higher sample volume throughput, different filter pore sizes, and improved depth ratings, promise to enhance their effectiveness in future deployments. This capability, combined with the use of preservatives like DNA/RNA Shield (Zymo Research), which can preserve DNA at ambient temperatures (4 - 25°C) for up to two years, positions these samplers as a valuable tool for long-term and broadscale biodiversity monitoring. As these technologies are refined, they are likely to become increasingly integral to the management and conservation of marine ecosystems, providing critical data for assessing biodiversity and informing conservation strategies.

Overall, our study demonstrates the promising potential of autonomous eDNA samplers for biodiversity monitoring, particularly in sensitive and remote marine environments across seasons [25] [26]. The ability of these devices to effectively capture and preserve eDNA from a wide range of taxa, including both common and unique species, emphasizes their utility for comprehensive biodiversity assessments. The observed differences in species richness and composition between the autonomous and traditional FAS methods highlight the importance of optimizing sampling protocols, such as filter pore size and water volume, to maximize detection across diverse taxa.

The successful operation of these samplers in challenging coastal conditions, coupled with their ability to minimize biofouling and maintain sample integrity, provides a strong foundation for further development and deployment. The potential to use these units for minimally invasive, long-term monitoring in offshore, deepwater, and remote locations—such as Arctic regions—presents a significant advancement in the field. By enabling extended sampling periods and reducing the need for costly research vessel missions, autonomous eDNA samplers offer a scalable and efficient solution for broad-scale biodiversity monitoring efforts in aquatic environments.

ACKNOWLEDGMENT

We thank Katie Thistle and Shawn Roach (DFO) for their assistance in deploying and recovering the eDNA samplers in the Bedford Basin. This study was funded by the Innovative Solutions Canada program and funding from the DFO Marine Conservation Targets program. DNA sequencing was provided with support from the Tula Foundation/Hakai Institute.

REFERENCES

[1] Naeem, S., 2002. Ecosystem consequences of biodiversity loss: the evolution of a paradigm. Ecology, 83(6), 1537-1552.

[2] Oliver, T.H., Isaac, N.J., August, T.A., Woodcock, B.A., Roy, D.B. and Bullock, J.M., 2015. Declining resilience of ecosystem functions under biodiversity loss. Nature Communications, 6(1), 10122.

[3] Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenuil, A., Corinaldesi, C., Cristina, S., David, R., Dell'Anno, A. and Dzhembekova, N., 2016. Implementing and innovating marine monitoring approaches for assessing marine environmental status. Frontiers in Marine Science, 3, 213.

[4] Dunham, A., Dunham, J.S., Rubidge, E., Iacarella, J.C. and Metaxas, A., 2020. Contextualizing ecological performance: Rethinking monitoring in marine protected areas. Aquatic conservation: marine and freshwater ecosystems, 30(10), 2004-2011.

[5] Balbar, A.C., Daigle, R.M., Heaslip, S.G., Jeffery, N.W., Proudfoot, B., Robb, C.K., Rubidge, E. and Stanley, R., 2020. Approaches for assessing and monitoring representation, replication, and connectivity in marine conservation networks. DFO Canadian Science Advisory Secretariat (CSAS).

[6] Jeffery, N.W., Lehnert, S.J., Kess, T., Layton, K.K., Wringe, B.F. and Stanley, R.R., 2022. Application of omics tools in designing and monitoring marine protected areas for a sustainable blue economy. Frontiers in Genetics, 13, 886494.

[7] Government of Canada 2023. Federal Marine Protected Areas Protection Standards. Ottawa.

[8] Gold, Z., Sprague, J., Kushner, D.J., Zerecero Marin, E. and Barber, P.H., 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. PLoS One, 16(2), e0238557.

[9] He, X., Stanley, R.R., Rubidge, E.M., Jeffery, N.W., Hamilton, L.C., Westfall, K.M., Gilmore, S.R., Roux, L.M.D., Gale, K.S., Heaslip, S.G. and Steeves, R., 2022. Fish community surveys in eelgrass beds using both eDNA metabarcoding and seining: implications for biodiversity monitoring in the coastal zone. Canadian Journal of Fisheries and Aquatic Sciences, 79(8), 1335-1346.

[10] He, X., Jeffery, N.W., Stanley, R.R.E., Hamilton, L.C., Rubidge, E.M. and Abbott, C.L., 2023. eDNA metabarcoding enriches traditional trawl survey data for monitoring biodiversity in the marine environment. ICES Journal of Marine Science, 80(5), 1529-1538.

[11] Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G. and VanMorter, S.M., 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. ICES Journal of Marine Science, 78(1), 293-304.

[12] Hendricks, A., Mackie, C.M., Luy, E., Sonnichsen, C., Smith, J., Grundke, I., Tavasoli, M., Furlong, A., Beiko, R.G., LaRoche, J. and Sieben, V., 2023. Compact and automated eDNA sampler for in situ monitoring of marine environments. Scientific Reports, 13(1), 5210.

[13] Thomas, A.C., Nguyen, P.L., Howard, J. and Goldberg, C.S., 2019. A self - preserving, partially biodegradable eDNA filter. Methods in Ecology and Evolution, 10(8), 1136-1141.

[14] Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H. and Kondoh, M., 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society open science, 2(7), 150088.

[15] Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T. and Machida, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology, 10, 1-14.

[16] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857.

[17] Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. and Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, 13(7), 581-583.

[18] Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, et al. 2022. vegan: Community Ecology Package. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

[19] Wickham, H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

[20] Jeffery, N.W., and Stanley, R.R.E. 2024. dfo-mar-mpas/DOT_SR_eDNAExperiment: Pre-publication release https://zenodo.org/doi/10.5281/zenodo.13126692

[21] Yamahara, K.M., Preston, C.M., Birch, J., Walz, K., Marin III, R., Jensen, S., Pargett, D., Roman, B., Ussler III, W., Zhang, Y. and Ryan, J., 2019. In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater vehicle. Frontiers in Marine Science, 6, 373

[22] Delauney, L., Compere, C. and Lehaitre, M., 2010. Biofouling protection for marine environmental sensors. Ocean Science, 6(2), 503-511.

[23] Deiner, K., Lopez, J., Bourne, S., Holman, L.E., Seymour, M., Grey, E.K., Lacoursière-Roussel, A., Li, Y., Renshaw, M.A., Pfrender, M.E. and Rius, M., 2018. Optimising the detection of marine taxonomic richness using environmental DNA metabarcoding: the effects of filter material, pore size and extraction method. Metabarcoding and Metagenomics, 2, e28963.

[24] LeBlanc, F., Belliveau, V., Watson, E., Coomber, C., Simard, N., DiBacco, C., Bernier, R. and Gagné, N., 2020. Environmental DNA (eDNA) detection of marine aquatic invasive species (AIS) in Eastern Canada using a targeted species-specific qPCR approach. Management of Biological Invasions, 11(2).

[25] Truelove, N.K., Patin, N.V., Min, M., Pitz, K.J., Preston, C.M., Yamahara, K.M., Zhang, Y., Raanan, B.Y., Kieft, B., Hobson, B. and Thompson, L.R., 2022. Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. Environmental DNA, 4(4), 972-984.

[26] Sigsgaard, E.E., Nielsen, I.B., Carl, H., Krag, M.A., Knudsen, S.W., Xing, Y., Holm-Hansen, T.H., Møller, P.R. and Thomsen, P.F., 2017. Seawater environmental DNA reflects seasonality of a coastal fish community. Marine Biology, 164, 1-15.

.