Hardware Article

Programmable Autonomous Water Samplers (PAWS): An inexpensive, adaptable and robust submersible system for time-integrated water sampling in freshwater and marine ecosystems

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ABSTRACT

Water chemistry conditions in freshwater and marine environments can change rapidly over both space and time. This is especially true in environments that are exposed to anthropogenic impacts such as sedimentation, sewage, runoff and other types of pollution. It is critical in studying these systems that researchers have tools capable of accurately collecting water samples across relevant spatial and temporal scales. Here we present an inexpensive, open-source Programmable Autonomous Water Sampler (PAWS) that is open source, compact, robust, highly adaptable and submersible to 40 m. PAWS utilizes a time-integrated sampling approach by collecting a single sample in a syringe slowly over minutes to days. Once analyzed, data from the sample collected represents and integrated average of water chemistry conditions over time. Due to its adaptability and low cost, PAWS has the potential to improve the spatial and temporal coverage of many freshwater and marine studies.

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Specifications table

Hardware name	PAWS – Programmable Autonomous Water Sampler
Subject area	Environmental, Planetary and Agricultural Sciences
Hardware type	Field measurements and sensors
Open Source License	CERN–OHL-W
Cost of Hardware	$300.00
Source File Repository	https://doi.org/10.17632/htkn8ky6zz.1

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Hardware in context

Modern human activity is responsible for numerous unprecedented chemical inputs into freshwater and marine ecosystems, including, but not limited to, heavy metals, pesticides, herbicides, petroleum products, industrial waste, emerging contaminants (e.g. pharmaceuticals, personal care products, legal and illegal drugs), plastics, and nutrients (e.g. nitrogen and phosphorus from fertilizer, agricultural runoff and sewage) [1–8]. Each of these pollutants have their own effects and impacts on aquatic systems, and therefore deserve close study. The shared protocol used in studying and understanding these diverse parameters in aquatic ecosystems is the collection and analysis of water samples. In this paper, we present a novel method of collecting water samples through the use of a low-cost, open-source Programmable Autonomous Water Sampler (PAWS).

Water chemistry and environmental conditions in aquatic ecosystems can be highly variable in both space and time, with conditions changing rapidly in response to even small changes in factors such as precipitation, depth, tides, currents, wave action and animal behavior [1]. One of the primary challenges associated with monitoring the health of freshwater and marine aquatic systems, is the resource-intensive nature of collecting data at high enough spatial and temporal resolutions to capture these changes [9]. The three most common methods of collecting water chemistry data are: 1) bottle sampling at discrete timepoints (grab sampling) followed by laboratory analysis, 2) automated water sampling followed by laboratory analysis, or 3) in-situ analyzers which are deployed in the environment and perform analyses autonomously. These methods support the collection of data with either high spatial or temporal resolution, but due to cost and/or logistical challenges, often cannot feasibly provide both [9,10,11]. Because of their low cost, ease of use, and adaptability, PAWS aims to allow for the collection of water quality data from highly dynamic aquatic systems with high spatial and temporal resolution (Table 1).

Historically the most widely used method to collect water quality data is through periodic manual sampling in bottles, or similar containers. Collecting a single water sample is relatively simple and allows for a variety of highly accurate lab-based analyses to be conducted on the same sample. Though collection of bottle samples has a relatively low-cost and low effort per sample, it only provides data regarding the single moment in time the sample was taken. Filling in data gaps by increasing the spatial or temporal resolution of bottle sampling requires both large sampling efforts and potentially large budgets depending on the sampling locations [9,10]. Once collected, samples will need to be transported to and analyzed in a lab. This can escalate costs rapidly depending on the location (in-house vs external lab) and method of analysis. As a result, many long-term, multi-year, water sampling regimes only collect samples on weekly to monthly intervals. Relying on this small number of samples to determine the flux of chemical contaminants can result in vast over, or under, estimates [9,10]. As bottle sampling is limited to collection times with human compatible working conditions including weather, river or sea state and site access, sampling efforts may miss important episodic events like short period, high intensity storms. Manual sampling at depth can be accomplished using Niskin or Van Dorn style samplers, though this type of sampling is also depen-

Table 1

PAWS compared to other sampling options. PAWS provides a sampling tool that can be appropriate in situations, such as high energy environments, where other options are not available.

	Bottle Sampling	Commercial Samplers	SAS	PAWS
Initial Cost	**Low to Medium,** basic equip inexpensive, but manual samplers can be >**$500**	**Medium to High,** $2.5 k - $50 k per unit	**Low**, $220 per unit	**Low**, $300 per unit
Sampling Effort	**Low to High** single sample low, increases rapidly with additional samples/sites	**Low**, autonomous collection	**Low**, autonomous collection	**Low**, autonomous collection
Number of Samples	**Variable**, depending on sampling effort	$\leq$ **24** discrete samples per unit	**2** discrete samples per unit	**1** integrated sample per unit
Sample Volume	**Variable**, depending on sampling equip	**375 ml to 9500 ml / sample**	$\leq$ **900 ml / sample**	**60 ml / sample**
Cost per sample	**Low**, depending on sampling effort	**$104 to >$2000**	**$110**	**$300** for an integrated sample
Cost per 24 h of sampling	**Increasingly higher** effort/cost to add additional timepoints	**$104 to >$2000** (1 sample per hour)	**$2640** (1 sample per hour)	**$300** for an integrated sample
Spatial Resolution	**Low**, increasingly higher effort/cost to add additional sites	**Low**, high cost to add additional sites	**High**, low cost to add additional sites	**High**, low cost to add additional sites
Depth Rating	**Surface to Feasible Rope Length**	**Surface to >5000 m**	**55 m**	**40 m**
Robustness	**Medium**, can be difficult to sample in challenging conditions	**Medium to High**, range from weather sealed to deep depth rated, may not be suitable for high energy environments	**Medium,** depth rated but not suitable for high energy environments	**High**, depth rated and suitable for high energy environments
Synchronized sampling at multiple sites	**No,** not without highly coordinated efforts with multiple people	**Yes** by deploying multiple units	**Yes** by deploying multiple units	**Yes** by deploying multiple units

dent on accessibility and safe working conditions. An additional limitation of bottle sampling is the inability to easily collect samples from multiple location simultaneously, making it difficult to capture the same short episodic events across research sites.

In freshwater studies one solution to this limitation has been the deployment of ISCO-style samplers, large sampling systems which use an electric pump to collect water into a sequential rosette of a fixed number of sample bottles over a user-programmable interval [12]. While these samplers do offer autonomous collection of a limited number of discrete samples, they are large (dia: 69 cm × height: 51 cm), heavy (~15 kgs), and expensive (~$2.5 k to $5 k) for the most basic and compact versions. Furthermore, these systems, as well as existing lower-cost open-source alternatives [13] are not waterproof, limiting their use to studies where they can be deployed on land adjacent to the water they are sampling. As such, they often need to be deployed inside of locked enclosures to improve resistance to both weather and tampering. Though ISCO samplers have become a standard in freshwater science for good reason, the non-submersible nature of their design limits their potential applications considerably. Depending on the study, integrated sampling using a submersible system like PAWS may offer a durable, cost-effective alternative.

To address the needs of marine scientists, numerous submersible autonomous sample collection systems are commercially available, or have been developed by researchers. As with many depth capable oceanographic instruments, commercial submersible systems are quite expensive ($35 k - $45 k) [14]. There is a high coast associated with depth rating and for many freshwater or coastal studies, these systems are essentially over capable as these studies are often conducted in <30 m. Recent open-source, researcher developed sampling systems are considerably less expensive than their commercial counterparts [14–16] and are more appropriate for shallow deployments. However, these systems often employ multiple housings, exposed moving pump components, and/or external sample collection bags or bottles. Though this increases the possible sample volume and aids in reducing the system cost, it also increases the footprint, complexity, vulnerability to damage and tampering, and potential failure points of the systems. As such, these systems may not be capable of sampling in high energy (e.g. fast flowing rivers, or in a subtidal area with wave action) or debris laden environments (e.g. a river during a storm surge, or a wastewater channel) without sustaining damage [14,16]. Of these sampler projects, PAWS is closest in terms of cost and capability is the Subsurface Automated Sampler (SAS) for ocean acidification research [16]. The SAS is capable of collecting two separate samples in bags up to 900 ml using one of two sampling regimes 1) At a set time and date, or 2) once daily. SAS would be appropriate if larger sample volumes or more discrete samples were needed, whereas PAWS would be more suited to collect a much smaller time integrated sample in a high energy environment.

In the last few decades, the deployment of in-situ analyzers, waterproof electronic packages deployed in the environment that measure, record, and often transmit data in real time, have become an alternative to physical collection of samples for lab-based analyses [17]. In many cases, this has revolutionized aquatic research. In-situ analyzers can collect data with high temporal resolution in both freshwater and marine ecosystems. As no lab analysis is required, data is collected in near real time and is often transmitted wirelessly. There are however instances where sampling and lab analysis are still preferable. In-situ analyzers have a high upfront cost. For example, a single basic SUNA nitrate sensor for shallow freshwater deployments costs >$32 k [18]. Though the cost per data point of these systems decreases rapidly over time, the upfront cost, as well as maintenance costs, might still be too much for some research programs. The upfront costs are even higher if the goal is to study multiple parameters as each parameter requires its own probe. Additionally, in-situ analyzers are not available for every parameter [16] or are not sensitive enough for a given system. For example, high quality ammonium probes have a detection limit of 0.2 mg/L [19] whereas ammonium concentrations in tropical waters are often <0.02 mg/L [20]. Finally, in-situ analyzers often rely on hardware, firmware, and software that is proprietary, making instruments difficult to repair, troubleshoot and modify in the field. Many analyzers must be sent back to the manufacturer for maintenance and calibration, something that may not be feasible while operating in remote areas.

Generally autonomous water sampling systems and in-situ analyzers are designed with either fresh water, or marine environments in mind. As scientists studying the interface of freshwater and marine ecosystems in remote locations, we saw a need for a device that combined the autonomous nature of in-situ analyzers with the simplicity and adaptability of manual water sampling. In response to this need we developed PAWS, a low-cost, user-friendly, and highly adaptable autonomous water sampler. This device is open source making it easy to construct and repair. In addition, its compact size (11 cm × 61 cm), inconspicuous, durable, and streamlined housing, and depth rating of 40 m allows it to be deployed in a wide range of aquatic environments including high energy or heavily trafficked areas as well as small cryptic spaces (Fig. 1).

The PAWS system is designed around the concept of time-integrated sampling. This technique is regularly used in eco-toxicology studies using chemically absorptive materials, membranes or devices such as Chemcatchers® [21,22]. In passive sampling, the chemical structure of the sampler allows molecules or compounds of interest, such as trace metals, PCBs, herbicides and pharmaceuticals to sorb onto the surface of the sampler. Upon retrieval, the compounds are washed off the surface and analyzed in a lab. Using this method, concentrations of pollutants in aquatic environments are calculated by assessing the amount of a given compound retrieved from a sampler over the period of time it was deployed. As sampling efficacy is determined by the chemical compatibility between the sampler and the compound of interest, many compounds are not suitable for passive sampling [23]. PAWS shares the time-integrated strategy with passive sampling, but rather than relying on absorption properties PAWS collects a single, continuous water sample over the deployment period. In this way PAWS is not selective, any dissolved compound, small suspended particles, or microbiota can be sampled.

To accomplish time integrated water sampling, PAWS uses a syringe pump-like mechanism programmed to collect water at a continuous rate. When it is recovered, the sample collection chamber contains an integrated water sample over a period

Fig. 1. PAWS on a pier ready for deployment. The black bands are SCUBA diving ankle weights. The dive weight attached to the green rope hits the seafloor before the sampler does allowing PAWS to be anchored above the bottom reducing the possibility of particulate matter blocking the sampler's inlet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of a few minutes to >100 h (even longer deployments are possible with a larger battery). Continuous sample collection in this way offers several benefits over bottle sampling or other manual sampling techniques(Table 1). Integrated sampling captures an overall average of water chemistry over a given time period. This includes large chemical fluxes from short period, high intensity rainstorms that may fall between bottle sampling intervals. The tradeoff with integrated sampling however is the loss of the fine scale temporal resolution offered by high frequency manual or automatic sampling, or in-situ analysis. The integrated sample includes an averaged picture of water chemistry including short period high or low intensity events, but it will not indicate the timing and absolute concentrations associated with them. If a study is only concerned with longer term averages of water chemistry PAWS can provide a significant reduction in sample collection cost as compared to bottle sampling by reducing effort and person-hours. Additionally, PAWS can also provide a reduction in analytical cost as compared to bottle sampling, or autonomous rosette sampling, as a single PAWS sample represents an integration over a period that may require multiple spot samples. Less overall number of samples results in lower analytical costs per study.

Hardware description

One important goal for these samplers was to make them as easy and inexpensive to construct as possible. To achieve this, we designed the system using mostly components that are readily available off the shelf. The few mechanical pieces that are custom made are designed to be fabricated using a laser cutter and a 3D printer, as opposed to machine tools such as a mill or a lathe as is commonly the case for underwater housings. This reduces the cost and ease of production as 3D printers and laser cutters require little training to operate and are increasingly becoming available in many libraries, maker spaces and research labs. Alternatively, laser cut parts could be cut out carefully using a bandsaw or jigsaw, and a drill. Any of the custom parts could also be made inexpensively by one of the many local or web-based services that have these tools, as opposed to traditional machine shops which generally have a high overhead due to expensive tooling and a highly trained workforce. Furthermore, all the firmware is open-source and written in Arduino, one of the most accessible and widely used programming languages for hardware control, making it easy to adapt the samplers to new use cases.

PAWS is comprised of three principal component blocks: 1) the sampling mechanism, 2) the power and control system, and 3) the pressure housing.

The sampling mechanism

Syringe pumps are a well-established tool for precise dosing or sampling in laboratory settings. Recently, there have been several published open-source syringe pump projects for a variety of lab applications [24–26]. To our knowledge however, PAWS is the first open source, submersible environmental water sampler built around a syringe pump-like architecture (Fig. 2). Some syringe based water sampler designs have used a rosette of spring actuated syringes to collect a series of discrete samples [27,28]. In contrast, syringe pumps allow for precise control of the sample collection rate in a single syringe. Designing PAWS using this architecture allows for precise collection of an integrated water sample over an extended period of time in a relatively small and inexpensive package. There are numerous additional benefits to building a sampler around a syringe. Syringes are readily available, inexpensive and can come pre-cleaned and sterilized. Though slightly more expensive
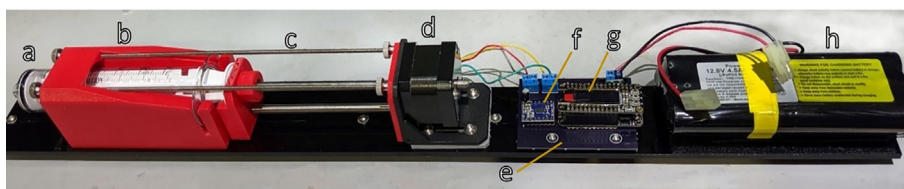
Fig. 2. PAWS sampling mechanism, and power and control system out of its housing. a) 60 ml polycarbonate syringe. b) 3D printed syringe cradle. c) Stainless steel threaded rods lock the syringe in place and provide additional bracing. d) NEMA 17 non-captive linear stepper motor actuator. e) PAWS printed circuit board. f) Pololu DRV8880 stepper motor driver carrier. g) Adafruit Feather M0 Express microcontroller with an OLED screen shield. h) Powerizer 12.8v 4.5Ah LiFEPO4 Rechargeable Battery Pack.

than the common disposable polypropylene syringe, PAWS utilizes 60 ml polycarbonate syringes which have a longer lifespan, higher pressure rating, lower moisture and gas permeability, and a high thermal tolerance which allows them to be repeatedly steam, gamma, and/or EO sterilized.

After experimenting with, and breaking, a handful of established syringe pump designs, we decided to develop our own system for securing the syringe. When inserted into the sampler, the syringe slides into a 3D-printed carriage and locks into place with a quarter turn (Fig. 3). We designed this carriage to hold the syringe securely while the sampler is under high pressure while also allowing the syringe to be easily removed without the use of any tools. The height of the carriage and its angled outside corners are also designed to secure the sampling system inside the housing. The syringe plunger sits into a 3D printed flange that attaches to the sampler motor shaft and rides on a stainless steel rod running between the syringe carriage and the motor mount. Once the syringe is in place, two threaded rods slide into the front of the syringe carriage, over the syringe tabs, and into the motor mount (Fig. 4). These serve a dual purpose, 1) as extra locks to keep the syringe in place and 2) as tensile rods to stabilize the forces exhibited on the system when under pressure.

The sampling mechanism is driven by a non-captive NEMA 17 hybrid stepper motor linear actuator. With this type of actuator, the motor turns an internal nut which drives a threaded shaft forward or backwards, depending on the direction of rotation (Fig. 4). In general, these actuators are very durable and precisely controllable. The additional benefit of the non-captive style motor in this application is that it allows the force of the differential between the housing's internal pressure and the outside water pressure to be directly centered on the motor shaft. This reduces the shear stress on the shaft and motor and allows the motor to run at a lower current. Once the pressure differential is greater than the friction of the o-ring in the syringe, the pressure differential is driving the sample collection with the motor acting like a finely controlled brake. At even shallow depths, PAWS is not drawing in a water sample, but rather letting more water into the syringe in a slow, controlled manner.
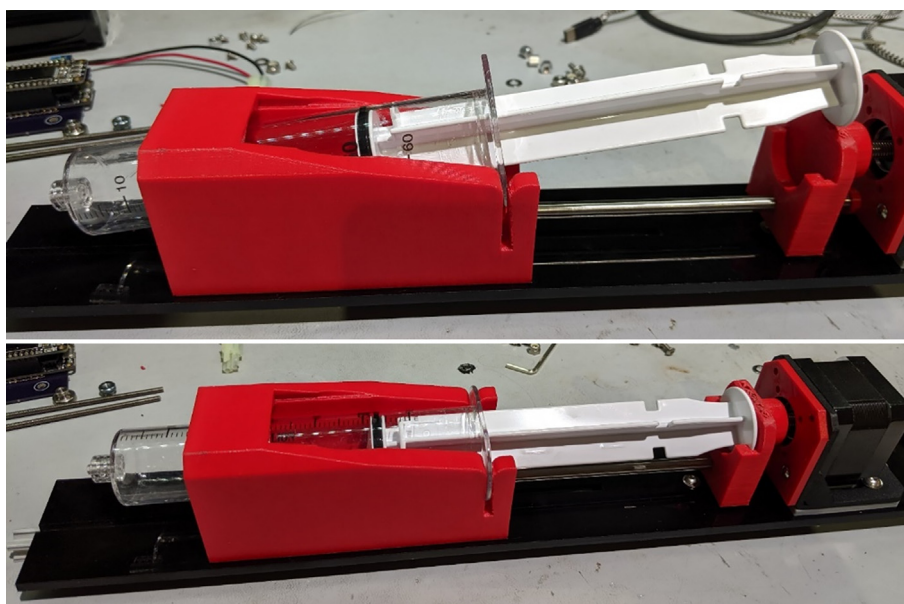

Fig. 3. The syringe slides into the syringe cradle and locks into place with a quarter turn.
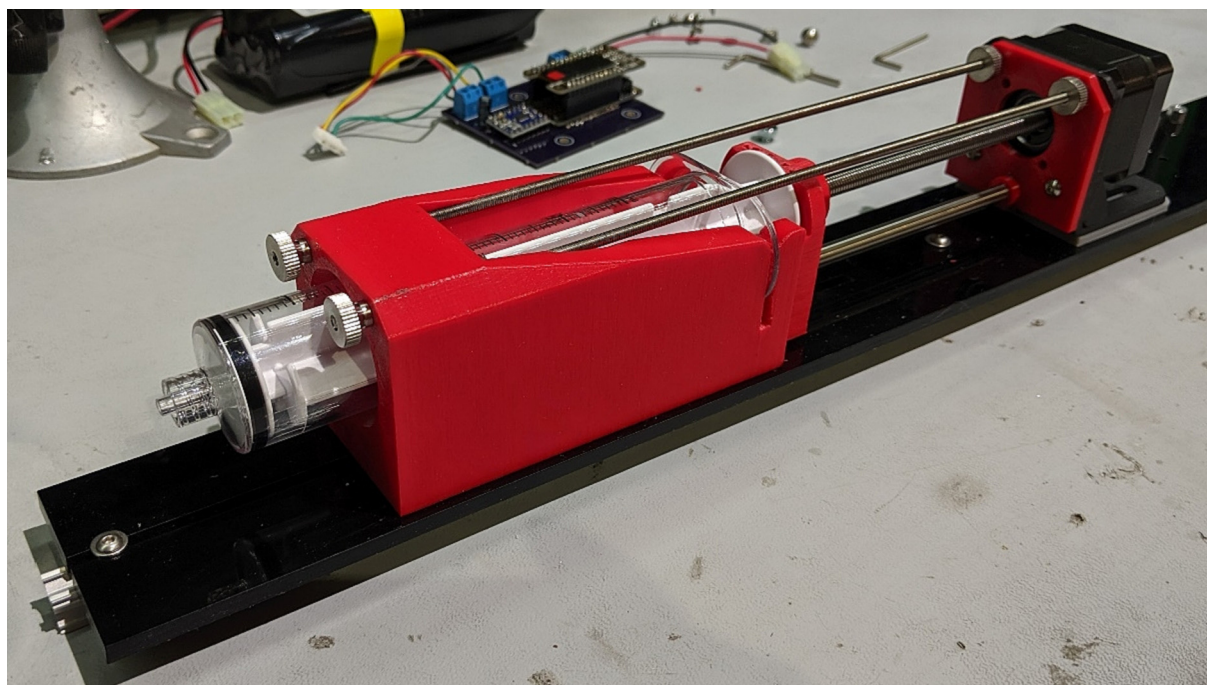
Fig. 4. PAWS sampling mechanism built around a 60 ml polycarbonate syringe and a NEMA 17 non-captive stepper motor. The red and grey components were 3D printed on a PRUSA i3 MK3S filament printer using ABS filament. The black tray is made from laser cut acrylic and mounted on an extruded aluminum rail. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The power and control system

PAWS is controlled using a microcontroller and a stepper motor driver mounted on a custom printed circuit board (PCB) (Fig. 5). The Adafruit feather M0 Express was selected for this project because it is a low power and highly versatile micro-controller in a very small package. The Feather has a plethora of configurable analog and digital I/O pins, can be programmed in Arduino or Circuit Python, and Adafruit offers numerous compatible modules ("wings"), such as the screen (OLED) we incorporated into PAWS, which easily stack on top of the Feather. The OLED wing allows the user to access the PAWS menu and program a sampler without connecting it to an external device (Fig. 5). Once the microcontroller is programmed it com-municates with the stepper motor driver to wake up and trigger the motor to step to its next position based on the user defined parameters. We also appreciate the extensive and detailed documentation, user guides, sample code, and support Adafruit provides online for all of their products.

Power is provided by a 12.8 V, 4.5 Ah LiFePO$_4$ rechargeable battery pack. From the battery power is sent to the Feather through a step-down voltage regulator (Pololu D36V6F3) which takes any battery voltage above 4 V and outputs 3.3 V to the microcontroller and screen. Full battery power is sent to the stepper motor driver through the PCB and finally to the stepper motor via a bank of screw terminals. The Pololu DRV8880 was selected as the stepper motor driver for its dynamic current scaling capability. Because stepper motors draw current whether they are stepping or not, the dynamic current scaling func-tion allows for PAWS to reduce the power consumption of the motor between steps by more than 90 %. While stepping the system can draw up to 455 mA, but in between steps, the system draws only 40 mA. For each step, we energize the motor at full power for 10 ms. Each step rotates the internal nut 1.8 degrees and it takes 10,000 steps to collect 60 ml of water in the syringe. The time it takes to fill the syringe is controlled by varying the delay between steps. Regardless of the duration of sample collection, the system is only running at full power for a total of 0.03 h (1.7 min) consuming just 0.014 Ah of battery capacity. This leaves 112 h of battery capacity for an initial start delay and/or sampling. In other words, if set to sample immediately after setup, PAWS could collect 60 ml over 112 h (4.7 days), or it could wait in the field for up to 112 h and then collect 60 ml over one minute, or any combination in between. Longer deployments are possible with larger battery packs. This would likely require lengthening the housing by cutting a longer length of PVC tube.

Unless the user has access to PCB fabrication equipment, the custom PAWS PCB is the only part of the system which will have to be ordered from a fabrication house such as OSH Park. That said, the role of the PCB is to provide location to securely mount the electrical components, as well as a way to streamline and organize connections between components (Fig. 5). A careful and motivated user could assemble the PAWS control circuitry on a perfboard.
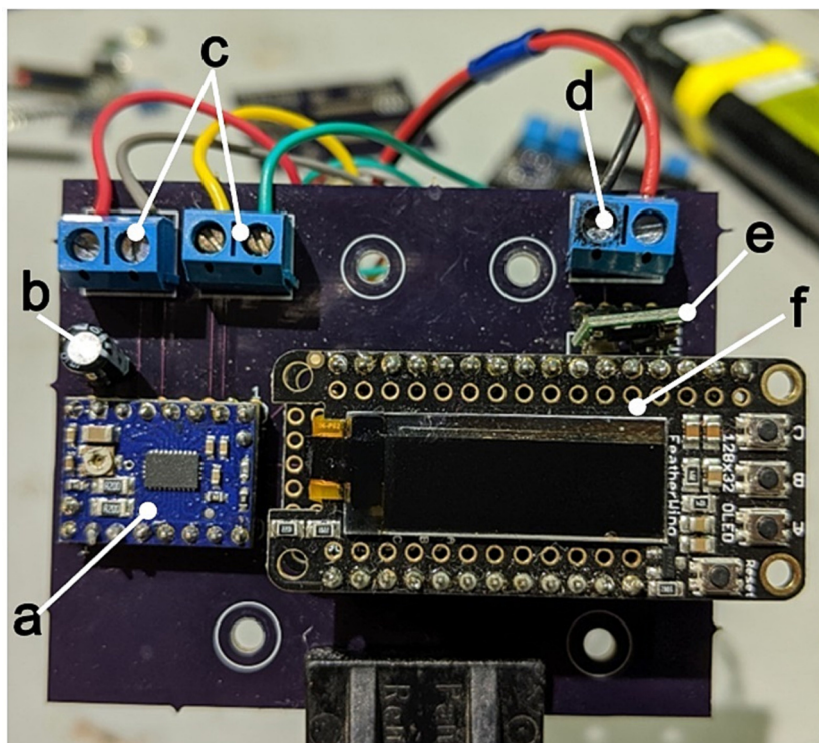
Fig. 5. PAWS printed circuit board. a) Pololu DRV8880 stepper motor driver. b) 100 uF capacitor. c) Stepper motor control wire connectors. d) Battery power connector. e) Pololu 3.3v step down voltage regulator. f) Adafruit Feather M0 Express with OLED screen "Featherwing" shield including menu navigation buttons.

The pressure housing

The housing for the samplers is constructed from 3 to inch schedule 40 PVC pipe capped at each end by PVC unions, components which are readily found at most local hardware stores (Fig. 6). PVC unions, with one of the flanges replaced by an acrylic disk, make for an excellent housing door as they are already equipped with a face type o-ring seal [29]. One of the PAWS acrylic doors has a hole into which an IV valve is epoxied. This acts as a connection between the tubing and filters on the outside of the housing, and the syringe on the inside. The other door is made of a solid piece of acrylic. The overall cost of the housing could be reduced by ~$40 if this solid door and its union were replaced by a simple PVC endcap glued in

Fig. 6. PAWS housing with PVC union threaded endcaps and collars. The front and rear doors (left and right respectively) are made from laser cut clear acrylic. The pass through in the front door is an IV valve epoxied into a laser cut, or drilled, hole through the center of the disc.

place, however we liked the utility of being able to open the housing from the battery side as well. These housings, with a generous safety factor, can be deployed up to 40 m depth, which encompasses a majority of applications in freshwater and coastal marine ecosystems. We believe that the system is capable of withstanding higher pressures than we tested. The IV valve epoxied in to the acrylic door is likely the weak point in this housing design. Further testing is needed to ascertain the maximum depth rating of this configuration. In general, we have found this housing design to be extremely useful and adaptable to a wide variety of applications (see Fig. 9 for an example). Due to the ready availability of PVC unions and tubing in a range of sizes, this design can be used to build capable housings both quickly and inexpensively. Housings for deeper deployments could be constructed using schedule 80 PVC components, and thicker acrylic doors.

Sample preservation

Depending on the goals of the study, any number of different tubing and filtration configurations can be easily and securely connected to the sample syringe via the standard Luer lock fitting. Because of the ability of PAWS to collect water slowly over time rather than pushing a large volume of water through a filter all at once, even filters with small pore sizes work well with this system. For example, we deployed PAWS in moderately turbid seawater for nutrient analyses (see 7.3 Field Deployment). For this study, we attached a 0.15 um prefilter to exclude any particulates and microbes which could alter nutrient concentrations in the sample. The system performed as expected even with the small pore size of the filters. If the study is focused on microbial community analyses paired with water samples, Sterivex type filters could be used. For measurements of total suspended solids, or just the exclusion of large particles, GF/F filters could be connected. For sediment pore water studies, Rhizons will connect directly to the Luer lock fittings on the sampler. If chemical preservation of the sample is required, the sample syringe or a length of intake tubing could be prefilled by a small volume of a fixative such as formalin, mercuric chloride, sulfuric acid, or ethanol.

Design files

Design files summary

Design file name	File type	Open source license	Location of the file
Tray	*.stp. / dxf*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Syringe Carriage	*.stp /.stl*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Solid Door	*.stp /.dxf*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Plunger Flange	*.stp /.stl*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Motor Spacer	*.stp /.stl*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Door with Pass Through	*.stp /.dxf*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Bracket Attachment	*.stp /.stl*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
PAWS Board	*.brd*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Firmware	*.ino*	*CERN-OHL-W*	*https://doi.org/10.17632/htkn8ky6zz.1*
Firmware Read Me	*.txt*		*https://doi.org/10.17632/htkn8ky6zz.1*

Design files descriptions

Tray – Tray to mount and connect all the components. .dxf provided for laser cutting. This part could be cut using a bandsaw, table saw, or jigsaw and a 1/8" drill if the file was printed onto a template.

Syringe Carriage – This component holds the syringe securely in place. .stl provided for 3D printing. We used a PRUSA i3 MK3S filament printer to print this part in ABS.

Solid Door – Acrylic disc for the rear endcap. Seals against the PVC union's o-ring. .dxf provided for laser cutting. This could also be cut using a bandsaw or jigsaw.

Plunger Flange – This component connects the plunger flange to the shaft of the linear actuator. Slides along a stainless steel rail. .stl provided for 3D printing. We used a PRUSA i3 MK3Sfilament printer to print this part in ABS.

Motor Spacer – Raises the height of the motor bracket off the tray. .stl provided for 3D printing. We used a PRUSA i3 MK3S filament printer to print this part in ABS.

Door with Pass Through – Acrylic disc with a center hole for an IV valve. Seals against the PVC union's o-ring. .dxf provided for laser cutting. This could also be cut using a bandsaw or jigsaw, and a 7/16" drill.

Bracket Attachment – Mounts to the front of the stepper motor bracket to support the stainless steel rail. .stl provided for 3D printing. We used a PRUSA i3 MK3S filament printer to print this part in ABS.

PAWS Board – Custom PCB to mount and connect the electrical components.

Firmware – Firmware for the PAWS system. Programmed in the Arduino IDE.
Firmware Read Me – Provides important notes for writing, implementing and modifying the firmware.

Bill of materials

Bill of materials is available in the Mendeley Data repository.

Build Instructions

See Detailed Build Instructions in Appendix 1.

Operation Instructions

PAWS was designed for easy deployment and operation in the field with minimal prep in the lab. The PAWS syringe cradle was designed so the 60 ml polycarbonate syringe will slide in easily and rotate ¼ turn to lock into place (Fig. 3). When inserting a new syringe, it is critical to ensure that the plunger of the syringe is seated in the plunger flange. Two M3 stainless steel threaded rods and thumb nuts are used as a security measure to hold the syringe in place under pressure as well as provide some structural support for the sampling mechanism (Fig. 4). These rods slide through the two holes at the top on the front face of the syringe carriage, over the syringe. Two thumb nuts are then threaded onto both ends of both M3 threaded rods (total of four nuts). The inner two nuts are spun until they are approximately 1 cm in from the end of the rod. Then, the rod ends are screwed into the upper stepper motor mount holes and tightened the nuts against the stepper motor bracket. Finally, the remaining two nuts are thightened against the front face of the syringe cradle.

Users interface with PAWS controller using the OLED shield (Fig. 5). In addition to the screen, the shield has three individually programmable buttons (A, B, and C) and a reset button. In the provided code, the buttons are programed to navigate the PAWS menu system, adjust deployment and delay periods, and start the device. The reset button can be used at any point to restart the menu system with the default duration and delay settings. PAWS turns on immediately once the battery has been connected. After a brief welcome screen, the PAWS menu system goes into the sampling duration page. Here the user can adjust the amount of time it takes to fill the entire syringe (60 ml). In the current firmware, potential durations range between 1 min and 24 h. The duration can be increased by 1-minute increments by pressing button A, and 1-hour increments by pressing button B. The counter cycles back to 0 after 60 min, or 24 h, respectively. As such, the sample collection rate can be set between a max of 60 ml/min and a minimum of 60 ml/24 h or 0.042 ml/min. If longer sampling periods are needed, it is possible to change line 182 in the code to reflect the desired number of hours (up to 112 h with the current battery). To make set-up faster, we set this value a maximum of 24 h as all our sampling regimes were 24 h or less. See appendix 2 for a graphical description of setting the sampling duration.

Once the desired deployment period is selected, the user presses button C to advance to the delay menu. In this menu, the user can adjust the length of time PAWS delays before starting sample collection. Potential delays in the provided code range between 0 min (immediate start) and 24 h. Again this can be adjusted by modifying line 182 of the code. Once the desired delay has been selected, button C is pressed. This advances the PAWS menu to the start screen which instructs the user to press C to start. If the delay is set to 0-minutes, sampler collection will begin immediately when the user presses C, a useful feature for bench testing. Otherwise pressing C here will start the delay countdown timer (not displayed). If at any point the user would like to stop operation, C can be pressed again. The countdown or sampling will stop immediately, and the user will have the option to press C one last time to return the sampler to its "home" position. The homing function keeps a running tally of steps taken and when activated will reverse the motor at full speed the same number of steps. The homing function is also available once PAWS has completed a programmed sample collection. Homing is useful for resetting the system to a sampling ready state but could also be used to expel a collected sample without removing the syringe. See appendix 2 for a graphical description of setting the sampling delay and using the homing function.

Once the sampling duration and delay functions have been set, the tray can be slid into the pressure housing. The tray and the syringe carriage lock the sampling system into the housing by bracing against the inside walls of the housing tube. A short luer extension tube is connected between the syringe and the pass through on the pressure housing door. Once this connection is made, the door can be put in place and the locking collar screwed into place. Note – users should make sure that the o-ring is clean and greased with a thin layer of silicone prior to putting the door in place. Now any external tubing and/or filters can be connected to the exterior Luer lock on the acrylic door. After retrieving the sampler, it is important to remember that part of the sample will be contained in any length of tubing between the inlet and the syringe.

PAWS are approximately 5 lbs (2.25 kgs) positively buoyant in seawater. As such, it is important to secure the samplers in a method that is appropriate for the deployment environment. In low energy environments, a PAWS will sit on the benthos with a couple of dive weights zip-tied onto the pipe section of the housing. Ankle weights for drysuit diving also fit very snugly around the housing (Fig. 1). In more energetic or turbulent environments it might be necessary to secure the samplers using a post, bracket, or sand anchors.

Validation and characterization

Pressure design and testing

All of the components of the PAWS system which handle pressure internally, including the extension tubing, pass through, and syringe, are rated by the manufacturer to at least 175 PSI (119 m water depth) working pressure. We used the Under Pressure™ housing and vessel design software from DeepSea Power and Light to calculate the pressure rating of the external housing [30]. The calculated failure pressure for the 3″ schedule 40 PVC pipe is 398 PSI (273 m water depth). The calculated failure pressure for the acrylic doors is 191 PSI (131 m water depth).

The syringe mechanism was tested for failure in a lab setting under a 60 PSI (40 m water depth equivalent) pressure differential for 24 h. We then turned on the PAWS system and simulated water sampling at this pressure over a 24-hour period. In addition, we conducted a repeat stress test of the sampling mechanism consisting of 50 rapid cycles between 0 and 60 PSI. The pressure housing was tested for one hour at a depth of 21 m in seawater, and during multiple deployments of up to 12 h at 1 m depth in seawater. No leaks, mechanical damage, or signs of stress were observed in any of these tests. At the time of publication, we have not conducted failure testing on the sampling mechanism. Though we believe it is capable of withstanding higher pressure, we have rated the pressure limits of the system based on the successful 60 PSI tests that we performed.

Simulated environment testing

To test the efficacy of the PAWS system to integrate changes in water chemistry over an extended period of time, we deployed a sampler in a lidded tank containing approximately 44 L of artificial seawater made from tap water and Instant Ocean®. Over 8 h, the tank was spiked with phosphorus (Seachem Flourish®), a common macronutrient of concern in both marine and freshwater systems. In an effort to simulate the heterogeneity of natural systems, we varied the volume of phosphorus added between 0 and 10 ml. At hours 0.5, 2.5, and 4.5 the tank was dosed with 5 ml of Seachem Flourish®. At hour 1.5 the tank was dosed with 10 ml. No phosphorus was added at hours 3.5, 5.5, 6.5 and 7.5. The tank was stirred with a small paddle for at least a minute after each addition and then again before sampling. On the hour, three samples were collected by hand adjacent to the PAWS inlet and analyzed using a Hanna Instruments "Checker" Ultra Low Range Phosphorus Colorimeter. After 8 h, the PAWS system was removed from the tank and the syringe removed from the sampler. The integrated sample from the syringe was then analyzed three times using the colorimeter and compared with the individual samples (Fig. 7). The mean concentration from the PAWS samples (0.314 ± 0.031 ppm PO_4) is an underestimate as compared to the integrated concentrations from the manual samples (0.341 ± 0.032 ppm PO_4), but the PAWS samples still fall within the standard error of the mean of the manual samples (Table 2).

Discussion

Real world field studies are often limited by time, personnel, or budget constraints which do not allow for high frequency bottle sampling (e.g. hourly). This becomes increasingly more difficult when the number of sampling locations in a study increases. Supposing that our test system could not be sampled hourly, but on 4-hour or 8-hour intervals instead, the PAWS samples do a much better job at capturing a mean PO_4 concentration that is closer to the hourly mean as compared to means calculated from samples collected at lower resolutions (Table 2). PAWS cannot capture the high frequency variability of a system, such as that seen at hour 3 of the tank test (Fig. 1). It is possible that this variability occurred in the test system as a result of chemical interactions between the added phosphorus and the dissolved salts from the Instant Ocean® resulting in a form of phosphorus not detectable by the colorimeter. It could also be the result of insufficient mixing, despite the small tank size and mixing after additions and before sample collection. Similar high frequency variability will also occur in natural systems in the form of inputs, deposition, blooms, or chemical reactions. However, unless a study is particularly concerned with these short-duration events, smoothing the variability using an integrated sample may provide a more representative picture of long-term trends that is not as skewed by extreme events.

Field deployment

We deployed a PAWS system alongside three benthic flux chambers in a subsea sediment deposition zone at the mouth of a river in Mo'orea, French Polynesia. Benthic flux chambers consisted of a piece of 10 cm inside diameter by 61 cm long clear tubing that was driven at least 5 cm into the sediment. The upper end of the tube was capped with an o-ring sealed lid that included a septa port for extraction of water samples using a syringe, and a magnetic propellor to mix the fluid within the chamber prior to sampling [31] (Fig. 8). The goal of this study was to assess what, if any, flux of nutrients (nitrogen and phosphorus species) might be occurring between the sediment and the surrounding seawater. Over 12 h, the benthic chambers captured seawater exposed to sediment while the PAWS collected ambient seawater for comparison. For this deployment, the PAWS was programmed to fill the syringe over 12 h and was equipped with a 0.15 uM prefilter on a 15 cm long inlet tube. Once the study area had settled after installation of the flux chambers and sampler, time zero water samples were collected out of the septa of the chambers and directly adjacent to the PAWS inlet using syringes. After 12 h, samples were again collected from the septa of flux chambers (after mixing with the magnetic stirrer), and adjacent to the sampler inlet. The
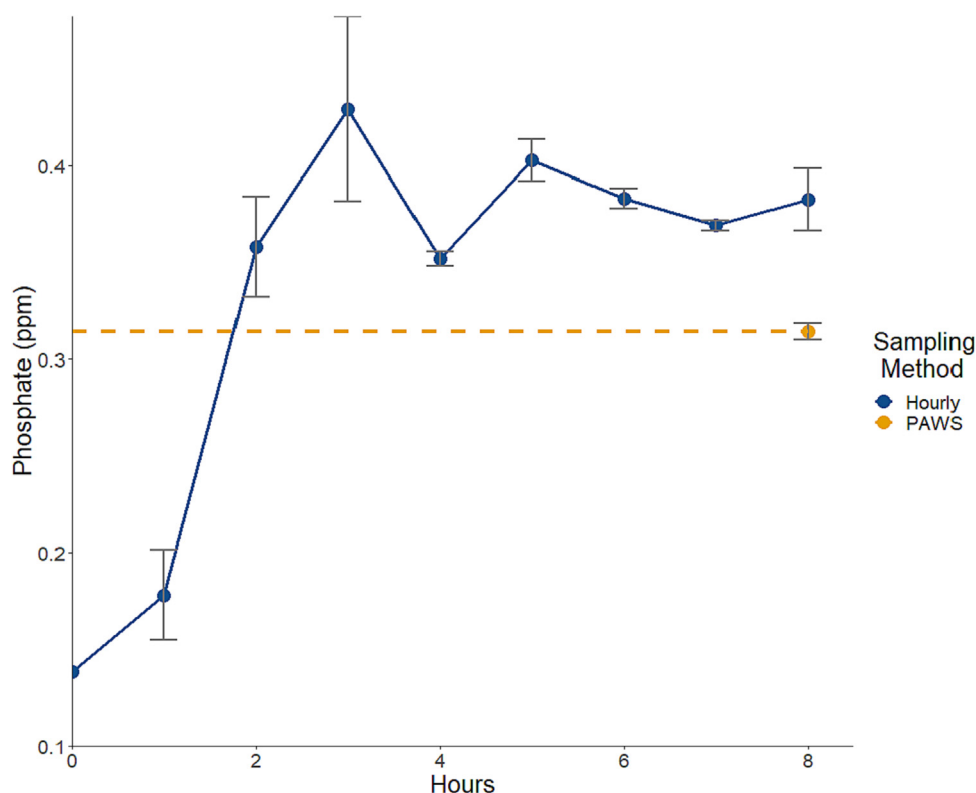
Fig. 7. Mean phosphate concentrations, with standard error, from test tank samples collected manually (blue dots) as compared to the integrated sample collected by PAWS (yellow dot). The dotted yellow line indicates the integrated phosphate concentration over the sampling period derived from the PAWS sample. The solid blue line indicates the interpolated trends in phosphate between individual bottle samples. The standard error bars reflect the variation in the three samples collected at each bottle sampling timepoint (with the exception of t = 0 which had only one sample), and the three sub-samples that were analyzed from the PAWS syringe. The ~ 44 L tank was spiked with a 5 ml dose of Seachem Flourish® Phosphorus at hours 0.5, 2.5, and 4.5, a 10 ml dose at hour 1.5, and 0 ml at hours 3.5, 5.5, 6.5 and 7.5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Means and standard error (SE) for PO_4 concentrations from PAWS compared to bottle sampling at different frequencies.

Sampling Method	Mean PO_4 Concentration (ppm)	SE
PAWS	0.314	± 0.031
Hourly	0.341	± 0.032
Every 4 h	0.306	± 0.031
Every 8 h	0.260	± 0.028

sampler was then retrieved along with the flux chambers. Back in the lab, the PAWS syringe was removed and the sample was transferred to a storage bottle. All samples were sent to Oregon State University for analysis.

Discussion

Unfortunately, the results from this study cannot be shared as the samples were lost in a lab fire [32]. However, the study provided an excellent field test for the PAWS system, as the mechanics of system performed faultlessly. Additionally, the study design presents a good example of how PAWS can be used in the field. In this case, we feel that the sample collected by PAWS would have provided a better comparison to samples collected from the flux chambers, as opposed to bottle samples. This is because both the flux chamber and the PAWS samples are integrated over time, representing an average concentration, or accumulation, of nutrients. Comparing the flux chamber samples to bottle samples, which only provide instantaneous values of the ambient water conditions could skew results as the bottles may be collected during or between peaks and valleys in nutrient concentrations. Despite the loss of the samples, this test deployment, in addition to the lab testing, provided collaborators with sufficient confidence in the system to deploy them in their studies. PAWS will be deployed in the near future to track nutrient cycling inside the cryptic structures of coral reefs.

Fig. 8. Benthic flux chamber (left) and PAWS (right) deployed at 1 m water depth in Mo'orea, French Polynesia. The flux chamber consists of a 10 cm clear tube capped with a sealed lid (grey) which contains a manual magnetic stir propellor (center white) and a rubber septum for sampler collection with a syringe (edge white). The chamber is driven into the seafloor to collect potential chemical fluxes over a 12-hour period. The PAWS system was deployed alongside three flux chambers to collect a comparison sample of ambient seawater.

Additions and future developments

PAWS was designed as a functional base unit that allows easy opportunity to expand and build upon. Inherent in the Adafruit Feather architecture is the ability to add functionality by stacking compatible boards or "wings". Wings could be added to PAWS control system that would allow it to be controlled and communicate over ethernet, wifi, or LoRa protocols. The Feather microcontroller can also communicate with numerous external devices simultaneously using a multitude of communication protocols including I2C and SPI. This functionality allows for the easy addition of sensors (e.g. pressure or temperature sensors) that could trigger PAWS to start sampling in response to physical changes in the environment. For example, a pressure sensor equipped PAWS unit deployed in a stream could wake up and begin sampling when there is an increase in stream depth to capture an integrated water sample during a storm event. Towards this end, we have built and tested a prototype of a temperature and pressure data-logger designed around the Feather M0 express and deployed in the same type of housing used for the PAWS system (Fig. 9). Future work will integrate these two devices into one unit. Moving forward, we also intend to release an update to the PAWS firmware to improve power management and sleep functionality. This will make PAWS more efficient, reducing battery requirements for longer deployments.
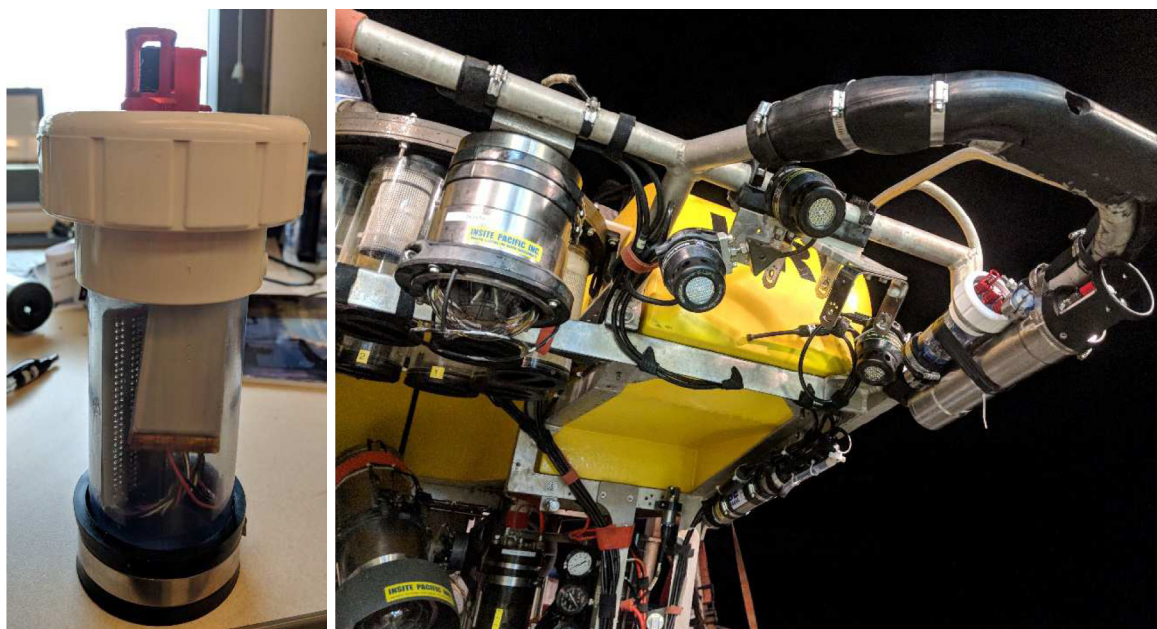

Fig. 9. Pressure and temperature logger built with the same general architecture as PAWS sitting on the bench (left). Both the logger and PAWS use an Adafruit M0 express microcontroller. The logger uses temperature and pressure sensors from Blue Robotics. This version of the logger was built in a 2-inch PVC housing with a PVC union and laser cut acrylic door on one end, and a rubber cap on the other. We tested the logger down to 1000 m alongside a commercial CTD package on the ROV *Hercules*. To handle the high pressures, the housing was filled with mineral oil and the rubber cap acts as pressure compensator. Future versions of PAWS will incorporate sensors for parallel data logging, or to act as a trigger. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conclusion

Research questions related to water chemistry impacts on aquatic and marine environments are exceptionally varied. They cover a wide range of chemical compounds, time and spatial scales, habitats, depths, flow conditions and organisms. To address these myriad questions it is essential that marine and freshwater scientists have an arsenal of tools at their disposal. It is especially important that these tools are able to provide data on the spatial and temporal scales relevant to rapidly changing and highly heterogeneous conditions. Here we present a Programmable Autonomous Water Sampler that adds an inexpensive, robust, and highly adaptable tool to the available arsenal. PAWS is capable of capturing an integrated picture of water chemistry conditions over a range of timescales with a price per unit that allows for coordinated widespread deployments either as a standalone sampling device or as a supplement to a larger research program.

CRediT authorship contribution statement

Kyle C. Neumann: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition. **Daniel La:** Methodology, Investigation, Validation. **Hyemin Yoo:** Software, Validation. **Deron E. Burkepile:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ohx.2022.e00392.

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