eDNA Sampler

Figure 1: eDNA Sampler being deployed in the Channel Islands in California in Dec 2019

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Contents

1 Introduction

We are making a low-cost compact underwater environmental DNA (eDNA) sampler. eDNA is a DNA collected from a variety of environmental rather than directly sampled from an individual organism. As various organisms interact with the environment, DNA is expelled and accumulated in their surroundings. The analysis of eDNA has great potential not only for monitoring common species, but also for genetically detecting and identifying other extant species that could influence conservation efforts.

In the future, we expect there to be mass-producible samplers that could be deployed in various manners (i.e. towed by vehicles or carried by Scuba divers) at multiple locations. Keeping track deployments will be very difficult once there are more than a few. Therefore, we propose to use RFID tags embedded filters, each of which corresponds to the deployment information. They can be scanned prior to deployment for the user to configure upcoming deployment as well as obtain filter specific data. This way, we will be able to keep track of all of the samplers online. In this iteration, we provide a proof of concept, where a local network can be established by a local host (e.g. Raspberry Pi or a personal computer). The host simultaneously runs a web application (in this case based on Django Framework) that lets users configure and retrieve data from the eDNA Sampler.

The eDNA project is a joint effort between the MIT Future Ocean Lab, Dana Yoerger's group and Govindarajan Laboratory at Woods Hole Oceanographic Institution (WHOI). Our eDNA Sampler enables the user to set automatic sampling conditions based on depth, temperature (ex: pump between 105m-110m depth) and end conditions based on flow rate and pumped volume (ex: stop pumping when the flow rate is less than 0.5L/min).

The goal of this documentation is to aid engineers, who will be involved with this project, to improve upon this iteration. One should be able to re-build the current iteration by following and understanding the documentation. Hopefully, significant improvements can be made for future iterations in terms of robustness and compactness. We first provide overall system architecture and break down into four components: flow-stack, electronics-stack, RFID readerand PVC shell. Then, we walk you through the process in building each components. We also provide the list of components as well as the user-manual for using this in the field.

2 System Architecture and Discussions

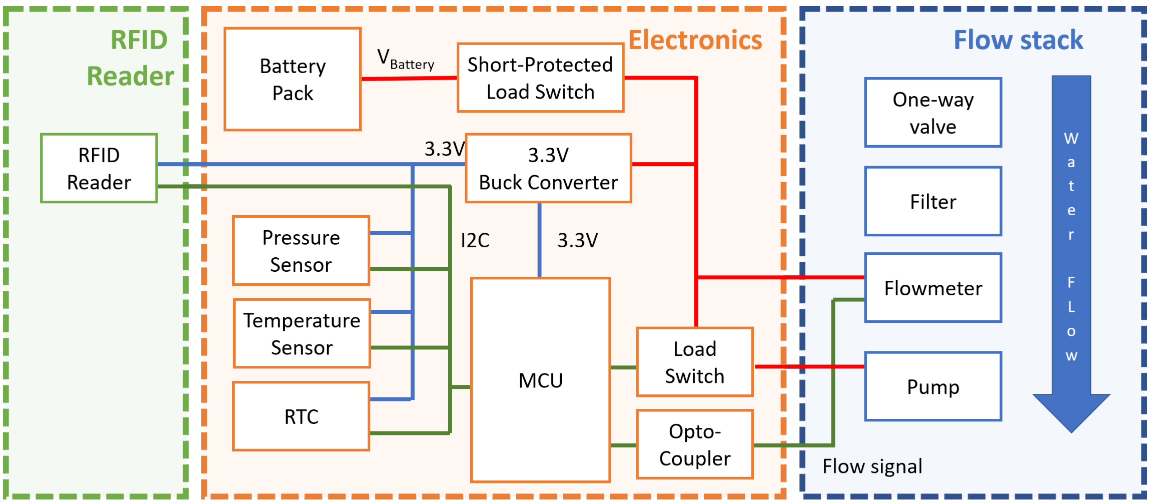


Figure 2: Overall system architecture in a block diagram. The PVC shell will enclose the flow-stack and the housing for the electronics-stack.

The eDNA Sampler can be broken down into four components: flow-stack, electronics-stack, RFID reader and PVC shell. The assembled components of the eDNA Sampler are shown in Fig.3. The flow-stack and the RFID readerare powered and controlled by the electronics-stack as shown in Fig.2. The off-the-shelf parts for the flow-stack come with specific electrical and mechanical constraints. These electrical requirements were met by the electronics-stack, which is independently housed. The PVC shell was designed to enclose the flow-stack and the housing for the electronics-stack. In each of the following sub-sections, we explain some specific design choices.

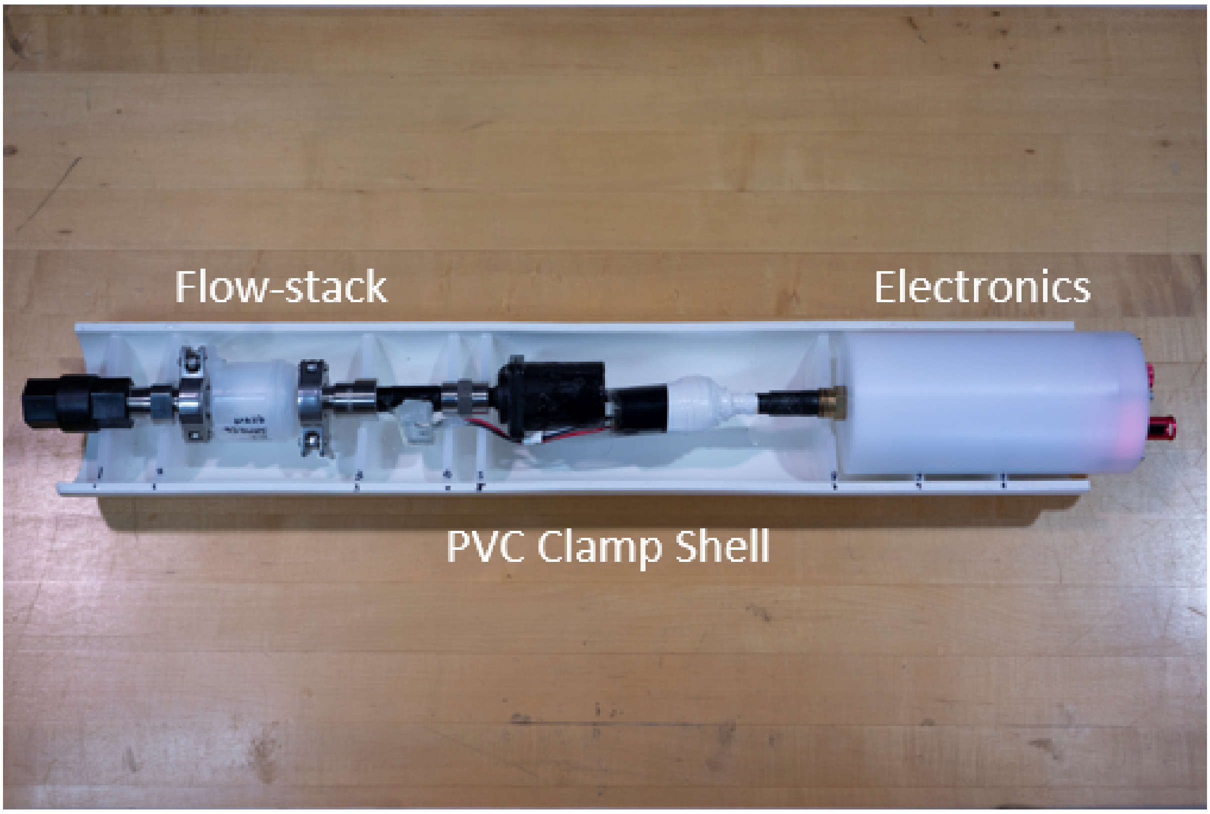
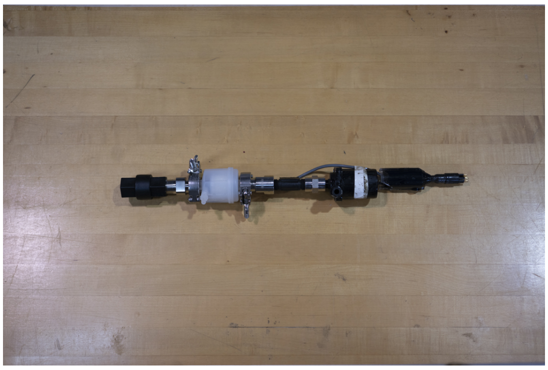
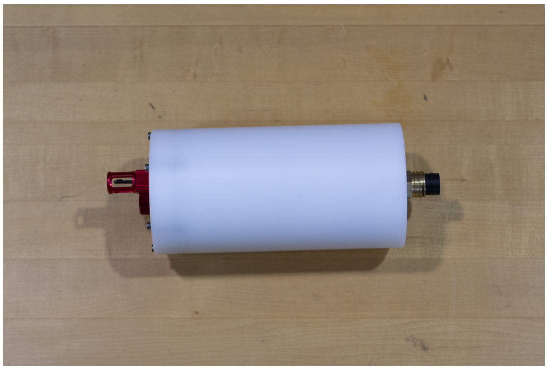


Figure 3: The internals of the eDNA Sampler inside of the PVC shell. The flow-stack and the housing for the electronics-stack are connected with series of adapters. They are clamped by the PVC shell so that the internals are protected and will not fall off accidentally.

2.1 Flow-stack



(a) The flow-stack fully assembled. It consists of (from left to right) a one-way valve, a filter, a flowmeter, a pump and a connector whose wires are potted in 3M Scotchcast. Each parts is connected with NPT adapters for their respective threads.



(b) The housing for the electronics-stack. It has a temperature and a pressure sensors on the endcap and an 8-pin connector on the other end.

Figure 4: The flow-stack and the electronics-stack that are housed inside the PVC shell

Fig.4a shows the flowstack of the system. Kleenpak filter capsule (1) is what has been commonly used by the eDNA researchers at WHOI. As for the flowmeter(2), the manufacturer does not specify its applicability in the ocean and under high pressure. Therefore, these flowmeters require pressure testing as well as calibration. The pump(3) has been used in other projects at WHOI and are used because they are mass-produced and pressure tolerant.

On a separate note, there are flowmeters, such as those shown in Fig.5 that run on the same principles

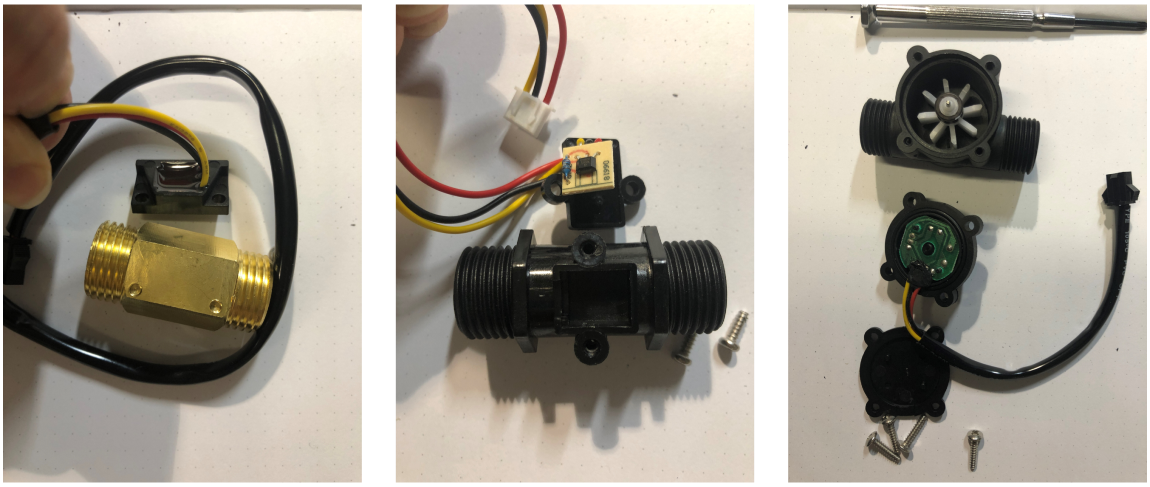


Figure 5: Other flowmeters available in the market. They seem to be usable at depth if the internal electronics are potted well. YF-B2(left), YF-S201C(center), and Digiten(right)

(hall effect sensor) but are significantly cheaper (price around approx. $10) than the ones that are currently used (approx. $110). They are: YF-B2, YF-S201C, and Digiten. The reasons that we did not pursue them in this iteration are: that they required potting the small electronics for pressure tolerance, and that they required G thread adapters, which are not readily available in the US.

2.2 Electronics

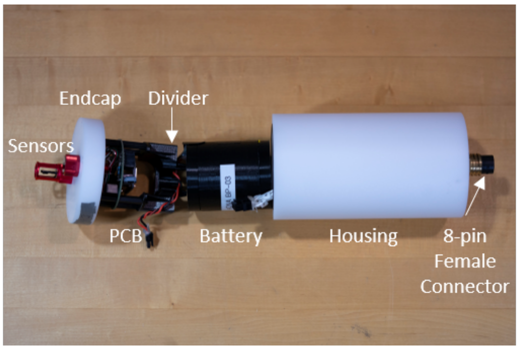
The electronics-stack (shown in Fig. 6a) can be broken down further into the following parts: power unit (the enclose battery pack shown Fig.6b), circuit (Fig.6c), sensors (Fig.6d) and wire connection to the flow-stack. The PCB (shown in Fig.6e and 6f), whose schematics and layouts are uploaded on the same Github project, is a hub which connects the power, sensors and MCU. The circuit channels the power to appropriate parts and deliver signals to and from MCU. The flowmeter and the pump draw the power directly from the battery pack (i.e. 12V - 15.5V) composed of four Lithium Ion batteries(4) with a battery protection board(5) and a standalone short-protection load switch(6) in between. On the other hand, the MCU and other sensors run at 3.3V, which is down-converted by a buck converter(7). The details on power and battery are further discussed in 2.2.2.

2.2.1 MCU (ESP8266)

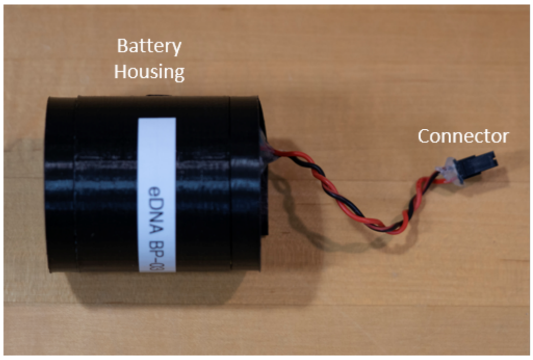
For the MCU, we used Adafruit HUZZAH ESP8266 breakout board(8) because it allows for wireless connection along with some basic functionalities for electronics control and logging the data to flash memory (upto 3MB). It is also programmable using the Arduino IDE, and thus we decided to follow the standard Arudino structure: setup and loop. To simplify the code, all of the work that requires wireless connection happens inside the setup code. This has a downside that the user needs to power-cycle (re-connect power) to re-configure the deployment. Once the configuration is ready, the MCU transitions into the loop section, where it waits to be submerged and triggers the pump when deployment conditions are met. It also logs the data every second. We also continuously measure the flow to check for any contamination of the sample. Since MCU is not equipped with an RTC, we have placed an external DS3231 RTC(9) to keep track of time.

We have, however, noticed a minor WiFi connection issue during our pool test. At one point, it was having difficulty connecting to a WiFi network. This problem was resolved when we replaced with a new MCU. While that is a solution, it seems to be a fragile embedded system.

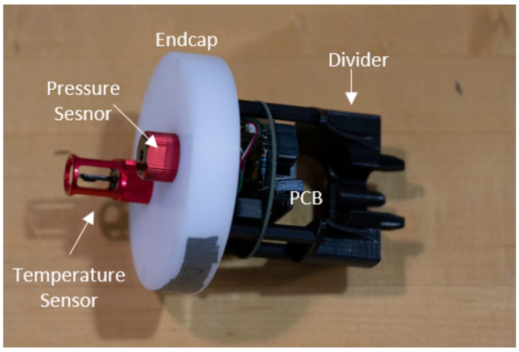
In addition, I think we overloaded the MCU's capability to handle Wi-Fi as well as the embedded system part of the entire system. It may be a good idea to use Teensy and ESP8266 to separate the roles (hardware and wireless).



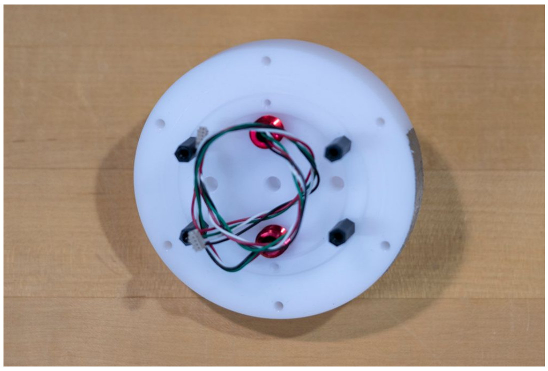
(a) The internals of the electronics-stack housing. It houses a battery and a control circuit.



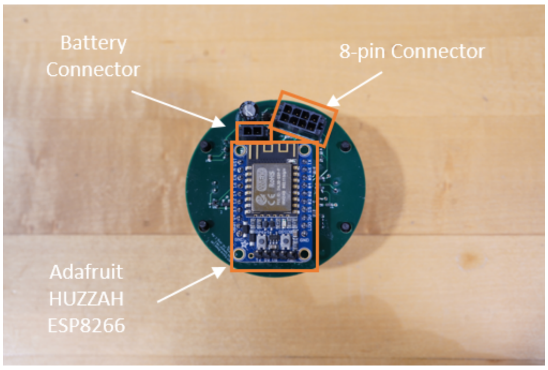
(b) The battery pack with two wires for voltage and ground.



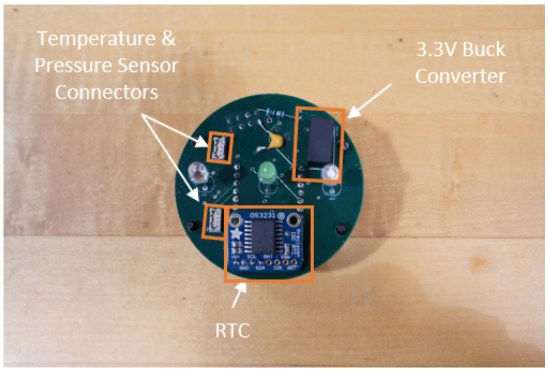
(c) The fully-assembled endcap with electronics mounted on the chassis. There is a spacer that mounts on top of the circuit that blocks from the battery to bounce around.



(d) The buttom layer of the endcap. The temperature and pressure sensors are screwed into the endcap. The PCB has female connectors that allow connections from these sensors.



(e) The top layer of the PCB, which consists of the MCU, load siwtches, connectors to the battery and 8-pin connectors.

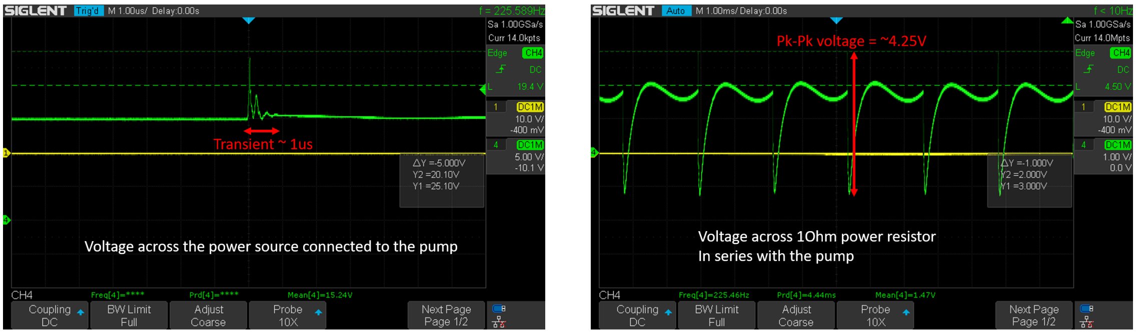


(f) The buttom layer of the PCB, which consists of LEDs, an RTC and 3.3V buck converter.

Figure 6: The electronics-stack

2.2.2 Power and battery pack

Both the flowmeter and the pump require power. We decided to use Lithium Ion batteries which provide high energy density so that we could save some space. The flowmeter is rated to operate between 5V and 24V at 8mA(2) and the pump at 12V at 1A(3). We ran some preliminary testing on the pump to understand its behavior at various voltages. The result follows that the higher the voltage, the more current consumed (i.e. higher power) as well as the faster pumping rate. The performance profile of an arbitrary pump across varying voltages is shown in Table.1 and Fig.8. It seemed unnecessary to fix the voltage at 12V, and thus, we decided to use the raw voltage out of the battery pack as long as the flown water volume is tracked by the flowmeter. The battery can only be charged outside of the electronics



(a) There is a voltage spike ($1\mu s$ long) when the inductive load switches from positive to negative voltage. The voltage was measured across the power supply.

(b) The current drawn, although on average 1.4A at 16V, has a specific waveform. The maximum current can reach as high as 3A.

Figure 7: The electrical behavior of the pump at 16V power supply. The voltage was measured across a 1Ω power resistor connected in series with the pump.

Voltage (V)	6	7	8	9	10	11	12	13	14	15	16
Avg. Current (A)	0.42	0.55	0.62	0.72	0.80	0.90	1.00	1.00	1.05	1.15	1.25
Avg. Power (W)	2.52	3.85	4.96	6.48	8.00	9.90	12.00	13.00	14.70	17.25	20.00
Flowrate (L/min)	1.06	1.50	1.81	1.95	2.05	2.40	2.29	2.67	2.67	3.00	3.16
Efficiency (L/J)	0.42	0.39	0.36	0.30	0.26	0.20	0.19	0.21	0.18	0.17	0.16

Table 1: A preliminary power and performance profile of an arbitrary pump of the same model at varying voltages.

housing.

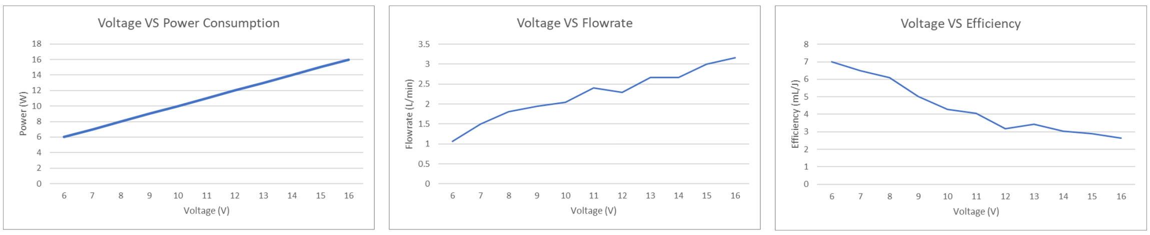


Figure 8: The graphs show the preliminary measurements of power consumption (left), flowrate (center), and efficiency (right) of the pump at varying voltages.

In the future, if one were to pot the entire electronics and thus requires pressure-tolerant parts, the electrolyte super-capacitor right next to the battery needs to be replaced with a ceramic capacitor. To do so, I would recommend putting another buck-converter that converts the battery voltage to 12V. The pump's transient response will be much smaller and smoother at lower voltage/power.

Our battery pack is composed of four Lithium ion batteries (LG 18650 3500mAh battery(4))(16.8V when fully charged and ends at 10V). The poles of the batteries were welded with pure nickel plates in-house. The battery pack is electrically protected by the protection circuit PCB and fully enclosed in 3D printed housing for physical protection. We have chosen a specific battery connector(10) with 3mm gap between power and ground wires to reduce the chance of short while crimping the wires. The exposed wires of the crimps at the end of the connector were also hot-glued.

Lithium Ion batteries require extra care to reduce any chance of hazard, so we have an extra layer of protection. A potential short could occur externally in the water. For example, if there was a potting issue in the flow-stack, the battery could be shorted. Hence, we have placed a smart load switch(6), which will detect an over-current at user-configured current and shut down automatically. Note also that since the pump runs a brushless DC motor, it is an inductive load. Therefore, we see voltage and current behaviors shown in Fig.7. Inductive loads can lead to negative voltage, and thus the load switch should also be able to handle a inductive load. The $1\mu s$ long voltage spike seen in the figure, which occurs at transient negative voltage drop, can be dampened significantly with an electrolyte capacitor with appropriate voltage rating. In our case, we placed a 100μs capacitor with 50V rating.

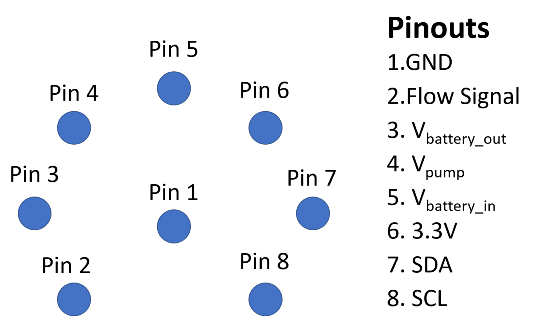
2.2.3 Sensors

Besides the parts in the flow-stack, a temperature sensor(11) and a pressure sensor(12) from BlueRobotics are exposed (Fig.6d) to the water. This allows for deployment configuration based on temperature (e.g. thermocline) and depth. The data from these sensors are logged along with the timestamp and the water flow by the MCU. These sensors communicate by I2C, and the PCB has connectors that the sensors can connect to.

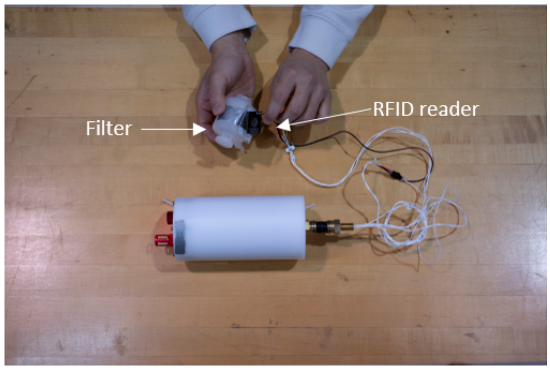
2.2.4 Housing

We have designed and machined a custom housing (Fig.4b out of 3” Delrin rod that could hold all of the internals and pressure tolerant down to 1500m. The housing uses a bore seal to make it water-tight. There are six screw holes in the inner layer of the endcap to use them for any chassis mounts. Two tapped holes on the endcap are for the temperature and pressure sensors. The housing body also has a hole at the tapped hole at the buttom for the 8-pin connector. We thank MIT Central Machine Shop for their detailed fabrication.

2.2.5 Connections to the flow-stack and RFID reader



(a) The pinout of the 8-pin connectors. It was designed this way so that both flow-stack and RFID reader could be connected to the same connector.



(b) The RFID readerplugged in with the electronics. The user will scan the RFID tag attached to a filter as shown.

Figure 9: pinouts of the connectors and the RFID reader

There are 8 pins available to the external units. Each of the pins are labeled in Fig.9a. There is a pin with short-protected battery voltage in(pin2) and another with the voltage out(pin3). The system turns on only when these two pins are externally connected. The small RC (super-cap and minimal resistance on the PCB trace) at the battery was also intended for debounce, but an placing a bigger resistance would be helpful. Very rarely, I would see the power turn on/off when I try to plug in. The signal line(pin4) from the flowmeter is optically isolated in the circuit such that if there is a short outside, the MCU is still protected by the opto-coupler(13). The power to the pump (pin5) is controlled by the MCU. In the case of connecting the RFID reader, three pins 3.3V (pin6), SDA (pin7) and SCL(pin8) are dedicated for the I2C communication.

2.3 RFID reader

The RFID readeris shown in Fig.9b. Initially, we wanted to place the RFID reader either inside the electronics-stack housing or potted along with the flow-stack. Both turned out to be challenging. We could not identify a reader that could be placed inside the electronics-stack housing efficiently and have a reachable reading distance. We also considered potting the reader, but we were not able to figure out the best way to pot the breakout board and antenna without voids. Therefore, we resorted to making a separate unit. We used Grove-NFC(14), a breakout board with an antenna for PN532 chip. There was another breakout board that claimed to be compatible with I2C, but it could only work with an SPI. While the MCU we used has SPI compatibility, we were short on available pins. I would recommend either finding a breakout board or customize a reader, especially if RFID will start to become a common component for other projects.

The RFID reader that is currently being used also has a poor failure handling library code. If it does not get initialized properly, which happens frequently unfortunately, it will through undefined error and restart the MCU. This is, however, tolerable, only because RFID reading is done in setup.

2.4 PVC shell

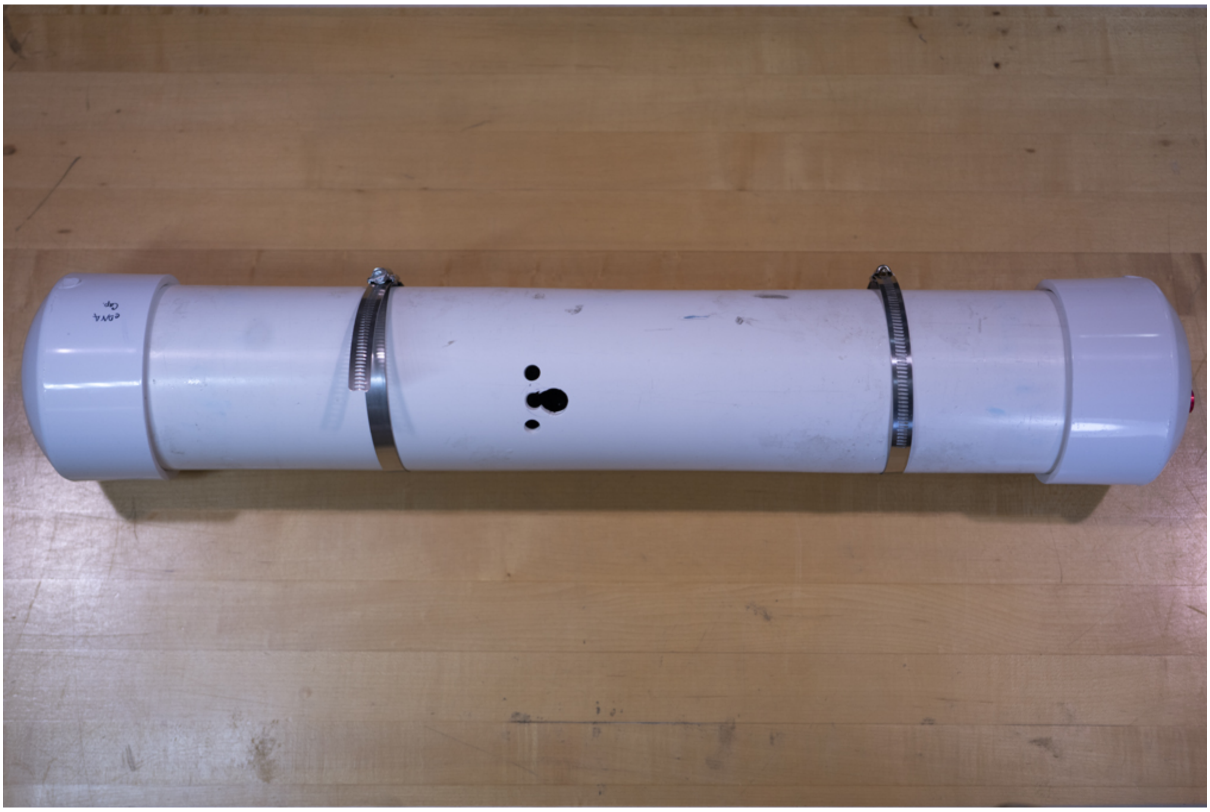


Figure 10: The fully assembed PVC shell that clamps in all of the internals.

The fully-assembled PVC shell is shown in Fig.10. The purpose of this to protect the internal components from any direct contact with other objects. We have decided to splice the PVC tubing in half and clamp the internals within. It is by no means a recommended method, but it was a relatively simple method. The halved tube will tend to bend inwards, deforming the original dimension. Hence, fitting the acrylic plate required some patient as they had the tendency to slip of fly off. When customized filter and flowmeter that will no longer require adaptors are made, we expect the length to shorten significantly.

3 How to build the eDNA Sampler

3.1 Flow-stack

The flow-stackas shown in Fig.12 consists of a 1/2" diameter one-way valve(15), a filter (Supor EKV - Mini Kleenpak Capsules(1)), a flowmeter (FTB-431(2)), a pump (Aubig DC brushless motor water pump(3)), and a connector (8-pin male bulkhead connector(16)). The control for the pump and flowmeter is connected to the 8-pin male bulkhead, joint and seal by scotchcast.The wires are connected to appropriate pins on the 8-pin connector according to Fig. 9a.

Each parts could be connected with varying NPT threads, which required adapters to connect each parts. The valve and the filter are connected with 1/2" NPT Male clamp adapter(17) while the filter and the flowmeter with 3/8" NPT Female clamp adapter(18). Both adapters have a gasket for Tri Clamp 3/4 in.(19) and are held together by Tri Clamp 3/4 in. sanitary clamp(20). The clamps came with their own wingnuts, which were too large to fit into our PVC shell. Therefore, we replaced them with

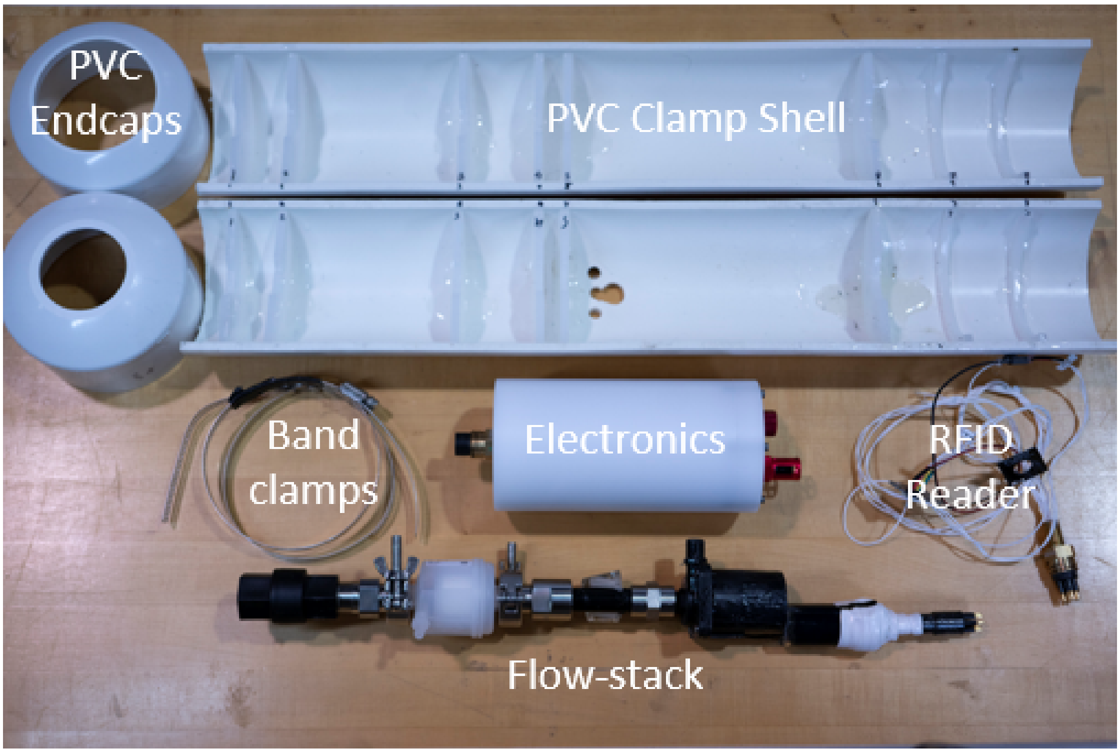


Figure 11: The display of all of the parts involved in the eDNA Sampler

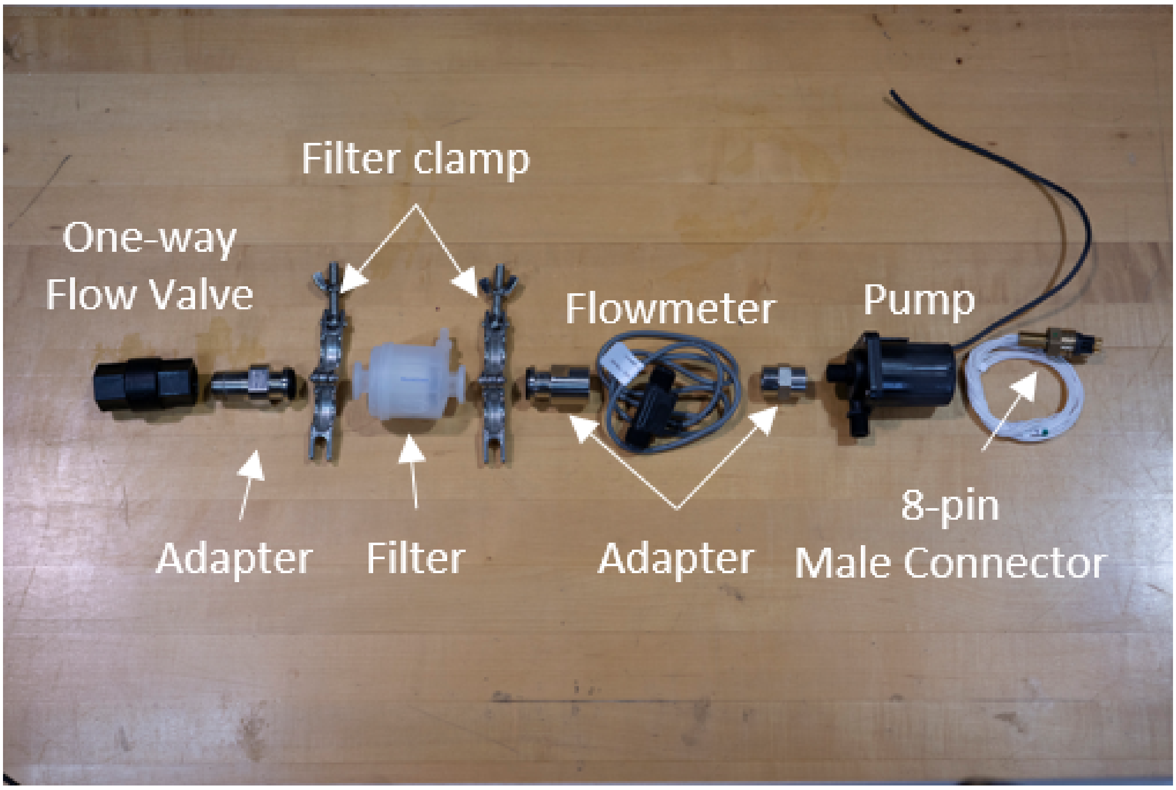


Figure 12: The display of all of the parts involved in the flow-stack

smaller wingnuts with 5/16-24 threading from McMaster-Carr. Finally, the flowmeter and the pump were connected via 1/4" to 3/8" NPT Female adapter(21).

We used 3M Scotchcast to pot the wire from the pump, flowmeter and the 8-pin connector after soldering the appropriate pins.

3.2 Electronics

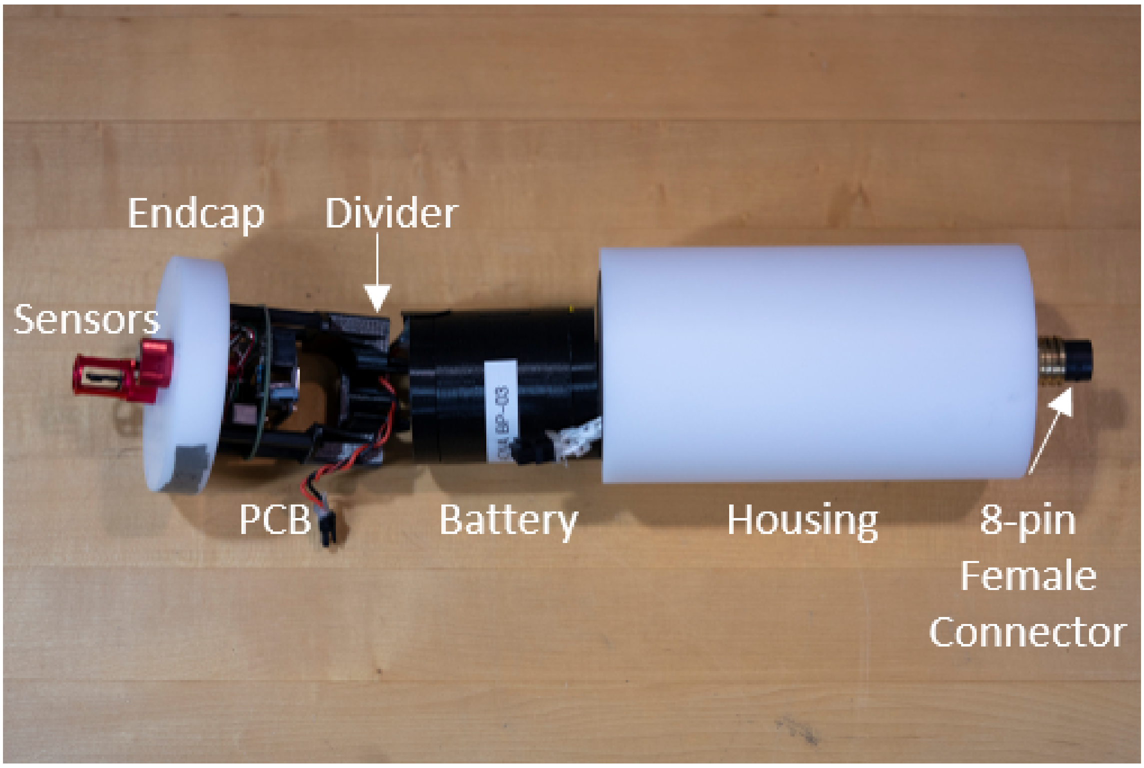


Figure 13: The display of all of the parts involved in the electronics-stack

In this section, we point to the source of design/drawings or describe the process of building each parts of the electronics-stack. The parts involved are displayed in Fig.13.

Battery Pack This requires 4 Lithium Ion (18650, 3500mAh) batteries, a battery welding station, some pure nickel strips, electrical wires, soldering station, a battery protection PCB (4-Series), 2-pin 3mm connector, a 3D printed housing and a glue gun. The batteries are connected in series to make 14.4V battery packs. The 3D printed housing is designed hollow in the center to let the 8-pin connector wires pass through. The disassembled components of the battery pack are displayed in Fig.14.

1. 3D print the housing	We provide the 3D model of the battery housing in the Github eDNA Sampler/Mechanical project directory.
2. Weld the batteries	Each batteries is connected to another by wire soldered onto nickle strips that are welded on each poles of the battery. We recommend welding them after placing the battery inside the housing.
3. Connect the batteries in series	
4. +/- wires out of protection PCB	A good length is about 8cm.
5. Crimp and fit connectors for +/- wires	We used 3mm Molex connector and crimps for 20-24AWG. Also, use glue gun to hold the wires at the connector permanently separate.
6. Connect the batteries and the protection PCB	Solder the poles of each batteries to appropriate pads on the protection PCB. This should also be inside the housing.
7. Close the housing	Put some glue using the glue gun on the exposed pads and wires on the protection PCB.

PCB We provide the EAGLE schematic and layouts for the current version of the PCB (with some corrections) in the Github eNDA Sampler/PCB project directory. The list of components are also given in 4.

Pressure Housing We provide the housing design specification in the eDNA Sampler/mechanical directory. The wires of the 8-pin connectors were cut to 16cm and are crimped and placed in the

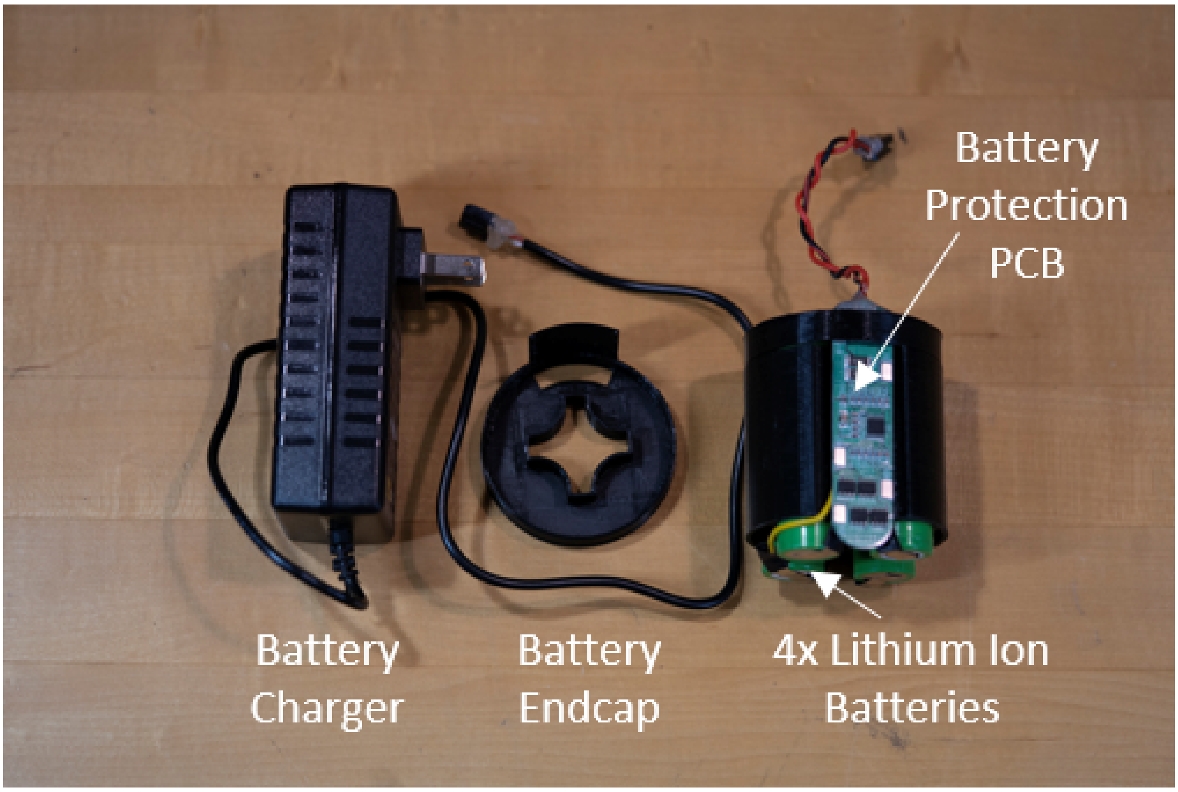


Figure 14: The battery pack (left) and the inside of the battery pack as well as its charger (right). The batteries are housed inside a 3D-printed housing.

8-pin female bulkhead(22). The pressure and temperature sensors are wired as follows: Red(3.3V), White(SDA), Green(SCL) and Black(GND).

Programming the MCU Clone the Github repository and install Arduino IDE along with required libraries mentioned in the README. To program the MCU, first plug in the serial cables as shown in Fig.15b, set the board to bootload mode (process described below).

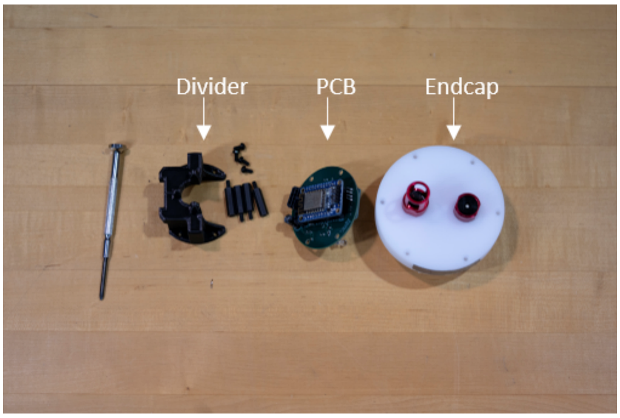
Now you can upload the code using Arduino IDE. In the IDE, please select Tools-¿Flash Size(3M SPIFFS).

Step 1.	Hold down the GPIO0 button, the red LED will be lit
Step 2.	While holding down GPIO0, click the RESET button
Step 3.	Release RESET, then release GPIO0
Step 4.	When you release the RESET button, the red LED will be lit dimly, this means it's ready to bootload

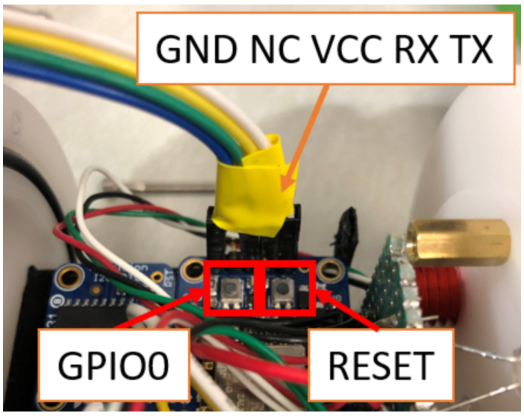
3.3 RFID reader

The RFID Reader(14) comes with UART communication configuration. To use the reader in I2C mode, copper trace between TX and RX on the back need to be cut using a razor blade. Then the header pins available were soldered onto the appropriate pins shown in Fig. 9a of the 8-pin connector(16). The two pins that connect the battery voltage lines (pin3 and pin5) are soldered together.

We also had to pot the RFID tags inside an epoxy to make it waterproof. We had a 3D model of the filter, so we 3D printed a mold that fits around the curvature of the filter. We taped the inside of the mold with an electrical tape and sprayed release agent. We poured the epoxy, and in half way, placed the RFID and filled up the mold. Once we had one RFID tag, we decided to make multiple copies of the mold out of silicon because it was difficult to take the potted tag out of the 3D-printed mold. We used a clay dough to make a (meta) mold for silicon molds. Afterwards, potting in silicon molds were simple and straightforward.



(a) Disassembled parts of the electronic puck. We use standoffs as chassis mount to the endcap



(b) Programming ESP8266 HUZZAH requires UART pins and pressing buttons in specific order

Figure 15: Electronics disassembled and pins for programming the MCU

(a) The acrylic plates and the half sliced PVC shell

(b) The acrylic plates were very slippery. Hence, we had to place a heavy brick on top of a bar across the plates.

Figure 16: The process of making the PVC shellclamps using acrylic plates

3.4 PVC shell

Shown in Fig. 3, we have sliced a 2ft long 4" PVC pipe (Size 4) in half and used epoxy to glue pieces of 1/4" thick acrylic plates that clamps down on the appropriate parts. During the epoxying process, the acrylic pieces often slipped out of the PVC pipe. Hence, we had to place some heavy cement bricks on top of 80/20 bar across all the acrylic pieces to hold them down. To hold the clamp shells together, we have used two band clamps and two endcaps (shown in Fig.16, whose centers are carved out using a lathe to expose the valve and sensors. The shell should also have a hole where there is a tube connected to the pump to let the water out. The endcaps are tight but can be popped out. Thus, we recommend drilling some holes on the end of the pipes and the endcaps to connect them with zipties.

4 Required materials

Item	Unit	Distributor	Manufacturer
Part A: Internal electronics and housing			
Part A.1: Hardware			
MCBH8F Micro Bulkhead Connector - 8 Socket Female	1	Amron Intl.	Amron Intl.
Pressure sensor 300m or 1000m	1	BlueRobotics	BlueRobotics
Temperature sensor	1	BlueRobotics	BlueRobotics
Part A.2: Battery			
LG INR18650 MJ1 Batteries	4	AA Portable Power Corp.	AA Portable Power Corp.
Battery Protection Circuit	1	AA Portable Power Corp.	AA Portable Power Corp.
Part A.3: Circuit components			
HUZZAH ESP8266	1	DigiKey	Adafruit
DS3231 RTC	1	DigiKey	Adafruit
BATTERY LITHIUM 3V COIN 12.5MM	1	DigiKey	Panasonic
DC-DC CONVERTER 3.3V	1	DigiKey	Traco Power
Blue LED	1	DigiKey	Cree
Red LED	1	DigiKey	Cree
Green LED	1	DigiKey	Lite-On
4 Pos. Connector for BlueRobotics sensors	1	DigiKey	Hirose
Load switch with short protection	1	DigiKey	TI
Load switch for pump control	1	DigiKey	TI
2-Pos Female Connector for battery	1	DigiKey	Hirose
2-Pos Male Connector for battery	1	DigiKey	Hirose
Crimps 20-24AWG	Multiple	DigiKey	TI
8-Pos Female Connector	1	DigiKey	Hirose
8-Pos Male Connector	1	DigiKey	Hirose
Opto-Isolater for Flowmeter Signal	1	Digikey	Toshiba
Capacitor 100uF 50V (C1)	1	Digikey	Rubycon
Capacitor 10uF 25V (C2)	1	Digikey	Rubycon
300pF C3	3	Digikey	Yageo
N-MOSFET	3	Digikey	Toshiba
BJT	3	Digikey	ON Semiconductor
Resistor 3.4k (R11)	1	Digikey	-
Resistor 11k (R10)	1	Digikey	-

Resistor 1.18K (R17)	1	Digikey	-
Resistor 520K (R12)	1	Digikey	-
Resistor 10K (R4)	1	Digikey	-
Resistor 1.5K (R5, 6)	2	Digikey	-
Resistor 35.7K (R3, 13, 14)	3	Digikey	-
Resistor 300 (R7, 8, 9)	3	Digikey	-
Part A.3: Housing			
O-Ring (146, 70A)	1 (pk. of 50)	McMaster-Carr	-
3.5” dia. Acetal rod	140mm long	ePlastics	ePlastics
Part B: RFID reader			
MCBH8M Micro Bulkhead Connector - 8 Pin Male	1	Amron Intl.	Amron Intl.
PN532 Grove NFC Platform	1	Digikey	Seeed Technology Co., Ltd
RFID Tag	1	DigiKey	Adafruit
Part C: Pump and flowmeter			
DC40-1250 Brushless DC Pump	1	Amazon	Aubig
3/8” and 1/4” NPT Thread male-male adapter(SS-5000-06-04)	1	Titan Fittings	-
FTB-431 Flowmeter	1	Omega	Omega
3/8” NPT Male clamp adapter	1	Glacier Tanks LLC	-
1/2 in. - 3/4 in. Tri-Clover Clamp	2	Buyfittingsonline	-
5/16-24 wingnut	2	McMaster-Carr	-
Kleenax filter	1	PALL	-
Male NPT Clamp Adapter (3/4” to 1/2”)	1	Dixon	-
one way valve	1	United States Plastic corp.	-
BUNA-N Gasket	1	Glacier Tanks LLC	-
3M Scotch cast splice kit	1	Digikey	3M
MCBH8M Micro Bulkhead Connector - 8 Pin Male	1	Amron Intl.	Amron Intl.
MCDC8F Micro Dummy Connector - 8 Sockets Female	1	Amron Intl.	Amron Intl.
Part D: PVC Housing			
4” PVC pipe	2ft	McMaster-Carr	-
4 Pipe Socket-Connect Female	2	McMaster-Carr	-
Pipe holder	2	-	-
$^1/_8$” Acrylic plate	1ft x 1ft	McMaster-Carr	-
UK U-09FL Urethane Adhesive	50mL	McMaster-Carr	-

5 Software

There are two software modules in this project: MCU and web application. When the MCU is in the setup stage, it communicates with the web application to update any configuration and upload data from deployment. The web application provides a front-end which allows the user to make configuration and retrieve data and log of deployment.

5.1 MCU

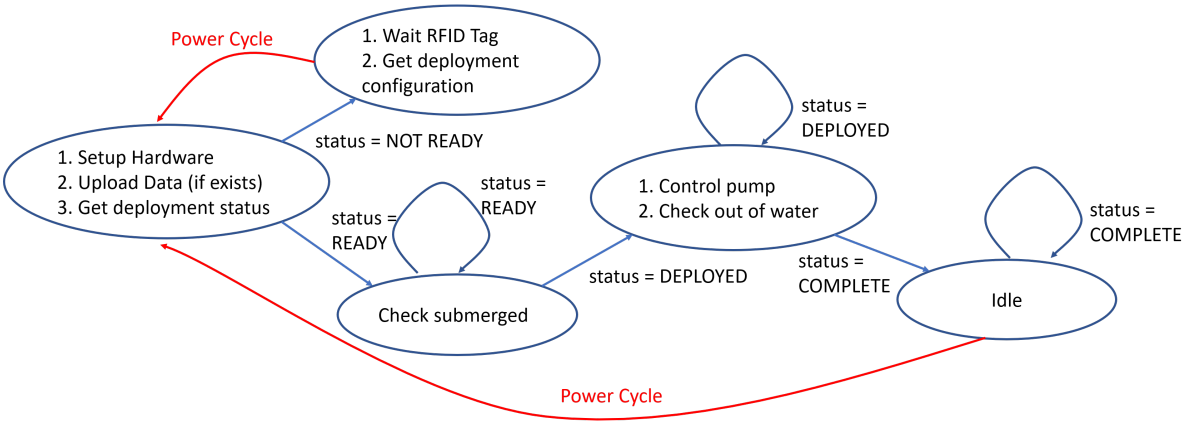


Figure 17: The state machine diagram of the MCU

Since we are following the Arduino programming practice, the code is divided into setup and loop. There is a flag "fSamplerStatus", which is the state of the sampler and the sampler follows the state machine shown in Fig. 17.

There are four modules of code (with their respective header files) that are used by the .ino file: "Sampler.cpp", "SamplerWiFi.cpp", "SamplerGlobals.h", and "SamplerHelper.cpp". For future users who wish to change the global settings, most of the global variables are gathered in "SamplerGlobals.h" file. "SamplerHelper.cpp" contains small functions (mostly controlling the LEDs) that are used to wrap small tasks. "Sampler.cpp" takes care of verifying user configuration, checking the pump triggering conditions and keep track of the situation. "SamplerWifi.cpp" handles all of the http calls that need to be made to communicate with the web application.

setup Upon setting up the hardware, it communicates with the web application to synchronize the time, upload existing data and log file, check if it is currently being configured for a deployment. When the device has no configured deployment yet, it will wait for an RFID tag to be scanned. It is important that the RFID readeris connected to the , not the flow-stack. Upon scanning, it will create a deployment on the web application and wait for valid deployment configuration from the user. Alternatively, if it already has a deployed configuration, it will create a data file and fall into the loop.

loop Every second, the loop gets called, where the sensor data are logged into the data file. When it goes deeper than 1m, the deployment has begun, and it will start controlling the pump (in this case, simply turning on/off the pump in the configured setting). When it is less than 1m submerged, it will consider the deployment to have ended. At this point, the MCU will continue to log the data and stay idle.

5.2 Web Application

We have implemented Django framework based on Python3 for a quick deployment. Fig.18 shows the page where the users can view the deployments in a list. When the user clicks "edit" or "view" (depending on whether the deployment happened or not), the user will be led to the deployment configuration page. On this page, one can edit and view the configuration of the deployment.

In order to setup the web server on RPI or a Linux-based server, please follow the instructions in the web application repository.

There are a few things to be cautious of before launching. Both the eDNA Samplerand the server need to be in the same network (local or web). The server's IP address and the port at which the application is run should be known. This information needs to be altered in "SamplerGlobals.h" so that

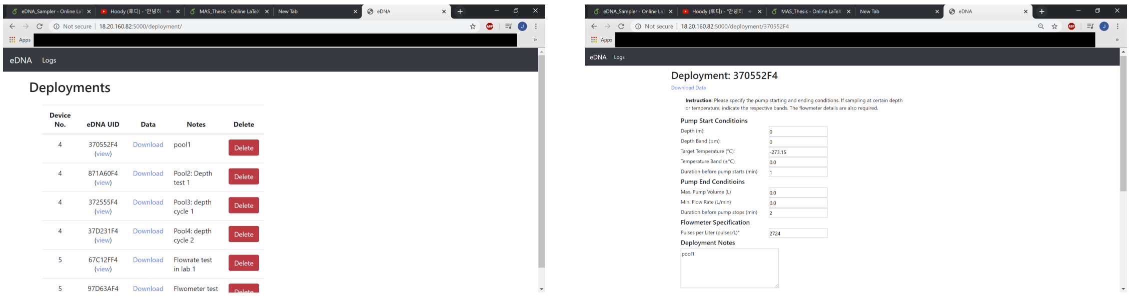


Figure 18: The web frontend examples

the ESP8266 can connect to the right network and communicate with your server. Also, the firewall had to off in our case for the server to talk with our personal computers (not in the case of RPI server).

However, nothing here is deployment-safe (e.g. security, robustness, etc...), and we highly recommend that the code for the web application to be re-designed.

6 User Manual

In this section, it is assumed that all of the relevant parts are potted, assembled, programmed and primed. There are three main components involved in preparation: (part a), RFID reader(part b), flow-stack(part c), and the PVC shell(part d).

	Before Deployment
Step 1	Plug in the RFID reader into the electronics housing. The red LED will turn on first. Wait until the blue LED blinks.
Step 2	Tap the the RFID tag attached on the filter onto the antenna. The blue LED should turn off, and the green LED should be blinking
Step 3	Go to the web application and edit the deployment configuration. Given that appropriate deployment configuration was added, the green LED should stop blinking.
Step 4	Unplug the RFID dongle and plug in the pump. The red LED will turn on first, and eventually, the green LED will turn on. You are ready for deployment.
Step 5	Assemble the PVC housing.
	After Deployment
Step 6	When the sampler is pulled out of the water, the red LED and green LED should be on.[1]To program for another deployment, you need to disassemble the PVC housing and plug in the RFID reader to repeat Step 1.

6.1 LED indications

The table below describes the indications by the LEDs.

LED	Status	Indication
Red	Static	Power is on
Red	Blinking	-
Green	Static	The deployment is properly configured
Green	Blinking	Waiting for deployment configurations
Blue	Static	-
Blue	Blinking	Waiting for RFID tag
All	Blinking	Connection to WiFi/sensors failed. Requires downloading log data.

[1] It is recommended to soak the device in fresh water if not going to be deployed again.

7 Test and Deployment

7.1 Unit test

To ensure that the pump is triggered and stopped at the right conditions, we have placed some unit tests in our code. "SamplerTest.cpp" contains these tests. They were test cases that consider scenarios for various deployment configurations.

7.2 Pool test

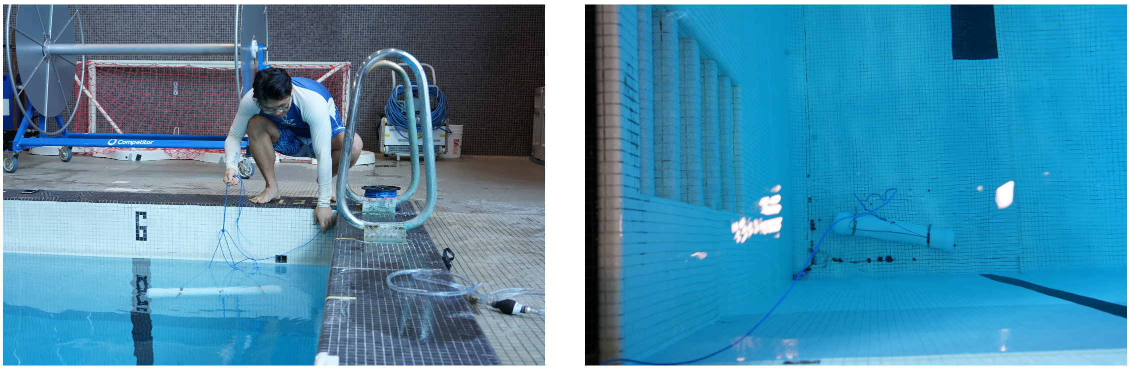


Figure 19: A round of test in the MIT Alumni pool. The pool was 4m deep.

On January 21st, 2020, we had a trial run in the MIT Alumni pool, max 4m deep, to test the operation in depth. First, the pressure sensor had to be calibrated to take into the account the 1m offset. We verified that the pump was triggered at correct depths and for the right volume of water / duration of time.

In the lab, we have also tested the pump stops pumping when the sampler starts to clog and the flowrate is reduced significantly (down to 2L/min from 5L/min) from a tap water. In all of our test cases, we used expired filters provided by the Govindarajan Laboratory at WHOI.

7.3 Test in the Channel Islands in California

Figure 20: Two eDNA Samplers were brought to the Channel Islands in California.

A ship of opportunity popped up at the Channel Islands in California, and we could take our eDNA Sampler for preliminary test deployments. We gained a lot of valuable experiences of deployment, device rigging, and field testing from this expedition. During this deployment, we had electronics that were soldered onto a protoboard, which caused numerous problems during handling.

We were at shallow water around Santa Rosa island, 25m maximum. The eDNA samplers were set to trigger between 5 to 15m, and stop when the flowrate reaches less than 0.2L/s. Both samplers were in the water for 10 minutes, maximum depth research is around 15m. While the pumps did not trigger at the right conditions, we discovered I2C (only when submerged!) issues and potential circuit flaw where short externally in the water could destroy the internal electronics.

There was another deployment at Catalina Island. We deployed the eDNA Sampler for 30 minutes, reached a maximum depth of 60m. The eDNA sampler was set to start sampling between the depth of

50-70m. Unfortunately, we saw that the pump was triggered at the right condition, but the data file never written.

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