**Title**
# PolyWAG: Autonomous filtered water sampling for eDNA

**Authors**
Kai Roy * Riley Prince * Marc Belinga * John Selker * Chet Udell

**Affiliations**
OPEnS Lab, Oregon State University, Corvallis, OR, United States

**Corresponding author's email address and Twitter handle**
Kai Roy - kaikurisakaroygmail.com
Riley Prince - princeri@oregonstate.edu
Marc Belinga - belingam@oregonstate.edu
John Selker - john.selker@oregonstate.edu
Chet Udell - udellc@oregonstate.edu

**Abstract**
Environmental DNA (eDNA) is an ideal way of researching aquatic environments and determining what species are present in an area the biodiversity of an area, and if any invasive or endangered species are present. Traditional sampling of eDNA consists of manually filtering water, which is labor and cost-intensive for remote locations. Furthermore, commercialized solutions are either expensive or require a field operator to function. We have built an eDNA sampler capable of autonomous multi-sampling for a greatly reduced price compared to existing technologies. Our PolyWAG eDNA sampler system is a water sampling device that collects DNA samples via 47mm filter and provides a non-invasive, safe and autonomous means of eDNA collection. The sampler can hold 24 filters and is designed to be easily replaced and reusable. A browser application is used for real-time monitoring, scheduling tasks, and data logging for time, pressure, flow, and filtered volume. Additionally, the sampler design is openly published, modular and is constantly being tested to help us optimize our software and hardware to give us the best results. The 13-step sampling sequence helps reduce cross contamination significantly. Our machine can be deployed for an extended period. It is completely autonomous and costs around $3800.

**Keywords**
Environmental DNA * Sampling * Arduino * Data Logging

**Specifications table**

| | |
|---|---|
| **Hardware name** | *PolyWAG* |
| **Subject area** | *Environmental, planetary and agricultural sciences* |
| **Hardware type** | *Field measurements and sensors* |
| **Closest commercial analog** | *Dartmouth Ocean Technologies' eDNA Sampler* |
| **Open source license** | CERN Open Hardware Licence Version 2 - Strongly Reciprocal<br>GNU AFFERO GENERAL PUBLIC LICENSE Version 3 |
| **Cost of hardware** | *$3800 (Cost of just components)*<br>*$6000 (Cost with labor included)* |
| **Source file repository** | *If you've uploaded your source files to an approved repository (OSF, Mendeley Data or Zenodo) write the DOI URL here. For exemple: http://doi.org/10.17605/OSF.IO/WGK7Q* |

Table 1:

## 1. Hardware in context

Environmental DNA (eDNA) is DNA derived from mucus, feces, gametes, and carcasses [1]. Many things can be learned once this DNA is put through sequencing. eDNA can be used to determine what species are present in an area, the biodiversity of an area, and if any invasive or endangered species are present [2]. eDNA sampling provides scientists and researchers a non-invasive, rapid, cost-effective and sensitive way to detect and quantify species in many environments.

Traditional sampling of environmental DNA consists of manually filtering water, often requiring one or more researchers to be on location for days or weeks [3]. The filtration process varies depending on the researcher, but it is common to pull a sample of water with a bottle and pour that water into a funnel containing a filter. This can be connected to a vacuum pump to expedite the filtering process. After the sampling process is completed, the filters need to be preserved and the setup cleaned to avoid cross contamination [3]. This process is labor intensive, cost intensive, and can be dangerous, especially for remote locations. While commercialized solutions to this problem exist, they either still require an operator to be on location or are very expensive. Smithroot's commercial solution offers a simplified process with additional data collection such as GPS location for a fair price, around $8,000 [4]. A disadvantage of this solution is that it is not fully autonomous, still requiring an operator to be on location to use the device [4]. An alternative is the DOT Sampler which is a fully autonomous solution that is capable of multiple samples (20+ samples) and is also submersible but comes at a cost of ∼$55,000 [5].

The solution designed by the OPEnS Lab is the middle ground of these two solutions. While it is not submersible (limiting its potential sampling environments), it is capable of autonomous, multi-sample operations for extended periods of time (approximately one month) for the cost of $6,000. The two core priorities for our design are its autonomous function and the cross-contamination. The autonomous function of the sampler is important for a handful of reasons. An autonomous system requires less researcher hours spent in the field. This has cost benefits from the reduced hours worked and safety benefits when sampling in hazardous environments.

## 2. Hardware description

The eDNA sampler we have developed is an autonomous multi-sampling device that collects eDNA samples from water via 47mm filter holders and provides a non-invasive, safe, and autonomous means of DNA collection. The sampler can hold 24 filter housing which are designed to be easily replaced and reusable. The sampler is controlled by a custom logic board with an Adafruit M0 Feather Wi-Fi microcontroller loaded with a webserver to act as the interface for the sampler's operations. This webserver hosts a browser application which is used for real-time monitoring, scheduling tasks, and data logging for time, pressure, temperature, flow, and sample volume. The data is located stored onto an SD Card for later data analysis.

The basic function of the sampler can be split into five main sections: Hydraulics, Sampling Procedure, Utilities, Electronics, and User Interface. The Hydraulics section describes how the sampler is physically arranged, the different devices connected to the system, and provides a general idea on how liquids flow through the system. This section is supported by the Sampling Procedure Section which covers what the PolyWAG sampler is doing during each stage of the process. The Sampling Procedure Section refers to the devices described in the Hydraulics section in order to describe what is happening in each state as well as the purpose of that state. The Utilities section is similar to the Sampling Procedure section, but covers the four main utility functions of the sampler. These are functions that are used by an operator during cleaning or setup of the sampler. The Electronics section gives a brief overview of how the custom control board functions. Finally, the User Interface sections gives a brief overview on what the can be found and one within in the UI. More specific details on the User Interface can be found in the Operation Instructions section

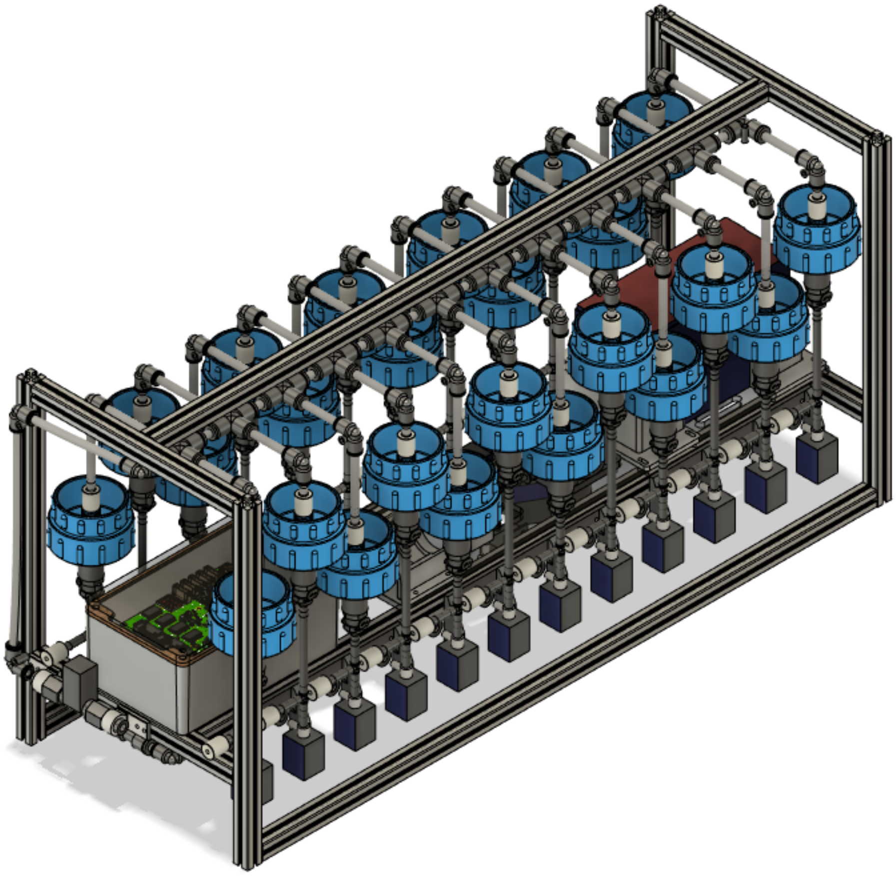

Figure 1: CAD Image of the complete sampler assembly

## 2.1 Hydraulics

The hydraulics of the sampler can be roughly split into the following sections:

- The Pump and Inputs
- The Lower Hydraulics
- The Filter Housings
- The Upper Hydraulics and Outputs

### 2.1.1 The Pump and Inputs

There are three inputs into the sampler: one for air, one for preservative, and one for water. The preservative input is connected to a hydration bladder where the preservative of choice can be stored. The sample water input has a prefilter at the front end of the tube to prevent debris from entering the sampler. Three valves are used to control the flow from these inputs with the air and preservative being regulated by solenoid valves and the water being controlled by a ball valve. These three valves connect into a single tube connected to the input of the peristaltic pump. The pump is capable of 400mL/min of flow under ideal conditions. The output of the pump connects directly into the Lower Hydraulic Rail.

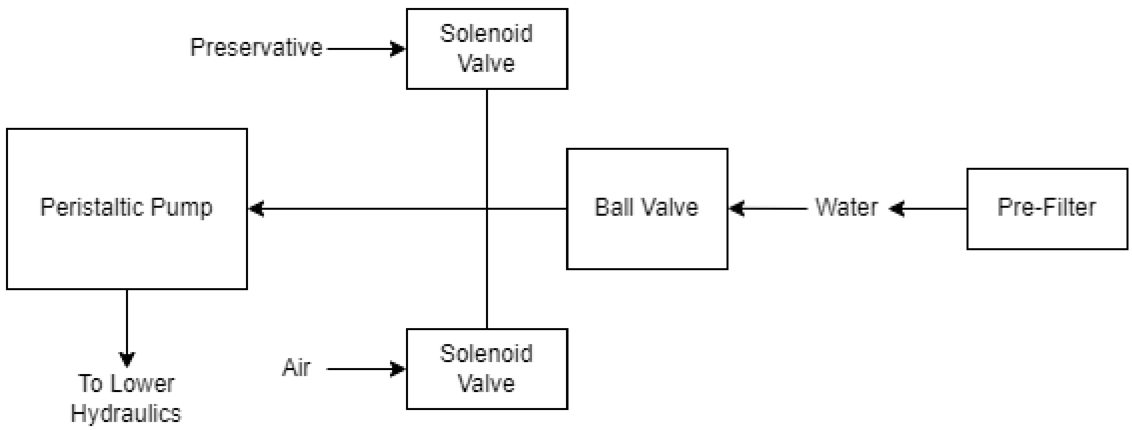


Figure 2: Pump and Input Hydraulics

#### 2.1.2 The Lower Hydraulics

The Lower Hydraulic Rail consists of 24 solenoid valves connected parallel to each other and controls which filter liquid flows through. These filter valves are split into two sets, one on each side of the sampler. In between these two sets of valves is a M32JM-000105-100PG pressure and temperature sensor. The temperature is logged for later use and the pressure is used for monitoring, stopping an operation if the pressure exceeds a certain margin. At the end of the Lower Hydraulic Rail is another solenoid valve which allows for the lower hydraulics to be purged of their current contents when necessary.

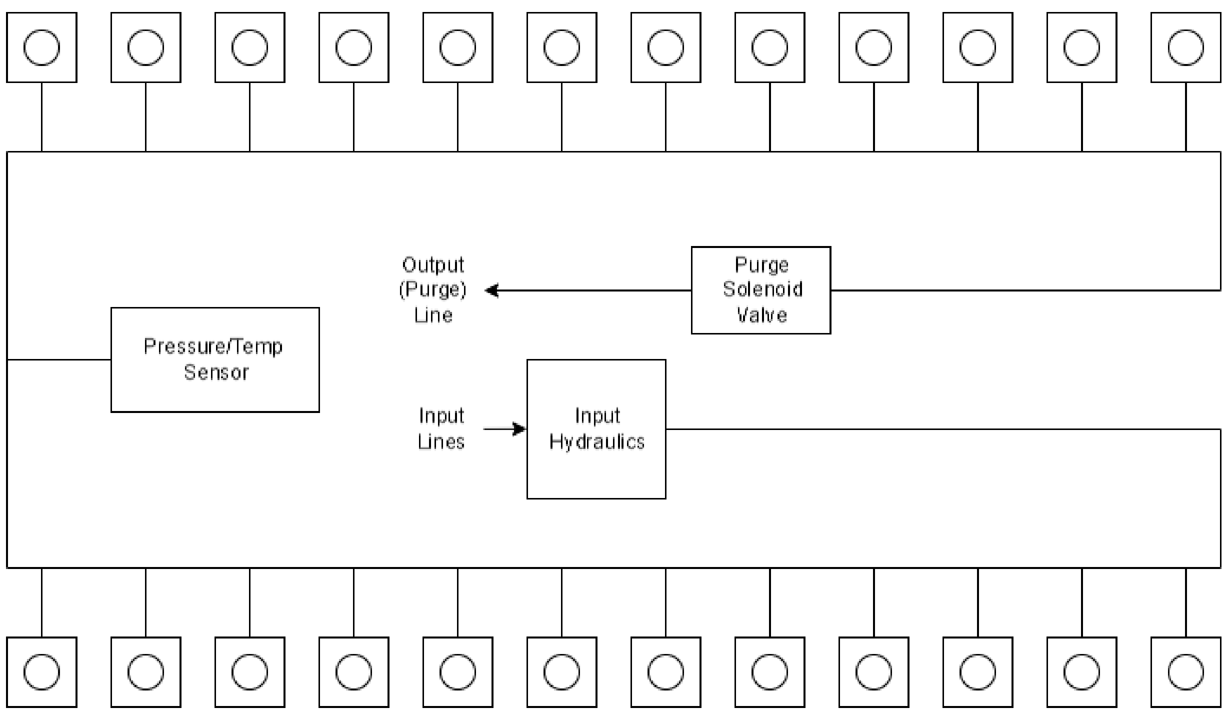


Figure 3: Lower Hydraulic System

#### 2.1.3 The Filter Housings

Downstream of each filter solenoid valve there is a tee connection that goes to a one-way check valve and a modified Advantec filter. The one-way check valve allows air into the solenoid valve that opens when the pump runs backwards. The Advantec filter is modified with a CPC quick disconnect and a one-way check valve. The one-way check valve is connected to the Upper Hydraulics and is used to prevent liquid from going backwards through the filter. The Upper Hydraulics simply connects the output of all the filters to one central line that goes through a flow meter and out of the sampler.

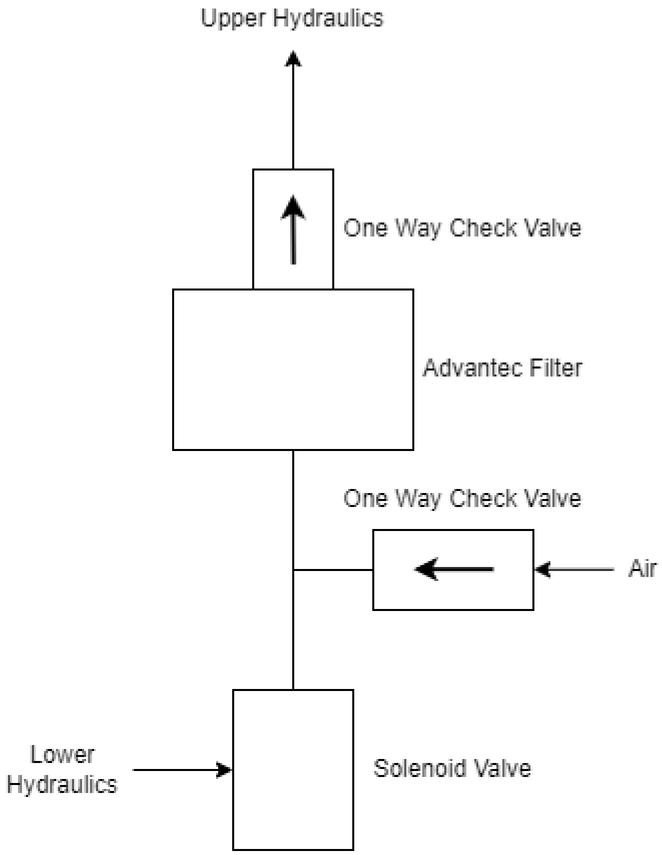


Figure 4: Filter Hydraulics

#### 2.1.4 The Upper Hydraulics and Outputs

The output of the filters connect into a single output hydraulic line. This line is the main output of the filters, any water that goes through the filters will end up going though this line. This allows for a single flow meter to be added that can measure the flow going through any of the filters. This flow meter is crucial as this is how the sampler knows how much water has gone through a filter. After the flow meter the output line goes outside of the sampler and lets liquids flow back into the environment.

2.2 Sampling Procedure

Having worked on multiple iterations of the sampler, we have decided to go with a 13-step sampling sequence that helps reduce cross contamination significantly. This sequence can be split into 9 unique steps: Idle, Prefilter Clear, Flush, Offshoot Clean, De-pressure, Sample, Preservative Flush, Preservative, Air Flush, and End.

1. Idle
2. Prefilter Clear
3. Flush
4. Offshoot Clean
5. Flush
6. Sample

7. De-pressure
8. Preservative Flush
9. Preservative
10. Flush
11. Offshoot Clean
12. Air Flush
13. End

#### 2.2.1 The Idle State

Idle is the default state of the sampler. The sampler waits for a signal from the RTC to move to the first/next state of the Sampling Sequence. If the sampler is not in sleep mode, this is when a client would interact with the UI to do a handful of tasks such as setting up a Sampling Schedule or using the other task utilities. If the sampler is in sleep mode, then only the RTC and supporting circuits are powered. This means there is no way to interact with the sampler without exiting sleep mode.

#### 2.2.2 The Prefilter Clear

Once the RTC sends the signal to start a sample procedure, the sampler enters the Prefilter Clear (PC) state. The purge and input ball valve are opened, and the pump is run in the backwards direction. This will allow for air to flow from the purge and out the input line. This is used to clear the prefilter of anything that might be clogging it, such as accumulated debris. This state runs for X seconds, before moving onto the next state.

#### 2.2.3 The Flush State

The Flush state prepares the lower hydraulics before the next state. The Flush state starts with the purge valve and the ball valving opening, then the motor starts to run in the forward direction. This fills the lower hydraulics with sample liquid and clears out/dilutes and liquid that remained from previous sample. The Flush state runs for the time specified when the Sampling Schedule is created. We recommend a Flush time of 6 minutes.

#### 2.2.4 The Offshoot Clean

The OC state closes the purge valve and opens the filter valve for the filter which is about to be used. The pump runs backwards for a few seconds. This clears anything that might be in the tube between the valve and the filter (what we refer to as the offshoot). The Flush state is run one more time before moving to the Sample state.

#### 2.2.5 The Sample State

In the Sample state, the system pushes the sample water through the filter. This is done by opening the filter solenoid valve and Ball Valve and running the pump in the forward direction. The system moves to the next state when the target Sample Volume is reached. This volume is measured by a Flow Meter on the filter output line. There is an additional condition that will end the Sample state, Sample Time. This time cutoff was added since the filter clogs, rapidly decreasing the flow rate during the sample process. To prevent the sample state running for too long, the time limit was implemented. Both conditions are set during task scheduling. Since the pressure greatly increases due to the clogged filter, the de-pressure state is used to reduce the pressure in the lower hydraulics to ensure that the valves can operate consistently.

#### 2.2.6 The Preservative Flush

The Preservative Flush state is nearly identical to the Flush state except the Preservative input valve is used instead of the ball valve. The lower hydraulics are saturated with preservative, preventing additional sample water that may have been stored in the lower hydraulics from going through the filter. If this water was allowed through the filter, then the Sample Volume would be inaccurate by the end of the sequence.

#### 2.2.7 The Preservative State

The Preservative state is like the Sample state except preservative is the input fluid instead of sample water. This state runs for a time specified by the user during scheduling.

#### 2.2.8 The Air Flush

Before the Air Flush, another sequence of Flush and Offshoot Clean states are run to purge the leftover preservative in the lower hydraulics. After these two states, an Air Flush (AF) state is run which is identical to the Flush and PF states but uses the air valve as the input instead of the other two inputs. This ensures that any liquid that is in the lower hydraulics is purged.

#### 2.2.9 The End State

In the End state, the system sets an RTC alarm for the time of the next sample. The system then moves into Idle and if the system was in sleep mode, then the system will go into its low power state.

2.3 Utilities

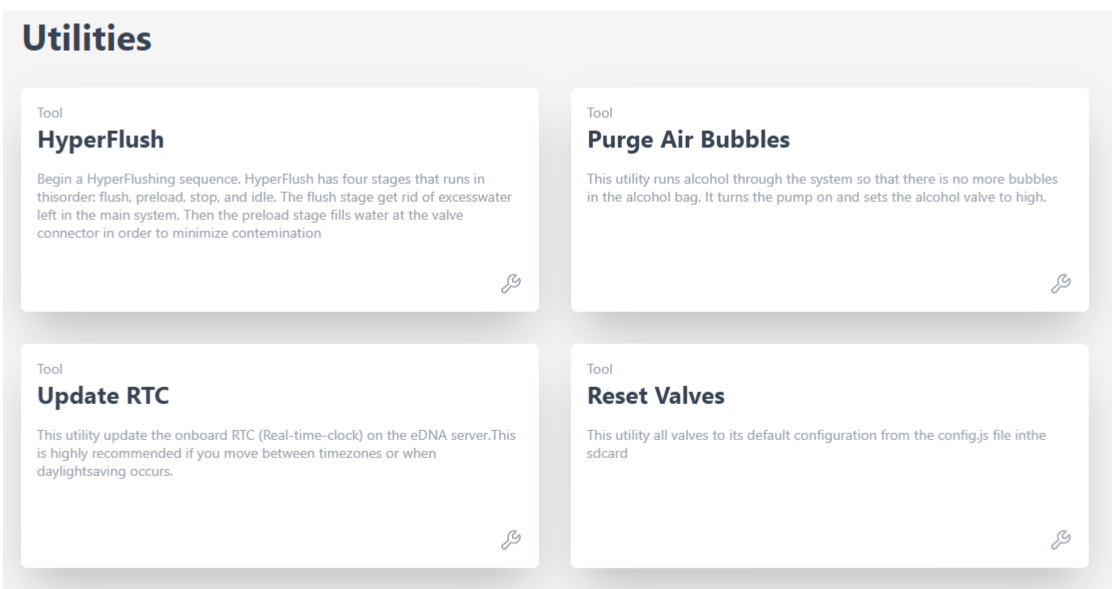


Figure 5: The utilities page in the PolyWAG sampler user interface

The HyperFlush utility runs water through every filter sequentially for a few seconds per filter. This is mainly used for cleaning out the system after a sample task (i.e., a set of 24 samples) to prevent any unwanted cross contamination. This utility can also be used to test the basic functionality of the sampler, as nearly every component is activated during this sequence.

The Preservative Air Purge (PAP) utility turns the pump on and opens the alcohol valve for 10 seconds. This runs some alcohol through the system and removes air bubbles from the alcohol bag. Often it helps to use this utility multiple times and to tilt the Preservative Bladder so that the air is near the port.

The Update RTC utility is needed to make sure that the time on the sampler matches your local time, so scheduling a task will remain accurate. Whenever the system is fully depowered (ie the battery is removed), or when new code is uploaded to the microcontroller, the RTC will need to be updated. It is also recommended that the RTC is updated when there is a daylight-saving change, or when you move between time zones.

The Reset Valves Utility is used when valves have been sampled that you want to be sampled again. This is required since the system 'locks' the filter valves when they have been used in a sample, this prevents samples from being corrupted accidentally. The code does not let you sample a valve multiple times without being reset to prevent messing up a sample. It is important to note that this utility will reset all valves, not a specific one.

## 2.4 Electronics

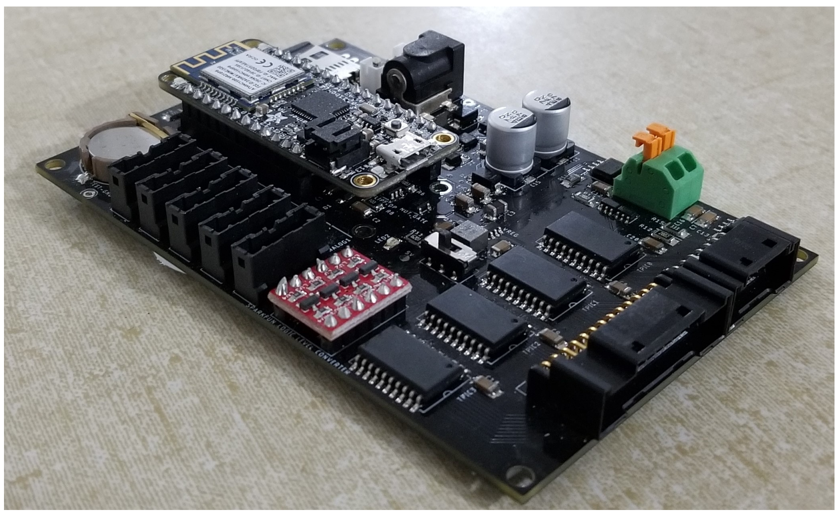

Figure 6: The main electronics control board for the PolyWAG sampler

The PolyWAG Sampler is designed with a custom electronics control board that can be split into 8-10 blocks with an Adafruit Feather M0 at its core. These blocks consist of the microcontroller/Wifi Block, Power, RTC, and sleep control blocks, and the output blocks consisting of the Shift Register, Pump, and Ball-Valve H-Bridge Blocks.

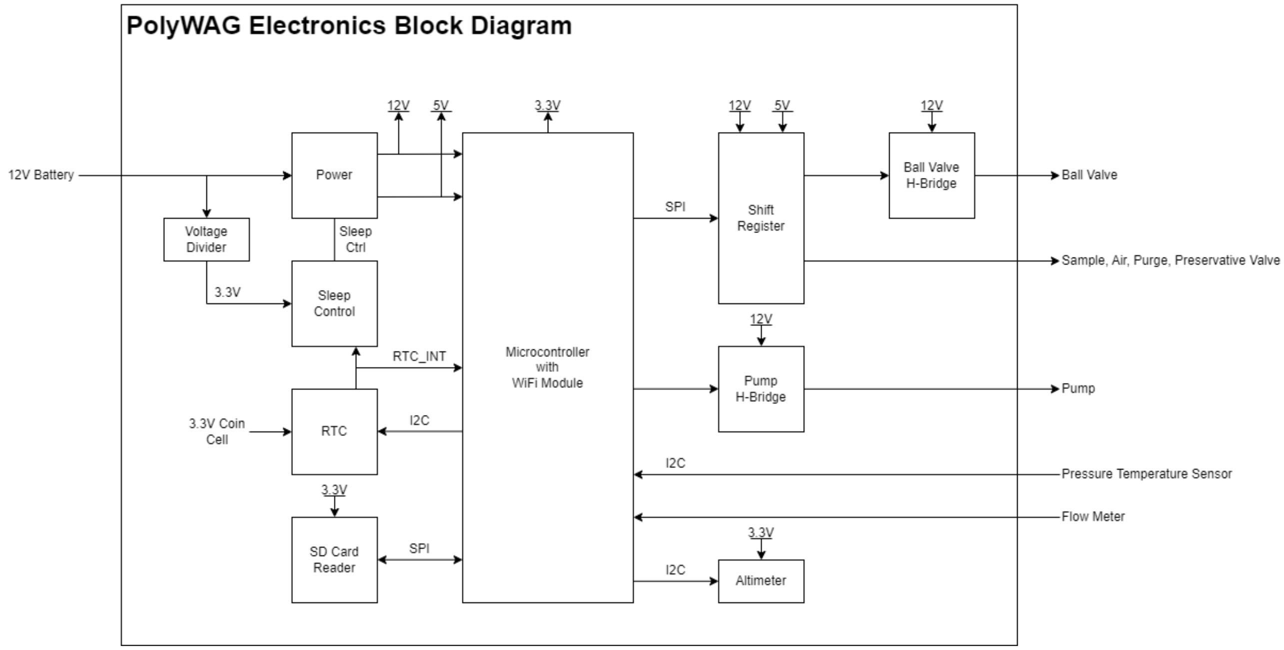


Figure 7: Block diagram of the main electronics control board

The power block consists of a reverse polarity current (RPC) circuit and a voltage regulator circuit. The RPC Circuit was added to protect the 12V battery from current flowing backwards through the system. While the battery has its own protection circuits, they lock the battery in the case of a short and need to be reset using the battery charger. The RPC circuit was added to prevent any “permanent” power loss while in the field. The voltage regulator circuit is a 12V to 5V regulator with an enable pin that connects to the sleep control circuit. This is used to save power during long term deployments.

The RTC and sleep control circuit are used to keep track of time and to save power respectively. The sleep control circuit controls the output of the power circuit and is constantly being power by a simple voltage divider circuit. It is basically a Flip Flop circuit that is reset when the RTC triggers an interrupt. The RTC circuit is used to keep track of the time between samples and is powered by a coin cell while power is off. This allows it to keep accurate track of time and signals an interrupt when its internal alarm is triggered. This interrupt is used to both turn power back on and to inform the microcontroller that it is time for a sample. If noise causes the sleep control circuit to reactivate power, the microcontroller will see that the RTC did not trigger the interrupt and will fall back into power saving mode.

The shift register circuit consists of 4 8-bit shift registers connect to the microcontroller via SPI. The shift registers are pull-down style shift registers where the ‘output’ pins are pulled to ground. This allows the shift registers to control devices that use higher logic voltages. This allows us to control the 27 12V solenoid valves with a 5V IC. The shift registers are also used to control the H-bridge for the Ball valve. The H-bridge for the pump is controlled directly by the micro-controller itself.

The board contains an SD Card circuit for data logging purposes. The data is logged every second and includes the current state, time, and data from the sensors. The sensors include an in-line pressure temperature sensors for monitoring the lower hydraulic line and a flow meter out the output for measuring volume.

The micro-controller of choice is an Adafruit Feather M0 WiFi. The WiFi version of the Feather M0 was chosen as the user interface requires the feather to host a web-server.

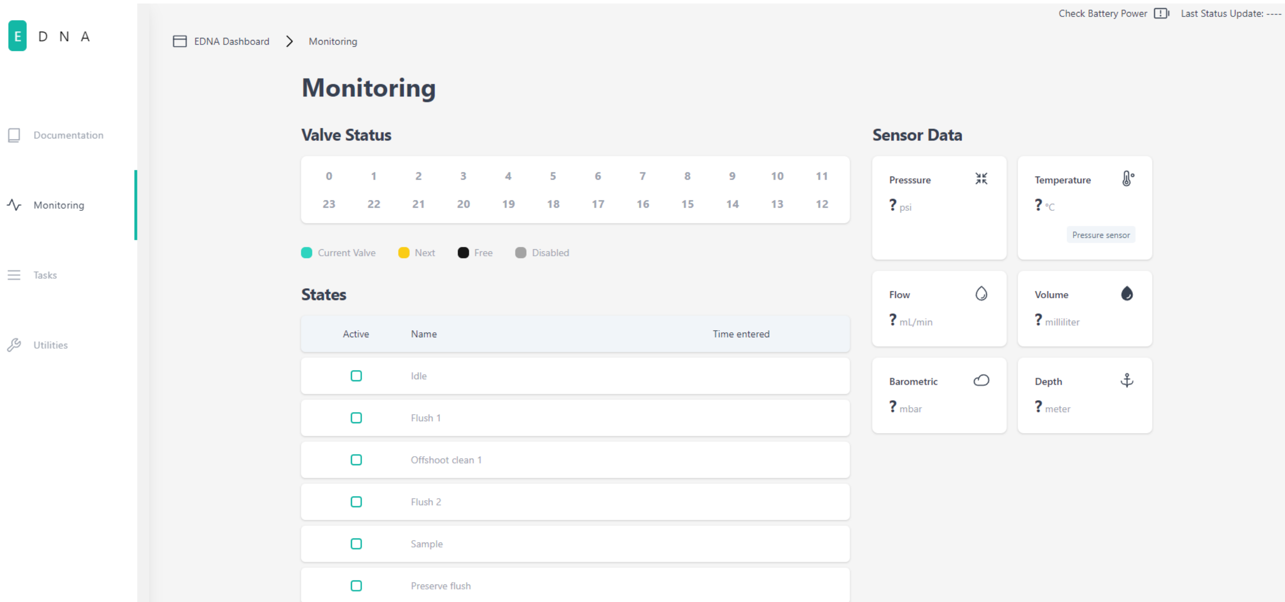


Figure 8: The monitoring page in the PolyWAG sampler user interface

PolyWAG Sampler hosts a webserver that can be connected to via a browser. This acts as the user interface for the system. There are three main sections that make up the user interface: monitoring, tasks, and utilities. The monitoring page displays the data from the sensors, the current state of the sampling procedure, and information on the sampling valves such as the current valve being sampled, and which valves are locked and unlocked.

The utilities page is used to activate the utilities mentioned earlier. The tasks page is where sampling tasks are created. Multiple tasks can be created, and each task is saved in memory for later modification and use. This page is also where tasks can be scheduled for sampling. Each task contains the information on which valves are being used as well as for how long each state occurs.

### 3. Design Files Summary

| Design filename | File type | Open source license | Location of the file |
|---|---|---|---|
| CAD Assembly | CAD file | CERN-OHL-S 2.0 | ... |
| Battery Bracket | CAD file | CERN-OHL-S 2.0 | ... |
| Sample Valve Mount | CAD file | CERN-OHL-S 2.0 | ... |
| Preservative Valve Mount | CAD file | CERN-OHL-S 2.0 | ... |
| Flow Meter Mount | CAD file | CERN-OHL-S 2.0 | ... |
| Tube Guide | CAD file | CERN-OHL-S 2.0 | ... |
| Central Assembly Mount | CAD file | CERN-OHL-S 2.0 | ... |
| Control Board Mount | CAD file | CERN-OHL-S 2.0 | ... |
| Electronics Box Lid | CAD file | CERN-OHL-S 2.0 | ... |
| Control Board Schematic | EDA file | CERN-OHL-S 2.0 | ... |
| Control Board PCB | EDA file | CERN-OHL-S 2.0 | ... |
| Switch Breakout Schematic | EDA file | CERN-OHL-S 2.0 | ... |
| Switch Breakout PCB | EDA file | CERN-OHL-S 2.0 | ... |
| UI Code | Software | GPL 3.0 | ... |
| Device (Server) Code | Software | GPL 3.0 | ... |

Table 2:

- The CAD Assembly is a CAD file with every major components. The tubing and minor things such as zip ties for cable routing are not included.
- The Battery Bracket is a 3D-Printed component to hold down the battery during transit. Paired with a Velcro strap, the battery does not move.
- The Sample Valve Mount is a 3D-printed bracket that holds four solenoid valves. There are six brackets in the sampler and each valve corresponds with a filter.
- The Preservative Valve Mount is a 3D-printed component that holds the preservative valve in place.
- The Flow Meter Mount is a 3D-printed bracket that holds the flow sensor to the frame.
- The Tube Guide is a 3D-printed components that helps hold the input and output tubes in place.
- The Central Assembly Mount is a laser-cut acrylic components that all of the ”central” components mount to. This includes the pump, input control valves, and the battery.

- The Central Board mount is a laser-cut acrylic components that the allows the main control board to mount inside the electronics box.
- The electronics box lid is a CNCed Acrylic components that replaces the metal lid but maintains the groove for the gasket.
- The Control Board Schematic and PCB are EDA files in the Autodesk EAGLE format for the sampler's main control board.
- The Switch Breakout Schematic and PCB are EDA files in the Autodesk EAGLE format for the sampler's main power switch.
- The UI Code is the codebase for the web application. The built UI files are stored in the sampler's SD Card.
- The device (server) code is the codebase for the sampler that is uploaded to the microcontroller. It handles all of the sampler's functions.

## 4. Bill of materials

Given the number of materials required to build a PolyWAG Sampler, The BOM will be located in an external file and can be found here

## 5. Build instructions

Given the complexity of the PolyWAG Sampler, a Build Instructions section of sufficient detail would be near a hundred pages long. This is why an external build guide document will be linked for those who are interested in knowing how one of these samplers are assembled. This file can be located here

## 6. Operation instructions

### 6.1 User Interface Setup & Browser Configuration

The codebase for the user interface (UI) is designed to be compiled into files that are put into the SD card so that the sampler machine can read. As a npm package, Node.js, specifically Node.js 10, and npm are required to run the scripts to compile. Information for installing both is available here. After downloading the codebase repository, it can be installed using the npm CLI commands install and compiled using the build script with the command run-script. The compiled files are created in the /dist subdirectory of the repository.

Note that the sampling machine uses the HTTP protocol, and not HTTPS. This means that depending on your browser, you will have to ensure that it does not fallback to HTTPS when trying to load the UI from the sampler. The WiFi network name and password are set within the configuration.hpp file of the sampler codebase, and the UI is always hosted on the IP 192.168.1.1 on that network.

Instructions beyond a technical description are available here.

### 6.2 Sampler Code Upload

The codebase for the sampler machine uses PlatformIO, an IDE for Microsoft Visual Studio Code. Downloading the codebase also requires downloading another repository, the framework repository, that is used as a Git submodule in the /lib subdirectory. Information on how to use PlatformIO is available here.

Instructions beyond a technical description are available here.

## 6.3 Sampler Cleaning

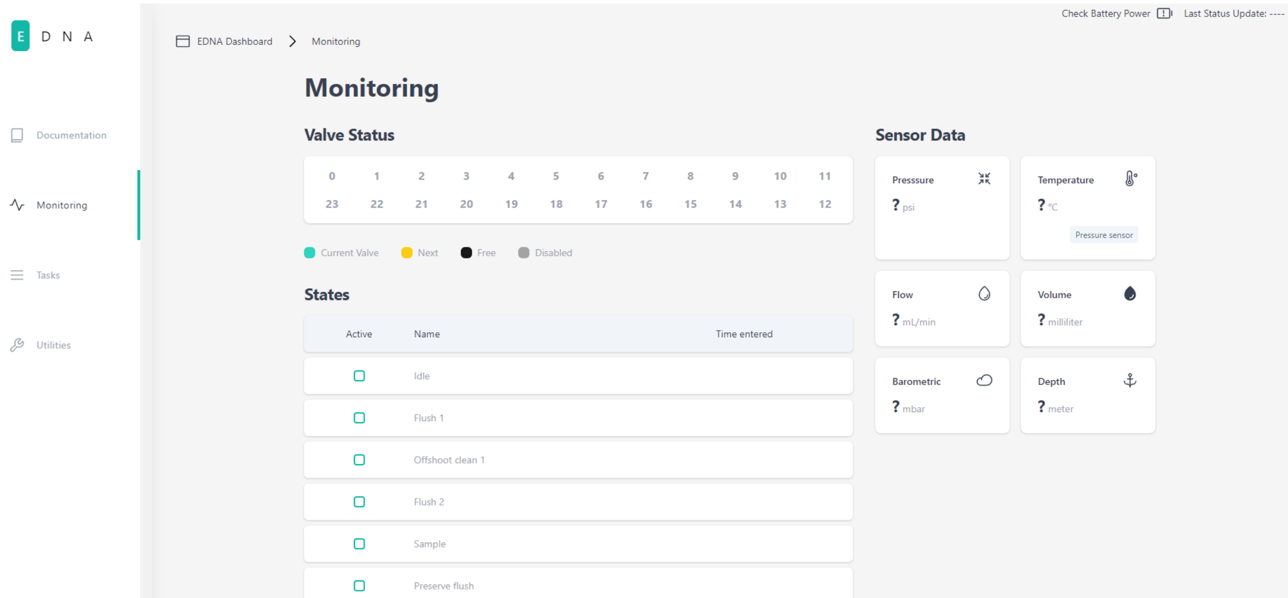


Figure 9: Screenshot of User Interface on Utilities Page

To ensure as little contamination as possible, the Hyperflush procedure can be used to clean the sampler. The procedure is available by connecting to the UI under the utilities tab. To clean the sampler, put all inputs and outputs into a container of bleach, and run the Hyperflush procedure completely five times. Afterwards, put the intake line into Ascorbic Acid and run the HyperFlush procedure five times. Finally, put the intake line into a DI water source and all other connections into a disposal system or sink, and again run Hyperflush five times.

## 6.4 Sampler/Filter Setup

Filter housings have a quick-connect plug on the bottom of their assembly, which allows for them to be quickly switched out with other housings as needed. The filter housings themselves are designed to be hand tightened and loosened to access the membrane filter inside. The valve layout that tasks use follows the diagram below:

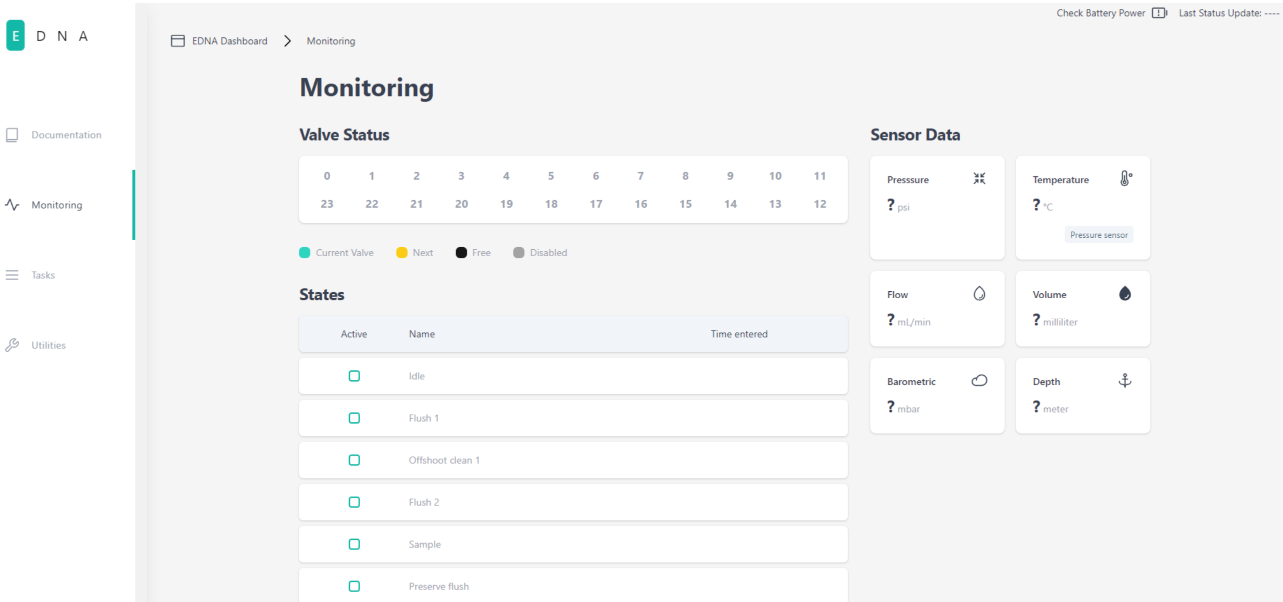


Figure 10: Visual indicator of filter labels

## 6.5 Task Configuration

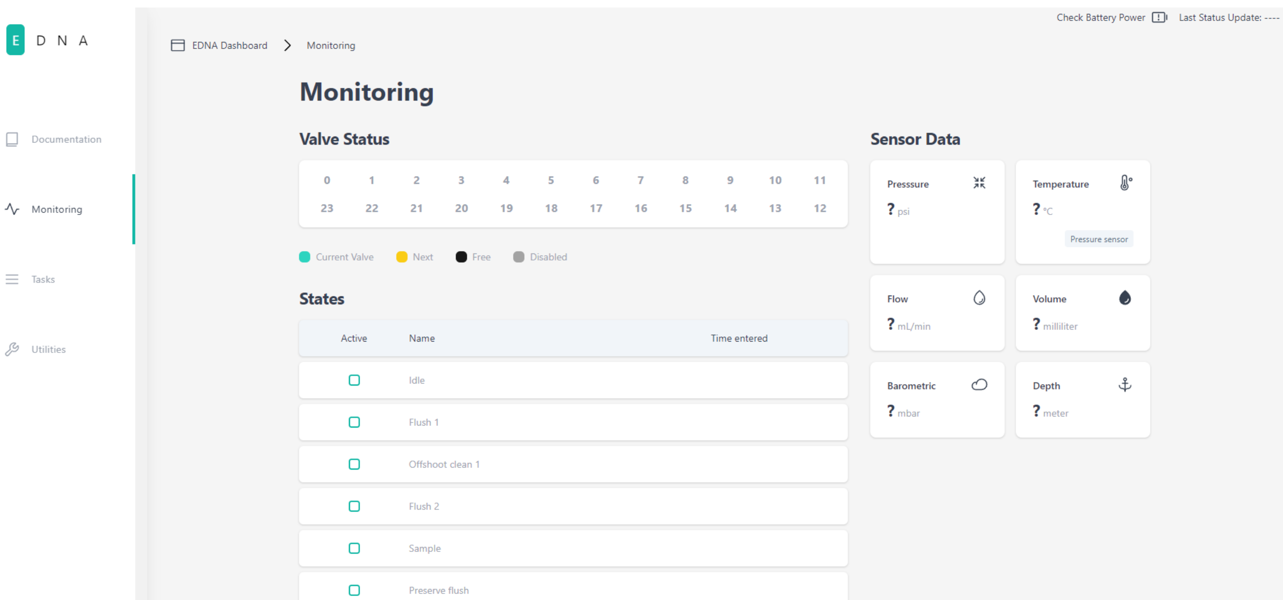


Figure 11: Screenshot of User Interface on Task Page

Scheduling a task is done with the UI by navigating to the tasks tab, and either creating a new task or modifying an old one. Tasks are sorted on the task tab by whether they are scheduled or inactive. Clicking on a task will bring you to the task configuration page, where you can set different parameters for the task. Scheduled tasks need to be unscheduled if they are going to be modified, otherwise they will be executed on the scheduled time. If a task has any parameters change, they must be saved before scheduled.

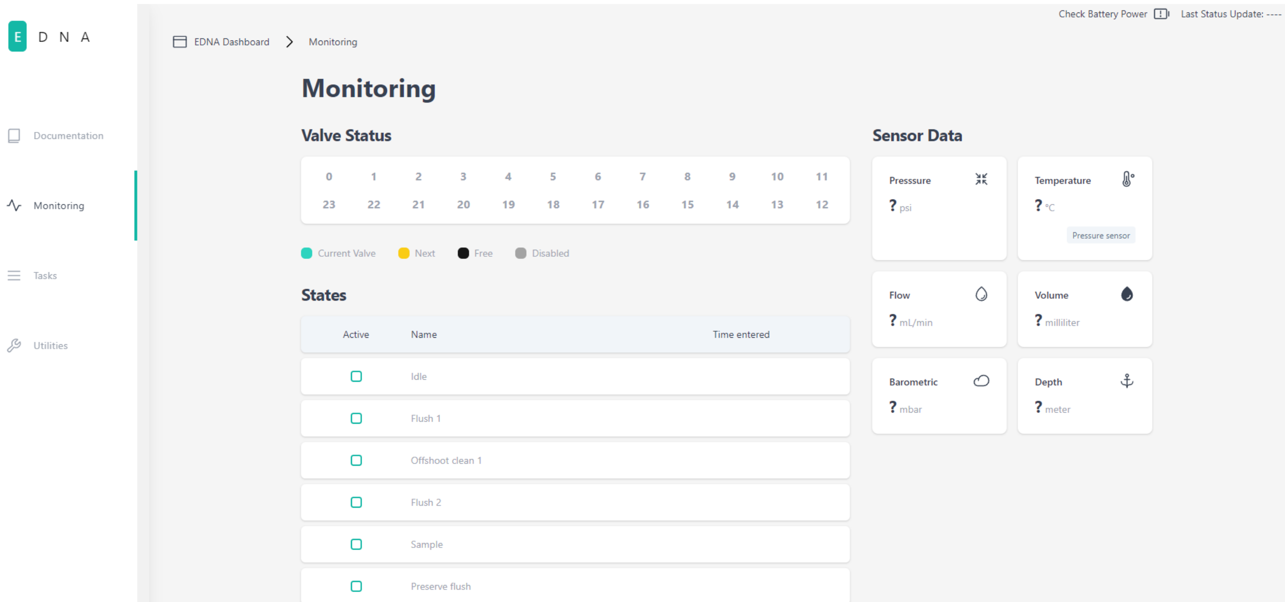


Figure 12: Screenshot of User Interface of a Task Configuration Page

Tasks can sample with multiple filters, with the ability to set the time between the ending of sampling one to the start of another sampling. Additionally, some states of the sampling procedure have a variable time that can be set by the user: flush, sample, dry, and preserve states. The sample state is unique in that the state will be considered complete depending on the time in the state, the volume of water sampled, and the maximum pressure reached while sampling. All three parameters can be set by the user depending on their use case.

## 7. Validation and characterization

The PolyWAG Sampler was tested by the Openly Published Environmental Sensing Lab at Oregon State University. It was characterized by a blend of isolated in-lab testing procedures as well as field tests on the Willamette River and the Irish Bend Covered Bridge In Corvallis, Oregon. To assess the capabilities of the sampler, the following evaluations were conducted: to establish the viability of field deployment at running water sources in Alaska.

### 7.1 Cross-Contamination Testing

The primary use case of the PolyWAG eDNA sampler is for use in water sampling to capture existing trace biological information in the form of DNA by means of filtration. In gauging the use case of the sampler for eDNA collection and characterization, we subjected the sampler to a lengthy cross-contamination study for detecting residual DNA in subsequent sample steps. Prior to sampling, a cleaning and sterilization process was conducted to eliminate any sources of contamination from previous testing. After running the sampling procedure, the results were evaluated by means of Polymerase-chain reaction (PCR).

The basis for these observations is the use of Alaskan Sockeye Salmon DNA. To obtain Alaskan Sockeye Salmon DNA, 1 filet of salmon (~500g) is placed in an 8 gallon container of DI Water and left for 24 hours. During this time, biological material diffuses into the bulk water. After 24 hours the remaining salmon mass is removed to ensure constant salmon DNA concentration across trials.

The sample cleaning procedure makes use of the sampler's HyperFlush utility to sterilize both the filter housings and the sampler hydraulic lines. The HyperHlush utility flushes each valve of the system sequentially with any bulk liquid connected to the input line. The cleaning procedure makes use of three HyperFlush cycles in the following liquid order: DI water, 6% bleach, 5% ascorbic acid. These cycles are repeated three times and followed by three subsequent HyperFlush cycles of DI water to purge all residual chemicals

The sampling procedure for cross-contamination characterization makes use of a sample of DNA followed by subsequent samples of DI Water. To establish experimental controls, the first sample of the study is a negative control containing DI water. This negative control gauges the success of the cleaning protocol, ensuring no prior contamination. Following this control, the process consists of three subsequent samples: Fish water (Positive Control) and two samples of DI water. Separate inlet lines are used for DI water and fish water to isolate the source of cross-contamination to the sampler itself, the lines being switched manually between samples. The data collected during this study consists of four trials performed using the following task settings on the eDNA UI: 1000 mL max sample volume, 4-minute max sample time, 10-second preservative flush, and 12-minute flush time (∼5L). After sampling, the samples were individually packaged and sent for PCR analysis.

For PCR analysis, the cellulose nitrate filters containing the salmon DNA are dissolved releasing the trapped DNA into solution. Sockeye Salmon primers were added to the DNA solution. During PCR the DNA is amplified on a scale of 109 times the original DNA concentration. From here, immuno-fluorescence is used to quantify the amount of DNA retrieved by measuring the intensity of light emitted by the sockeye-salmon DNA. These values are interpreted in terms of a CT score. CT scores between 0-40 CT indicates the presence of Salmon DNA, while a CT score >40 indicates no Salmon DNA is detected.

Using these processes 13 samples were taken using the sampler. This included 1 Negative control, and 4 sample trials.

| Sample Name | CT | Results |
|---|---|---|
| Negative Control | 39.65583038 | **Positive-Low Quantity(1/2, high Ct)** |
| Negative Control | Undetermined | |
| Positive Control | 29.15957832 | |
| Positive Control | 29.47208214 | **Positive** |
| Trial 1: Sample 1 | Undetermined | |
| Trial 1: Sample 1 | Undetermined | **Negative** |
| Trial 1: Sample 2 | Undetermined | |
| Trial 1: Sample 2 | Undetermined | **Negative** |
| Positive Control | 30.94010544 | **Positive** |
| Positive Control | 30.6325016 | **Positive** |
| Trial 2: Sample 1 | Undetermined | |
| Trial 2: Sample 1 | 39.86541748 | **Positive-Low Quantity(1/2, high Ct)** |
| Trial 2: Sample 2 | Undetermined | **Negative** |
| Trial 2: Sample 2 | Undetermined | **Negative** |
| Positive Control | 29.15957832 | |
| Positive Control | 29.47208214 | **Positive** |
| Trial 3: Sample 1 | Undetermined | |
| Trial 3: Sample 1 | 39.46964645 | **Positive-Low Quantity(1/2, high Ct)** |
| Trial 3: Sample 2 | Undetermined | |
| Trial 3: Sample 2 | Undetermined | **Negative** |
| Positive Control | 29.15957832 | |
| Positive Control | 29.47208214 | **Positive** |
| Trial 4: Sample 1 | Undetermined | |
| Trial 4: Sample 1 | Undetermined | **Negative** |
| Trial 4: Sample 2 | Undetermined | |
| Trial 4: Sample 2 | Undetermined | **Negative** |

Table 3:

The 4 trials conducted came to varying degrees of success. In trials one and four, the CT scores of the samples following the positive controls were undetectable. This indicates that there is no detectable DNA in the PCR-amplified sample. Samples two and three contained CT scores <40; however, in very low detectable quantities. In both cases, the sample immediately following the control are the samples in question. In the duplicate amplification one trial contained a CT score between 39 and 40 while the other was undetectable. This indicates a very faint presence of fish DNA between samples. The cause of this can be explained by two reasons. One is that the sampler is unable to prevent cross-contamination between trials. The other is that the cleaning cycle is not adequate for ridding all DNA in the sampler. The latter of the two seems more likely because of the negative control. Like the two barely detectable samples the negative control indicated a faint positive signal. This means one of two things: contaminated sample water or insufficient cleaning cycle. Based on these results the sampler does not justify evidence that the sampler prevents cross-contamination between samples. That being said there is evidence that the flushes between samples do minimize contamination. This is supported because sample two in all trials were identified as negative. Therefore, increasing the flush and cleaning times shows promise for mitigating cross-contamination issues.

### 7.1.1 Turbidity

To ensure the effective use of the sampler in non-ideal conditions, the sampler was analyzed by means of a flow analysis across a range of turbidity levels. The purpose of this test is to ensure adequate flow through the filter before it clogs. For eDNA purposes a minimum of 500 mL of filtered volume is required to accurately analyze the DNA concentration of the input stream. For testing, a 5.0 um filter and 75 um pre-filter are evaluated.

Based on water turbidity data for Alaskan streams in April, the turbidity of the water ranges from 30-80 NTU. To reproduce this range, a 2000 NTU formazin based turbidity standard was diluted to produce 3L of the following NTU standards: 50, 100, 250 NTU. These turbidity standards were mixed within a beaker with a 2-inch stir bar at the lowest speed that a vortex was observed. The 75 um system pre-filter was suspended two inches from the bottom of the beaker while sampling. Sampling was conducted and stopped once the flow rate dropped below 60 mL/min. This drop in flow rate indicates a filter clogging.

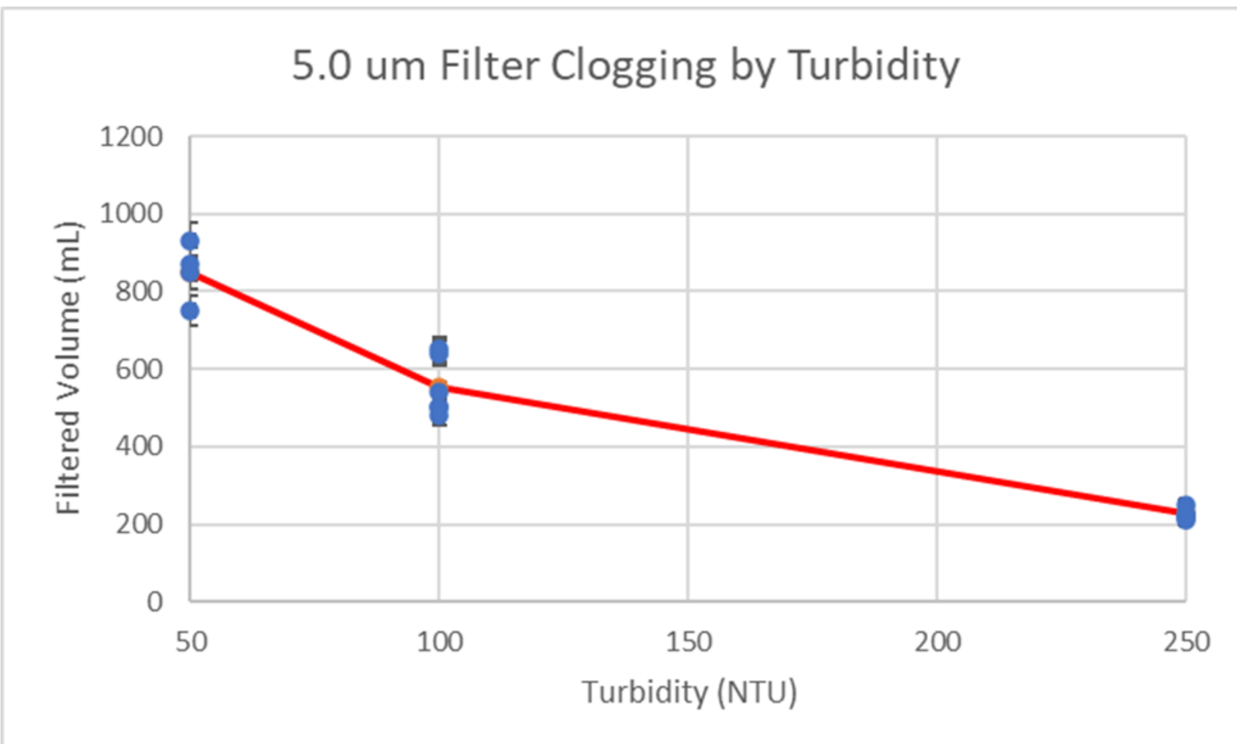


Figure 13:

To gauge the effectiveness of the sampler in the different turbidites the values are compared to the 500 mL minimum filtered volume. Greater than 500 mL of filtered volume indicates a successful sampler run at that water quality. As a result of this testing, the sampler is found to successfully operate in the range of 30-80 NTU. The data indicates the sampler can run in water qualities up to 100 NTU before samples under 500 mL are observed. Subsequent testing at 250 NTU indicates for lower-quality water streams, the sampler's function decreases substantially, only filtering 200-250 mL per filter.This indicates that the sampler's peristaltic pump is not able to build a high enough pressure gradient across the filter. Therefore for environments with lower water quality an increase in pump power is needed.

After conducting idealized laboratory trials, the sampler was placed in the field to test the flow for real-world applications. The sampler was placed in two locations in Corvallis, Oregon: the Irish Bend Covered Bridge on Oak Creek, and the Willamette Boat Landing on the Willamette River. In both locations, the sampler was deployed on the shore of the river with an inlet hose that ran to faster-moving water. For Oak Creek, the sampler's prefilter was staked 4 inches between the water level in the middle of the stream. For the Willamette River, the inlet hose was immobilized at the end of the Willamette landing boat launch dock. The purpose of this trial is to serve as a proof of concept of the device in real world water streams. In both Oak Creek and the Willamette River, three trials were conducted in series to investigate the effects of non-idealized real world conditions on the sampler. The success of these trials is based on the ability to achieve greater than 500 mL of filtered volume per sample. The two rivers provide variable conditions on the quality of the water. Oak Creek contains low river flow and high water quality, while the Willamette river was experiencing flooding with very murky, low water quality. Without a turbidity sensor, the relative turbidity of these streams were determined relative to the observed turbidity standards used for the lab turbidity testing.

| Filtered Volume Field Testing | | | |
|---|---|---|---|
| Trial | Location | Volume (mL) | Turbidity (NTU) |
| 1 | Oak Creek | 523 | <100 |
| 2 | Oak Creek | 750 | <100 |
| 3 | Oak Creek | 810 | <100 |
| 4 | Willamette River | 270 | >1000 |
| 5 | Willamette River | 300 | >1000 |
| 6 | Willamette River | 340 | >1000 |

Table 4:

As a result of these trials, the sampler performed in much the same way as the lab turbidity trials. For the lower turbidity water sources the sampler had no issues reaching over 500mL of filtered volume. For the higher turbidity sources the 500 mL filtered volume minimum was not met; however, this was expected of the trial. Altogether, these results confirm the use case of the sampler in low turbidity environments.

### 7.1.2 Flow Accuracy Testing

The kinematic viscosity of water varies with temperature. This indicates that the sampler will read different values for flow rate for different temperatures. To ensure the sampler the flowmeter provides accurate flow readings for the upcoming Alaskan trials, the sampler was calibrated to reach accurate flow readings between 3-5°C. For accurate eDNA measurements the accuracy of the filtered volume must be within 10% of the true filtered volume. Using the 10% value as an end condition, several trials were conducted to determine the optimal volume constant for the flow meter pulses. In these trials, the outlet of the sampler is manually measured with a graduated cylinder and compared to the sampler's calculated value.The percent error was then calculated between the two quantities. To ensure accuracy across the sampler's operating range, the filtered volume was measured and compared at 250, 500, and 1000 mL for each volume constant. By averaging these values at each volume constant the average error per volume constant was discovered.

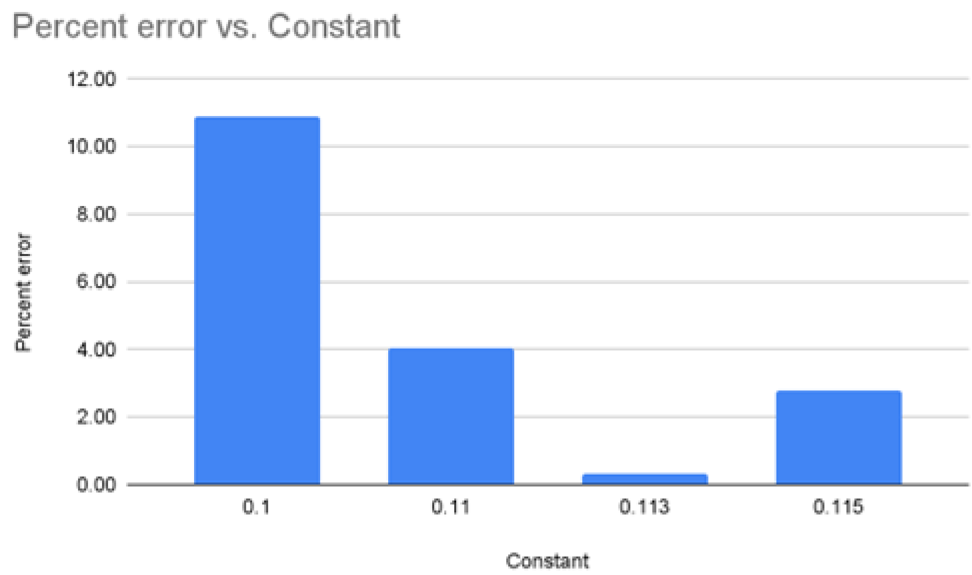


Figure 14:

The results determined from this process indicate the constant that is centered within the error. In other words, the smaller the bar the more the positive and negative errors negate each other. This essentially centers the solution in the error domain. The minimum error indicates an ideal volume constant at or near 0.113. This alone does not demonstrate the accuracy of the volume measurement. Rather this indicates the most normalized variability between the trials. When looking at the max error between trials the hard limit of 10% places these values in context.

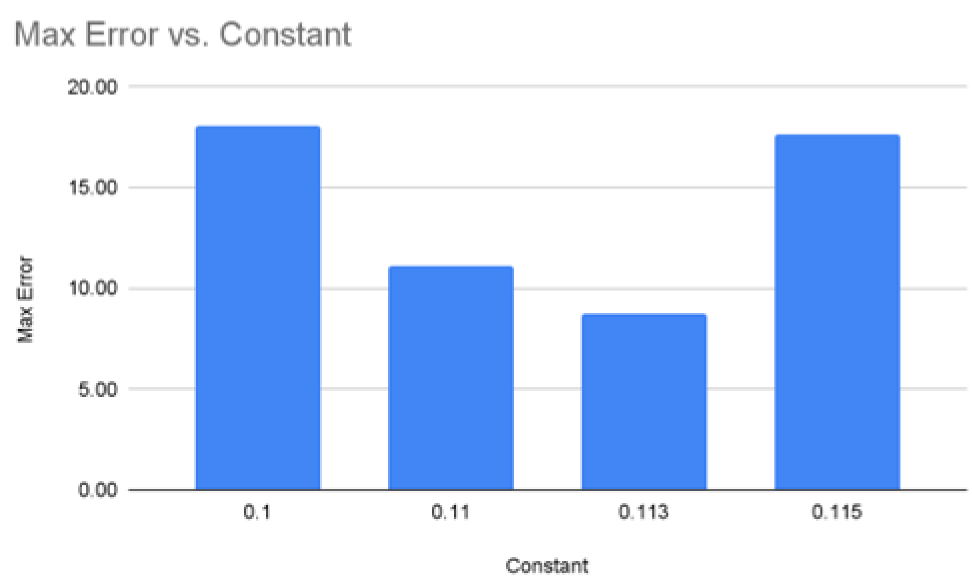


Figure 15:

When viewing the max magnitude of error, a convex solution with a minimum error between real and observed volumes. In this context, the minimum absolute error is also easily determined to be at volume constant 0.113. The max error at this measurement falls below the 10% threshold, indicating the sampler's viability for eDNA collection purposes.

#### 7.1.3 Battery Life Testing

Battery life varies with the ambient temperature of the surrounding environment [6]. To gauge the battery life of the sampler in a cold Alaskan environment the battery was placed in a freezer at 0°C. The battery was left for two weeks to simulate the two-week field deployment time in Alaska. After two weeks, the battery line was plugged into the sampler while remaining in the freezer. A full 24-sample procedure was conducted. To ensure normal operating conditions the full 24-sample procedure was used using the default sampling procedure. Because the battery did not die during this time the sampler was determined to have enough battery life for normal operation of the sampler.

## 8. Conclusion

In our paper, we introduced the PolyWAG eDNA sampler, an automated solution for collecting environmental DNA from aquatic environments. This system addresses the limitations of current eDNA sampling techniques, which are often labor intensive, costly, or limited in capability. Despite its advantages, we acknowledge areas for improvement to enhance its performance further. Our recommendations focus on increasing the sampler's capacity to handle larger volumes and higher turbidity, integrating an automatic calibration for water viscosity adjustments based on temperature changes, and employing more robust components such as stronger pumps and high-pressure rated solenoid valves. Additionally, we propose enhancements like a closed-loop bleach cleaning system for reducing cross-contamination, a pulse damper for pressure stabilization, and improved flow meter accuracy through noise filtering and firmware adjustments. These modifications aim to make the PolyWAG sampler even more effective and reliable for diverse field conditions, paving the way for its broader application in environmental DNA research and monitoring.

**Ethics statements**

The work does not use any human or animal subjects.

**Bios**

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