Use of Common Household Materials in Passive Environmental DNA Collection

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Abstract

Environmental DNA (eDNA) has been at the forefront of emerging biomonitoring technology for marine ecosystems. While traditional processes involve a labor-intensive, resource-heavy active filtration of water samples through a filter membrane, passive eDNA detection offers an alternative approach. Passive eDNA detection involves submerging various materials at a sample site to capture free-floating eDNA in the water. Specifically testing commercially available materials, this experiment was designed to make passive sampling more accessible and versatile in its future applications. Four different candidate materials were tested, and all had similar performances at successfully detecting rusty crayfish (*Faxonius rusticus)* eDNA in as little as five minutes of submersion time. However, contamination in the reagents used for the analysis reduces the confidence of the findings of the experiment.

1. Introduction

Species detection plays a critical role in ecosystem monitoring. Due to climate change, the threat of extinction and endangerment has further fueled the demand for efficient, noninvasive biomonitoring techniques. Conventional methods, like visual surveys and trapping of target organisms, are labor-intensive and quite invasive. Additionally, these techniques often yield biased results as it is difficult to quantify the true effects of human intervention in these studies.

Detecting eDNA offers an exciting alternative to biomonitoring. eDNA is any form of DNA, such as waste products, gametes, saliva, and blood, a target organism ejects into the environment (Díaz-Ferguson & Moyer, 2014). Because the source organism does not have to be caught in order to obtain eDNA, this practice is much more economical, noninvasive, and versatile in its applications. Requiring less equipment, time, and labor, eDNA detection is a rapidly growing field of research because of its potential to help monitor marine ecosystems more regularly and broadly (Simmons et al., 2016).

There are two primary methods of aquatic eDNA capture. Active filtration involves passing a water volume through porous filter membranes to isolate DNA fragments in the water sample (Chen et al., 2024). This procedure may be adapted based on the target species and resources available, however past experiments have cited its drawbacks as being equipment-intensive, consequently increasing the chances for contamination (Chen et al., 2024). Additionally, it is not ideal for turbid waters as the filter membranes have an increased chance of clogging (Chen et al., 2024). Lastly, the active sample reflects only a spatiotemporal snapshot of the marine

ecosystem at the time of sampling, providing additional constraints on when and where such samples should be collected (Chen et al., 2022). An alternative method is passive sampling, which involves submerging a natural or artificial membrane in water (Bessey et al., 2022). Requiring significantly fewer supplies and shorter processing time, passive sampling is better suited for high-density sampling in remote locations with harsher conditions (ie. high turbidity and strong currents) (Bessey et al., 2022). Furthermore, the variability of submersion time means surveying can span a longer time frame (Beng & Corlett, 2020). This can be especially useful for elusive species or organisms whose activity varies throughout the day, since simply leaving the materials submerged for longer amounts of time can increase the chances of detecting the target species' eDNA.

In addition, various cost-saving measures were tested throughout the experiment, from choosing common household materials as sampling membranes to using Ziplock bags for collecting sample waters. These alternatives would allow resources to be allocated to more expensive qPCR reagents and novel analysis techniques.

One challenge when dealing with eDNA, however, is that it degrades rapidly when exposed to environmental conditions, including but not limited to sunlight and chemical pollutants (Beng & Corlett, 2020). It is important to take the environmental context of both the sample site and the target species when designing a passive sampling experiment. For example, to increase the chances of eDNA capture, one might time the sampling to the species' mating season. Additionally, the sensitivity of the technology must not be underestimated and proper decontamination of all equipment, work surfaces, and samples must be undertaken to eliminate the possibility of false positive results (Bockrath et al., 2023).

In order to validate eDNA surveillance techniques, the target species and sampling site were chosen based primarily on convenience and efficiency. The rusty crayfish is a non-native, very aggressive species that was first introduced to the Finger Lakes region as fish bait (Larson et al., 2017). Distinguished by dark, rusty spots on either side of its carapace, the rusty crayfish poses danger to aquatic plants and fish, as they feed on fish eggs and invertebrate prey (U.S. Geological Survey, 2024). Visual surveys have proved the existence and abundance of the species in the region, which made the rusty crayfish an ideal target species for training and validation purposes. However, the same eDNA collection techniques could be applied to detect more cryptic species in the future.

2. Literature Review

In a study conducted by Bessey et al., 2022, researchers evaluated the ability of nine different membranes to capture fish eDNA in a laboratory setting (The Aquarium of Western Australia). The materials tested had various physical, chemical, and electrical properties and ranged from activated carbon sponge to cotton fibers in nylon bags. Multiple trials with different submersion times, starting from five minutes until 18 hours, were included. Five one liter active filtration

samples were also compared using 0.45um cellulose ester membranes. A simple yet effective apparatus was designed using the mesh pockets of a pearl oyster aquaculture frame.

The study found that all materials tested were successful in detecting fish eDNA, with comparable performances to active filtration methods. Furthermore, there was no significant trend between longer submersion times and higher eDNA concentrations. Using SEM imaging, the researchers found no consistent pattern between the nine materials tested that increased the potential for eDNA capture. In other words, the study concluded eDNA, which exists in many shapes and sizes, adheres unpredictably to available surfaces. Thus, the ideal material for passive eDNA collection depends largely on water conditions and characteristics of the target species. Lastly, this study found that all materials were successful at detecting more than half of the fish species present at the aquarium. A separate study conducted by Chen et al., 2022, corroborated the conclusion that passive sampling was effective at detecting more than 20 fish species in one sampling cycle. Therefore, passive eDNA sampling has applications for both single-species detection and surveying a broad range of taxonomic species.

3. Materials & Methods

3.1 Sampling

3.1.1 Sampling Site

Sampling was conducted at Fall Creek in Ithaca, NY. Beginning near Lake Como and flowing westward towards Cayuga Lake (New York State Department of Environmental Conservation), the water body was selected for its relative convenience and accessibility from the Cheong Lab, where the water samples were processed. Located about a 20 minute walk from the lab, the specific portion of the creek that was sampled is a popular swimming and fishing area for local residents. With relatively calm-flowing, low turbidity water, shaded riverbeds, and large slabs of rocks, Fall Creek is the ideal rusty crayfish habitat (U.S. Geological Survey, 2024). Furthermore, visual surveys of live and dead rusty crayfish confirmed the presence of the species in the creek.

3.1.2 Sampling Apparatus

Three different sampling methods were tested prior to and during the experiment. The first design involved clipping the various membranes to a mesh wire bucket that was placed in the creek for sampling. While this design was simple and constructed using commercially available materials, one limitation was the inability to adjust the orientation and submersion depth of the material when clipped to the bucket. To address this, the second design involved custom 3D-printed stands that secured the membrane between two plates. With adjustability for the height (ie. submersion depth) and angling of the material with respect to the water flow, these stands were lightweight and easy to manufacture with the help of Cornell's Rapid Prototyping Lab. However, because the creek experienced a lot of foot traffic, both the bucket and 3D-printed stand designs were prone to human tampering. One of the buckets left overnight was found outside the creek the following morning, and three 3D-printed stands could not be found.

The final design involved using binder clips to secure the materials to a small tree branch suspended across the water surface. Requiring the least amount of equipment, setting up the apparatus was extremely simple. By using nearby rocks to secure the branch, the materials remained fully submerged even as the water levels changed throughout the day.

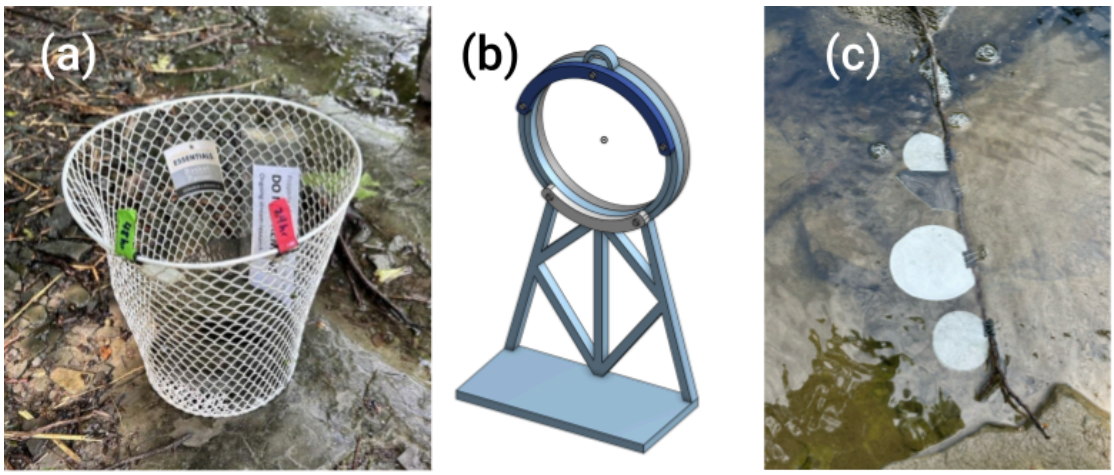

Figure 1. Three sampling methods. (a) Materials clipped to wire mesh bucket using binder clips. (b) Materials secured between plates of custom 3D-printed stand. (c) Materials clipped to tree branch and suspended across creek.

3.1.3 Materials

When selecting the materials to be tested, the primary consideration was making passive eDNA collection as accessible as possible. Consequently, the following inexpensive, commercial items were chosen: polyvinylidene fluoride (PVDF) filter paper (0.45um pore size), black KN95 face masks (0.3um pore size), and two types of 100% cotton pads.

In prior experiments, filter papers of different pore sizes and materials were tested. Specifically, active filtration samples were taken using a pure nitrocellulose blotting membrane (0.2um pore size) and a cellulose Whatman filter paper (11um pore size). However, the pure nitrocellulose membrane of 0.2um pore size was expensive and experienced lots of clogging due to debris in the water sample. The Whatman filter paper of 11um pore size was prone to ripping and extremely delicate when submerged for long periods of time.

In contrast, the commercially available PVDF filters exhibit strong UV and organic acid resistance, which is optimal when placed in sample sites with heavy sunlight for extended periods of time. Additionally, while pore clogging was a concern, a smaller pore size was selected because there was relatively little debris in the creek on the days of passive sampling. For the KN95 mask, a roughly 2” x 2.5” (5cm x 6.35cm) portion of the mask was cut out along a seam to ensure all layers of the mask stayed together. Lastly, the two cotton pads differed most noticeably as one (cotton pad A) contained a stitching around its border while the other did not (cotton pad B). Both cotton pads measured 2.25” (5.75cm) in diameter.

3.1.4 Submersion time

In addition to testing various material types, the optimal submersion time was also evaluated. The two submersion times tested were 5 minutes and 24 hours for each of the four materials mentioned above.

3.1.5 Sampling Procedure

Passive eDNA collection occurred on the week of July 8, 2024. Ensuring the materials were fully submerged in the water and the cross section of the material was oriented perpendicular to the flow of the creek, environmental data was taken at the site.

Returning to the sample site the following day, the tree branch with the four materials was successfully retrieved. Each of the four materials were then placed into a test tube of Longmire buffer to prevent DNA degradation while transporting the samples from the creek to the lab. Each test tube was labeled with the material type and submersion time. Nitrile gloves were used to fold and place the materials into the test tube. The same set of gloves were used to handle all samples.

In order to compare the efficacy of passive eDNA collection with that of active filtration, one active filtration sample and a corresponding negative field blank was collected at the scene. A sterilized sampling apparatus was brought to the sampling site, which included a Buchner funnel, hand pump, ring stand erlenmeyer flask fitted with a rubber stopper, tweezers, and tongs. To prevent contamination, nitrile gloves were used to handle the filter paper at all times.

To collect the negative field blank, the Buchner funnel was first lined with the PVDF filter paper (0.45um). Then, while continuously hand pumping, roughly 300mL of nanopure, autoclaved water (sourced from the lab) was poured in small increments through the apparatus. The hand pump was used to create a vacuum to help draw the water through the membrane and ensure the filter paper rested flat against the bottom of the Buchner funnel. Once the entire volume of water had been filtered, using a decontaminated set of forceps and tweezers, the filter paper was gently folded and placed in a pre-labeled test tube filled with Longmire buffer solution. After emptying the erlenmeyer waste beaker, the same procedure was repeated using roughly 1000mL of the sample creek water. Labeled as the active filtration sample, the filter paper was preserved in Longmire buffer. Once returning to the lab, all sample tubes were stored in a 4°C fridge until needed for DNA extraction.

Figure 2. Active filtration apparatus using a hand pump.

3.1.6 Decontamination Protocol

To prevent sampling contamination, the active filtration apparatus was thoroughly decontaminated between use. The Buchner funnel was taken apart and soaked in undiluted 8.5% bleach for at least half an hour. After soaking, the bleach was rinsed with nanopure water. Similarly, the tweezers and forceps were soaked in undiluted 8.5% bleach for about 20 seconds then rinsed with nanopure water when ready for use. For other pieces of equipment, including the ring stand, hand pump, and erlenmeyer flask, the standard decontamination protocol involved spraying the equipment with 70% diluted ethanol and patting dry with a paper towel.

3.2 DNA Extraction

DNA extraction occurred at a sterilized work station at the Cheong Lab. All work surfaces and pipettors were sprayed and wiped with 70% diluted ethanol. The samples were warmed in a 65°C water bath for about ten minutes and then vortexed for about five minutes. Using a DNA extraction protocol adapted from the Jim Casey Lab for the Qiagen kit, 20uL of Qiagen protease, 400uL of sample media, 200uL of Buffer AL, and 400uL of 100% ethanol was added to a reaction tube. The reaction tube was vortexed frequently to ensure a thoroughly mixed solution. Next, 550uL of the mixture was transferred into a spin column, which was micro-centrifuged at 6k x g for one minute. The filtrate produced in the collection tube was discarded into a biohazard container, and this process was repeated for the remaining mixture. Once centrifuged, 500uL of Buffer AW1 was added to the spin column and centrifuged under the same settings. After removing the filtrate, 500uL of Buffer AW2 was added and centrifuged at 17k x g for three minutes. Replacing the collection tube with a new one, the spin column was centrifuged at 17k x g for one minute. This short centrifuge process was repeated until no more solution was produced. The upper portion of the spin column was transferred to a labeled elution tube, to which 100uL of RNase free water was added. After incubating the column for five minutes at room temperature, the columns were centrifuged for the final time at 6k x g for one minute. The eluted DNA was stored at -20°C in a freezer until needed for qPCR a few days later.

3.3 qPCR Analysis

3.3.1 qPCR Plating

qPCR plating and analysis was performed in a separate room from DNA extraction to minimize the chance of contamination. Like the DNA extraction step, all work surfaces and pipettes were sprayed and wiped with 70% diluted ethanol. As an additional precaution, qPCR plating occurred in a biosafety cabinet. Because the samples, primers, probes, and standards are stored at -20°C prior to qPCR plating, the first step was to warm the test tubes by hand and centrifuge at maximum speed for about ten seconds to collect the solution at the bottom of the tubes. Next, the master mix was prepared by mixing Taqman reagent, RNAse-free water, FaRu forward primer, FaRu reverse primer, and FaRu probe, with the exact amounts calculated based on the number of wells needed for the plate. Following a plate map, 14uL of the master mix was placed into each well using a pipette. Once completed, the plate was covered with aluminum foil until ready to plate the samples and controls.

Eight serially diluted (1:10) standards were included as absolute quantification for eDNA copy numbers during the qPCR run. To prepare the standards, eight dolphin tubes were filled with 9uL of RNAse-free water and labeled from #1 to #8. 1uL of the standard was added to tube #1 and vortexed to mix. Then, 1uL of the solution from tube #1 was transferred to tube #2, which was then vortexed. This process was repeated for all tubes.

To check for PCR inhibition, spiked samples were also included in the assay. For every sample that was spiked, 0.5uL of the sample was added to a dolphin tube containing 4uL of RNAse-free water and 0.5uL of the standard. The spiked sample dolphin tubes were then centrifuged to mix the solution.

After the standards and spiked samples were prepared, plating involved adding 1uL of the respective solution to the corresponding well. All samples (including negative field blanks) had a corresponding spiked sample, and each sample, spiked sample, and standard was plated twice (each had two replicates). Two non-template controls (NTCs) were included to monitor contamination during the plating process, which involved adding 1uL of RNAse-free water to the wells. Rigorous practices were used to prevent DNA contamination, including using a new pipette tip for each well, using microcentrifuge tube opener to avoid creating aerosols when opening test tubes, and covering portions of the plate not being used with aluminum foil. Once the plating was completed, the plate was sealed carefully with a plastic film adhesive to prevent evaporation during the qPCR process. The plate was centrifuged at 400 x g for a minute to ensure the mixture is brought down to the bottom of the well. Once finished, all samples, primers, probes, and standards were returned to a -20°C freezer and work surfaces were thoroughly decontaminated with 70% ethanol.

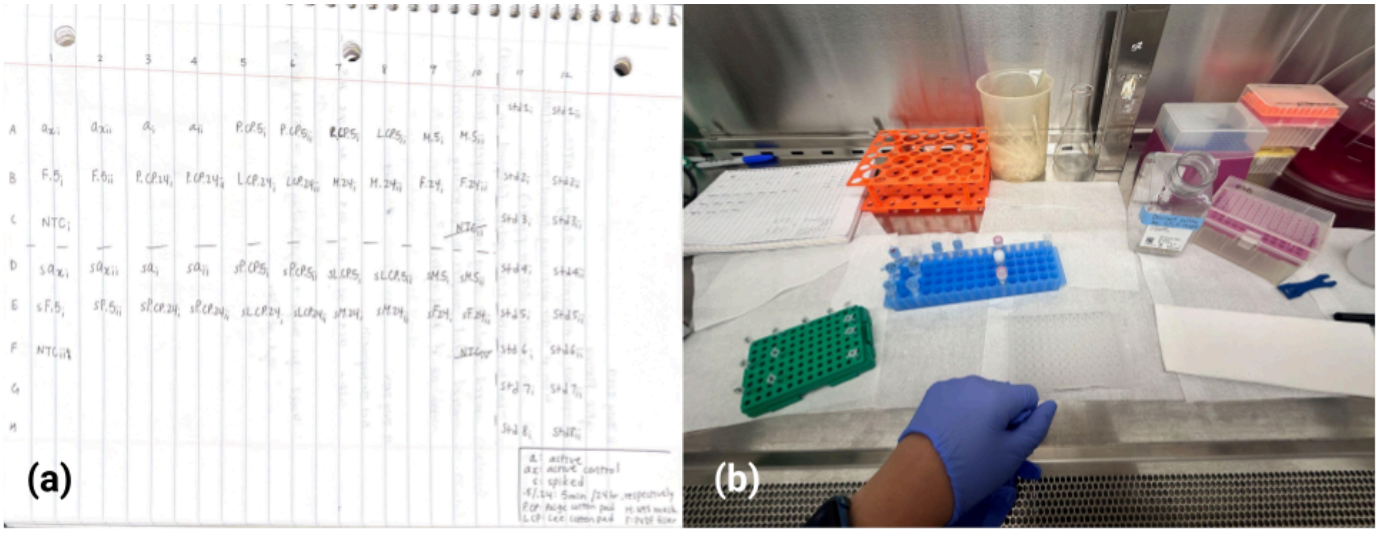

Figure 3. Preparation for qPCR plating. (a) Plate map for various materials and submersion times. (b) qPCR plating in biosafety cabinet.

3.3.2 qPCR Setup and Analysis

qPCR assays were performed using a QuantStudio 3 machine. Using the *QuantStudio Design & Analysis Software* (v1.5.1), the plate was mapped digitally into the software according to the plate map used during the plating process. The properties of the plate map were adjusted to be a 96 well 0.2mL block with a standard curve experiment type, TaqMan reagents, and standard run mode. After exporting the plate map as a .edt file into a USB hard drive, the USB hard drive was transferred to the qPCR machine. Once orienting the plate according to the map on the USB and sealing the plate, the qPCR analysis was run. The *QuantStudio Design & Analysis Software* (v1.5.1) was again used to view and analyze the qPCR results.

3.4 Gel Electrophoresis

Gel electrophoresis was used to determine the relative length of DNA fragments in select samples. To prepare the gel, a flask containing 1g agarose powder and 50mL 50x TAE buffer were boiled in a microwave for roughly a minute. The mixture was swirled and cooled until it reached 60°C. Next, SYBR green dye was added to the mixture, which was then poured from the flask into a gel mold box. Pouring slowly to avoid bubbles, a comb was added to one end of the gel to create the wells. The gel was then set for 20 minutes, after which the comb was removed from the gel.

To run the gel electrophoresis, the prepared gel was placed into the gel box with the wells aligned with the negative electrode. TAE running buffer and 20uL SYBR green dye was added to the box, fully submerging the gel. 10uL of the ladder was added to one of the wells, and 10uL of prepared samples were added to the other wells. To prepare the samples, 3uL of the sample, 1.67uL of the loading dye, and 5.33uL of water was mixed and vortexed. Using caution to avoid puncturing the gel, the electrophoresis was run at 60V for 60 minutes. The results were visualized on a UV light bed.

4. Results & Discussion

4.1 qPCR Results

4.1.1 Standards

The assay included eight distinct standards with a serial 1:10 dilution. Standard 1 was prepared by mixing 9uL of RNAse-free water and 1uL of the standard. Observing the amplification plot, the standard curves appear consistent in shape and evenly spaced between each dilution, except for standards 1 and 2. This indicates the qPCR machine delivers predictable and reliable measurements of DNA copy numbers in the samples.

As mentioned, the amplification plots for standards 1 and 2 did not have the same shape as the other standards. However, the multicomponent plot reveals that standards 1 and 2 reached saturation almost immediately, suggesting that the starting concentrations in these standards

were excessively high. This explains why the first two standards were inconsistent with the expected shape of the amplification curve.

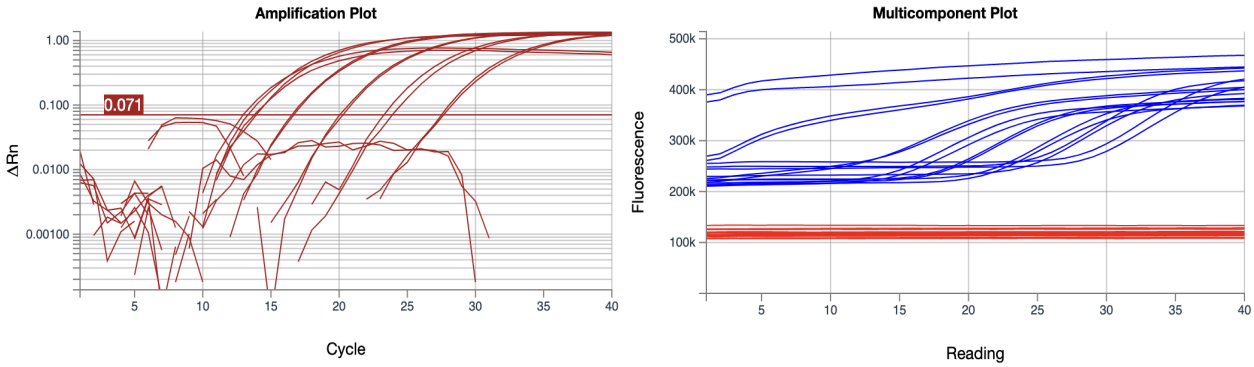

Figure 4. Amplification and multicomponent plots for standards serial 1:10 dilutions.

4.1.2 Negative field blank and active filtration sample

The amplification plot for the negative field blank revealed that the sample reached the detection threshold around cycle 32, meaning eDNA was present in the assay. It is unclear what the source of this contamination is precisely, as it could indicate inadequate decontamination of sampling apparatus or cross contamination during the qPCR plating process. As mentioned above, 10% undiluted bleach was used to decontaminate all field equipment and 70% diluted ethanol spray was used to decontaminate work spaces and sanitize nitrile gloves in between steps. The spiked negative field blank reached saturation threshold in roughly five cycles when examining the multicomponent plot, suggesting there was little PCR inhibition.

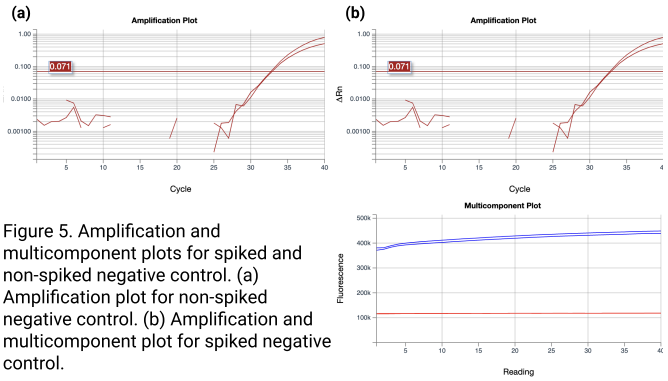

Figure 5. Amplification and multicomponent plots for spiked and non-spiked negative control. (a) Amplification plot for non-spiked negative control. (b) Amplification and multicomponent plot for spiked negative control.

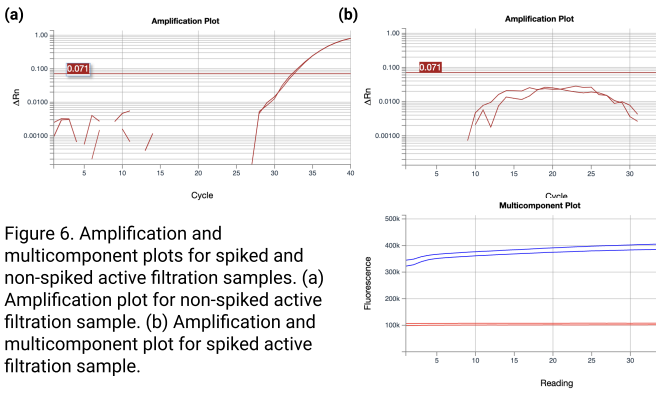

Figure 6. Amplification and multicomponent plots for spiked and non-spiked active filtration samples. (a) Amplification plot for non-spiked active filtration sample. (b) Amplification and multicomponent plot for spiked active filtration sample.

Similarly, the amplification plot for the active filtration sample and the spiked active filtration sample revealed it took roughly 32 cycles and five cycles, respectively, for the sample to reach the detection threshold. Because amplification was also observed in the negative field blank, it is not possible to conclude that the amplification in the active filtration sample is not a false negative result. More robust decontamination and qPCR plating protocols are necessary to make such conclusions. However, visual sightings of live crayfish near the sampling site on the day of sample collection suggest a false positive is improbable.

4.1.3 Non-template controls

Two non-template controls were placed in two separate regions of the plate. Ideally, no amplification should be observed in these samples because neither contained DNA-containing elements. In practice, however, both NTCs reached the detection threshold (in 27 and 32 cycles). This suggests contamination during the qPCR plating process, either in the reagents used or lack of precaution when handling samples and standards.

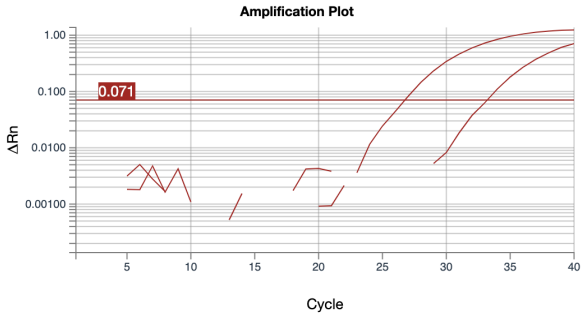

Figure 7. Amplification plot for NTCs.

A separate qPCR assay was designed to test the primer, probe, and mastermix solutions (used during the July 2024 assays) for contamination. Three separate samples were prepared, each isolating one of the aforementioned reagents used during the previous assay. A fourth control sample using entirely new reagents was also included. The amplification plot revealed the reagents used during the July experiments contained FaRu amplicons, though it is unclear whether they were synthetic amplicons or DNA from crayfish tissue samples. The primers had the highest copy numbers, followed by the probe, and lastly, the mastermix. Although the old mastermix sample did not reach the detection threshold after 40 cycles, there was non-negligible amplification observed in all three replicates, suggesting some amount of contamination. Only one of the three replicates using entirely new reagents reached the detection threshold after 37 cycles, while the others showed no amplification curve.

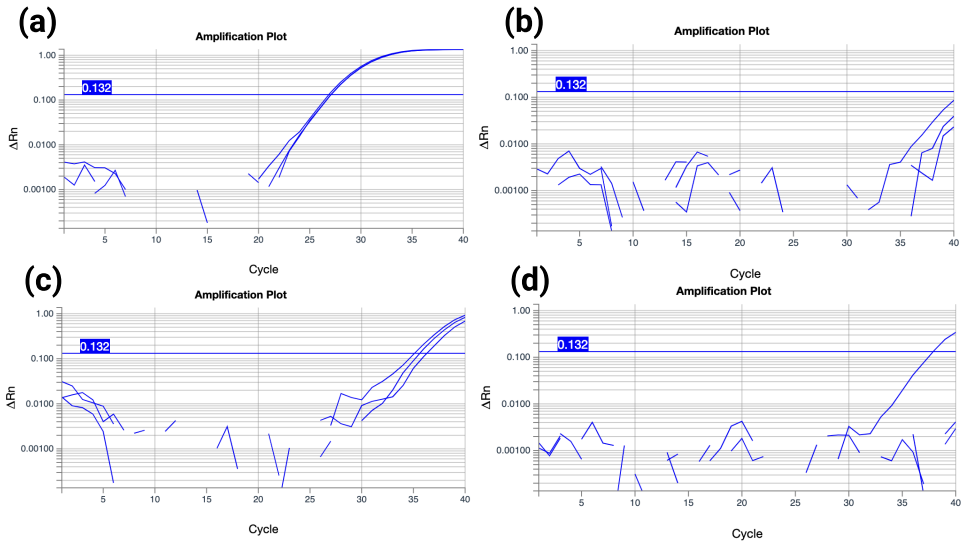

Figure 8. Amplification plots for individual qPCR reagents testing. (a) Isolated old primer. (b) Isolated old master mix. (c) Isolated old probe. (d) Entirely new reagents.

4.1.4 Passive sampling materials

Four passive eDNA collection materials were tested, each with two different submersion times. For a submersion time of five minutes, all four materials reached the detection threshold nearly simultaneously, at roughly 32 cycles. While these results suggest all materials tested were effective at passive eDNA capture in as little as five minutes of submersion, the contaminated qPCR reagents used during the assay would have inflated the copy numbers and does not eliminate the possibility that these results are false positives.

Similarly, amplification was observed for all four materials after 24 hours of submersion. Cotton pad A reached the detection threshold after 21-27 cycles, cotton pad B after 25-30 cycles, and both the KN95 mask and PVDF filter paper after 32 cycles. The most notable difference between the two submersion times was noticed for the two types of cotton pads, which had higher starting copy numbers when being submerged for a longer amount of time. The KN95 mask and PVDF filter paper had no significant difference in copy numbers for five minutes vs. 24 hour submersion times. Again, the use of contaminated reagents reduces the confidence of false positive results.

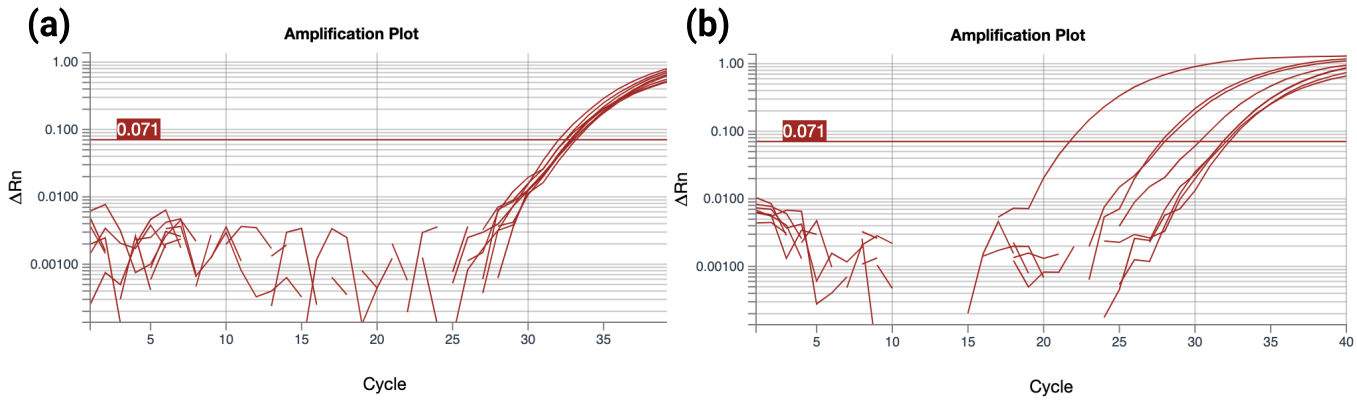

Figure 9. Amplification plots for passive sampling materials. (a) Five minute submersion time. (b) 24 hour submersion time.

4.1.5 Spiked passive samples

All eight spiked passive samples—all four material types and both submersion times—reached the detection threshold. The materials submerged for 24 hours appeared to have slightly higher initial copy numbers when spiked compared to those submerged for five minutes, which is consistent with the trends observed in the non-spiked samples. Overall, this suggests there was no significant inhibition during the qPCR assay.

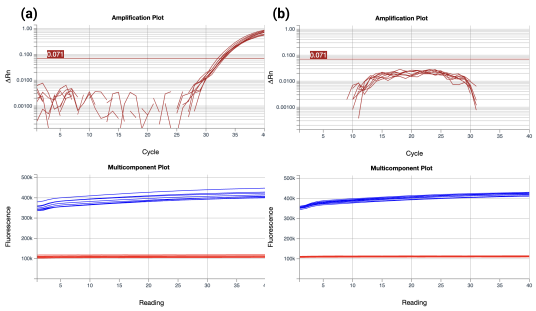

Figure 10. Amplification and multicomponent plots for spiked passive sampling materials. (a) Five minute submersion time. (b) 24 hour submersion time.

4.1.6 Gel Electrophoresis

A gel electrophoresis was run for three passive sampling materials (KN95 mask - 5 minutes, cotton pad #1 - 5 minutes, active sample) and the first four serially diluted standards. A ladder was included to provide a relative scale for the DNA fragment lengths. The results showed all passive sampling materials and standards contained DNA fragments of the same length, most likely FaRu DNA. Because all these samples also observed amplification of FaRu DNA during the qPCR assays, this gel result corroborates the results of the qPCR assay.

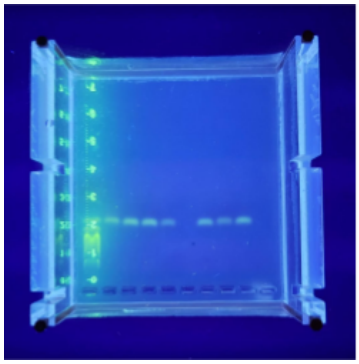

Figure 11. Gel electrophoresis testing and validation. From left to right:

1. ladder
2. std1
3. std2
4. std3
5. std4
6. empty
7. active sample
8. cotton pad #1 (five minutes)
9. KN95 mask (five minutes)

4.2 Discussion

While promising, these results must be analyzed critically in order to improve passive eDNA sample techniques for future use. It is not possible to conclude with full confidence that the four materials tested (two types of cotton pads, KN95 masks, and PVDF filter paper) are effective candidates for passive eDNA collection due to the contamination errors compounded throughout the sampling and qPCR assay process. However, it appears promising that all four material samples were measured to have some success at eDNA capture with as little as five minutes of submersion time. Repeating the experiment with more rigorous decontamination measures, careful plating techniques, using separate sets of pipettors for the standards and samples, and investing in brand new reagents would help strengthen conclusions regarding the effectiveness of passive eDNA sampling using accessible materials. These include soaking tools in stronger bleach concentrations for longer amounts of time (ie. one hour), disinfecting all pipettors with ethanol in between each plating step, and being more mindful of aerosol exposure when dealing with DNA-containing solvents.

It is also important to take the environmental context of both the sampling site and target species into consideration when designing passive sampling experiments. When attempting to set up passive traps further downstream, there were three instances of the passive trap being removed from the creek or disappearing entirely, either by human interference or strong currents. Furthermore, there were two instances where the material was not retained in the trap (ie. floated away in the current), and another two instances where a change in water levels caused the materials to no longer be submerged in the body of water. Most of these challenges arose while leaving passive traps overnight in the midst of an unexpected thunderstorm.

The passive sampling apparatus used in this experiment is ideal for shallow, calm water bodies. For sampling in large water bodies with strong currents, a studier and more robust design, such

as a tethering mechanism, is recommended. In addition, the optimal pore size and filter paper material may be unique to the target species and sampling location. When using the pure nitrocellulose membrane of 0.2um pore size, active filtration of a 1L water sample experienced significantly more clogging and took roughly 20 minutes. In contrast, the Whatman 11um filter paper was significantly faster at filtering the same water volume, taking roughly two minutes. Because a smaller pore size is more effective at gathering more eDNA (Liu et al., 2024), it is ideal for detecting elusive target species in temperate waters with little debris. The rusty crayfish targeted in this experiment was abundant, which meant a filter paper of larger pore size was successful at capturing crayfish eDNA. However, this would most likely not be the case when sampling for cryptic and rare species, like the crocea clam.

In conclusion, passive eDNA collection using common materials appears to be a promising field of exploration for efficient, low-effort, and cost-effective sampling methods for marine environments. Further testing is required to determine optimal sampling procedures, equipment, and decontamination measures based on the target species and sampling environment.

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