6. Operation instructions

6.1. User interface

Once the batteries are attached to the SASe circuit board the OLED screen will turn on and display the Status Menu (Fig. 1). To navigate through and within the SASe menu screens, use the four cursor keys and the enter button of the IR remote, the left and right cursors will navigate between menus from the top of the screen (Fig. 2). Settings are saved by selecting “ENTER SET” and pressing enter.

6.2. Calibration

The peristaltic pumps have an advertised pump rate of 0.2mL per revolution, but there is variability by pump head, so a calibration step uses the motor’s rotary encoder and user input to measure the actual pump rate and improve accuracy of sampling volume. To run the calibration, install a fresh Sterivex filter onto the filter luer lock connection, including the one-way check valves on either end of the filter (Fig. 3). Connect a piece of 1/8-inch tubing to the inflow connection of the sampling pump and put the other end of the tubing into a water cup. Direct the outflow of the filter into a graduated cylinder. With the Status Menu on the screen press the left cursor on the IR remote to navigate to the Prime Menu. Use the down cursor on the IR remote to navigate the cursor to the row labeled “RUN WATER”, then hit enter on the remote. The sampling pump will turn on and can be turned off by pressing enter on the remote again. The tubing and filter should be fully primed with water before starting the calibration to ensure accurate results. Position the outflow of the filter to flow into an empty graduated cylinder. Use the left cursor to navigate to the Calib Menu. Select “RUN CAL” and press the enter key on the IR remote. The sampling pump will turn on for 30 seconds. Measure the amount of water

transferred to the graduated cylinder when the pump stops. Use the IR remote and the up and down cursors to enter the volume of water collected on the “VOL:” line. Select ENTER SET and press enter to save the calibration. The screen will display <SAVED> to show it was saved. After calibration, the sampling pump has an accuracy of ±5% of the target volume (Fig. 4).

6.3. Set time

Once the calibration is set, the RTC on the microcontroller should be set. Navigate to the Time menu. The first row is the time in HH:MM:SS and the second row is the date in DD/MM/YY. Change the numbers using the up and down cursors to match up with the time and date. Select and press enter on “ENTER SET” to synchronize the RTC with set time. The correct time will now be displayed in the Status Menu.

6.4. Set sample mode, collection time, and date

The sampler can operate in two different modes: Once and Daily. Once mode will sample a set volume at a single given time and date and then wait in sleep mode until woken up and reprogrammed. Daily mode will sample a set volume at a given time every day starting on the date selected. Navigate to the Settings Menu and select “Mode”; use the up and down cursor to switch between “Once” and “Daily”. The next line allows the sample volume to be adjusted. The up and down cursors can be used to increase or decrease the volume in increments of 10 mL with an upper limit of 2000 mL. Once the mode and volume have been chosen, select “ENTER SET” and press enter to save the settings.

Navigate to the Pump Menu. The first line (T) is the time setting for an alarm to go off and

activate the sampling programming while the second line (D) is the date. Both options will always be available, but the date will only be displayed on the Status Menu for Once Mode. Daily mode uses the pump date to determine when daily sampling begins, but on the Status Menu only the time of sampling will be displayed. Use the up and down cursors to adjust the time (HH:MM) and date (DD/MM/YY) and then select “ENTER SET” and press enter to save the pump alarm time. Navigate to the Status Menu to review the input settings and confirm the current time.

6.5. Initiate sampling mode

After calibrating the sampler and inputting the settings the pump needs to be in a sleep mode to save battery life and initiate sampling tasks. Navigate to the Initiate Menu. Select “Press Enter” and press enter on the IR remote. The sampler will go into sleep mode. The time, date, and battery voltage will be logged on the dataLog.txt file on the microSD card when sampling starts. If the SASe is set to go off within 60 seconds of sleep mode being initiated “Wait...” will be displayed on the screen until the sampling starts. When the sampling mode begins, the sampling pump is activated and the screen will read “SAMPLING”. At this point the pump will run until the programmed volume of water has been filtered and the program cannot be interrupted with the reed switch. Once the sample has been taken, the sampling pump will turn off and the preservative pump will turn on. The screen will read “SYRINGE” and the preservative pump will run until five mL of preservative has been pumped into the filter housing. If the SASe is set to Once mode it will return to sleep mode and stay that way until woken up with the reed switch or until the batteries die. If it is in Daily mode it will add 24 hours to the sample time and date

and start sleep mode again, until the sampler is woken up with the reed switch or until the batteries die.

6.6. Interrupt sampling mode

While the SASe is in sleep mode, no changes can be made to the settings. To wake the sampler up from sleep mode and allow changes to be made, the reed switch on the face of the sampler must be activated. To activate the reed switch, press a magnet against the acrylic faceplate and move it back and forth in front of the black reed switch (Fig. 2). After 2-3 seconds, the sampler will come out of sleep mode and display "REED!" on the OLED before displaying the Status Menu.

6.7. Deployment

Before deploying the SASe the O-ring should be lubricated, fully charged batteries installed, and all securing screws and nuts carefully tightened to ensure a waterproof seal. Take care when lubricating the O-ring to ensure it is clean of dirt, hair, or other debris that could break the waterproof seal. Install a new Sterivex filter capped by check valves into the sampling cartridge and fill the preservative syringe. Secure the cover of the sampling cartridge using the #6-32 bolt and wingnut. Attach the filter connection and the syringe connection to the filter inflow check valve and the syringe, respectively (Fig. 3). Attach the filter cartridge to the SASe housing via the magnets.

To prevent an airlock in the tubing or compression of the filter air space when the SASe is deployed at depth, prime the filter with molecular-grade water prior to deployment. Use a clean

syringe to fill the filter or place the inflow of the sampling pump into the water source and use the Prime Menu's "RUN WATER" command.

The SASe is slightly negatively buoyant but it should be attached to a dive weight or to an underwater structure to prevent it from moving in waves or current. If attached to a solid structure, the SASe should be tightly secured to prevent wear on the housing. For long deployments, large stainless-steel hose clamps work better than zip ties which can stretch and loosen under stress over time.

Once a sample has been collected, the filter or the entire sampling cartridge must be replaced to continue daily sampling or set new dates and time for single sampling. New filters must have check valves and be primed with purified water. This filter/cartridge replacement can be done as many times as the battery life will allow, typically five to six times for 1000mL samples. Completed samples are returned to the lab for analysis. The preservative acts to preserve the DNA samples for weeks to months at room temperature [1, 2]. The check valves on the filter have a 2.9 psi cracking pressure to prevent sample contamination, loss, or dilution; however, the filters should be further sealed with luer lock caps after collection to ensure no sample is lost due to rough handling while awaiting laboratory analysis.

6.9. Downloading sample data

To retrieve sampling data, remove the internal armature from the housing and remove the microSD from the circuit housing. Download the two text files, sampleParam.txt and dataLog.txt. The sampleParam file retains the settings and sampling parameters as described

earlier. The dataLog file has a line of text for every sample taken which includes the sampling start time and battery voltage.

Example: 9:30:1,18/11/2019,10.74

Key: Hr:Min:Sec (Sampling started),Day/Month/Year (Sampling started),Voltage (volts)

The programming for the SASe requires these two text files to operate; do not remove the files from the microSD card when downloading the data and reinstall the card before storing or redeploying the SASe.

6.9. Cleaning

The SASe should be rinsed and cleaned with freshwater to remove any sediment, growth, and/or salt. The tubing for the sample and preservative pumps should be rinsed with molecular-grade water using the Prime Menu's "RUN WATER" and "RUN SYR." command. Press enter on either command to start its associated pump and press enter again to turn it off. To remove any biological material from the SASe sampling system, the pump tubing should first be flushed with freshwater and then soaked in a 5% bleach solution. To soak the sampling system, connect one piece of tubing to the inflow fitting and another piece of tubing to the syringe connection. Place the free ends of both tubes in a container of 5% bleach solution. Use the Prime Menu to run the bleach water through the tubing for both the sample pump and the preservative pump. Once the SASe tubing and the fittings are completely filled allow the bleach solution to sit for 30 minutes and then flush the tubing thoroughly with molecular-grade water to prepare the SASe for the next deployment.

6.10. Storage

After cleaning the SASe, it can be stored until future use. To extend the battery life, the power connection can be disconnected from the circuit board. While it is possible to have multiple deployments of the SASe off the same battery pack, it is recommended that fresh batteries are replaced whenever possible to limit the chance of partially processed samples, resulting from low or dying battery power. Once the coin cell battery voltage drops below 3 volts, it will no longer power the RTC. To avoid sample timing errors, the time on the SASe should always be checked and reset, if necessary, before deployment.

References:

[1] DNAgard® Tissues & Cells Handbook. Item No. 9101. Rev. 08/2011. [Online]. Available: https://www.biomatrica.com/download/hp-dnagard-tissue-handbook/?ind=1519868897797&filename=9101-0811.pdf&wpdmdl=580&refresh=5efb407ad50a71593524346 (Accessed 10.15.20)

[2] DNA/RNA Shield Zymo Research product description. [Online]. Available: https://files.zymoresearch.com/protocols/_r1100-50_r1100-250_r1200-25_r1100-125_dna_rna_shield.pdf (Accessed 5.27.21)

Figures & Tables:

Figure 1: The seven menus in the SASe user interface with the menu title in highlighted in yellow at the top. Left to right: Status Menu, Settings Menu, Pump Menu, Initiate Menu, Time Menu, Calib Menu, Prime Menu.

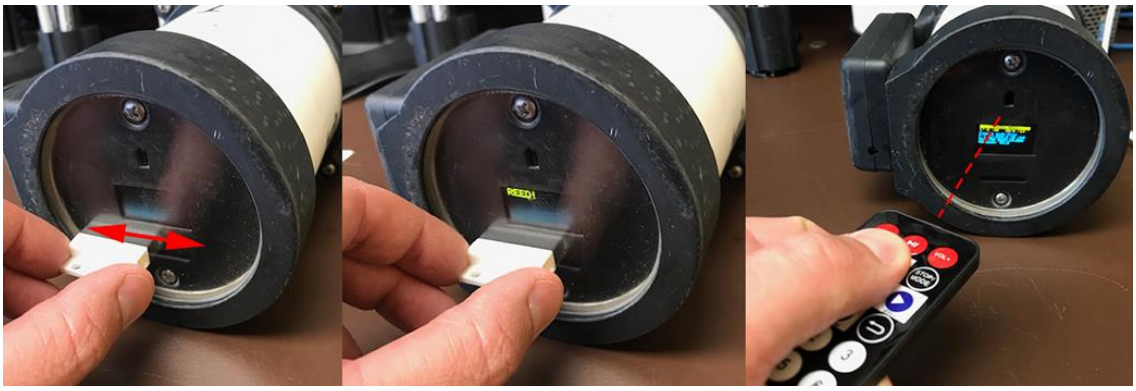

Figure 2: Moving a magnet across the face of the reed switch will wake the SASe from sleep mode. With line of sight, the IR remote can be used to communicate with the IR receiver for navigating through the SASe menus.

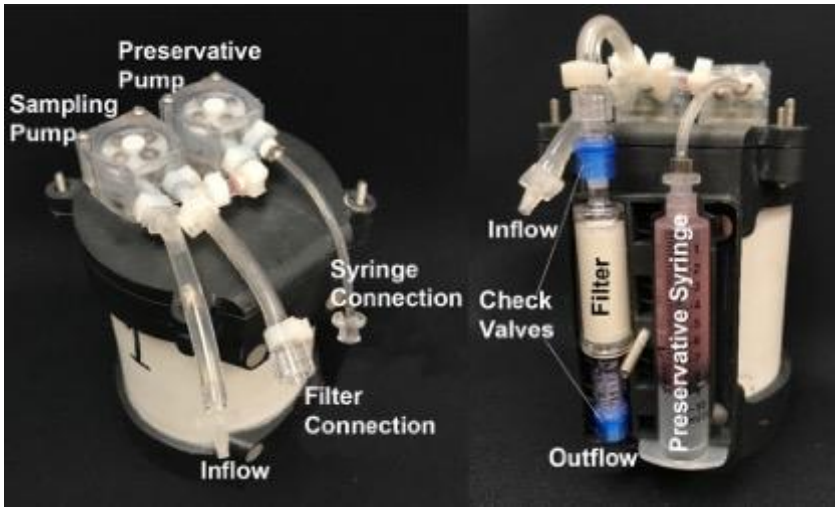

Figure 3: The external components of the SASe sampling system labeled for reference.

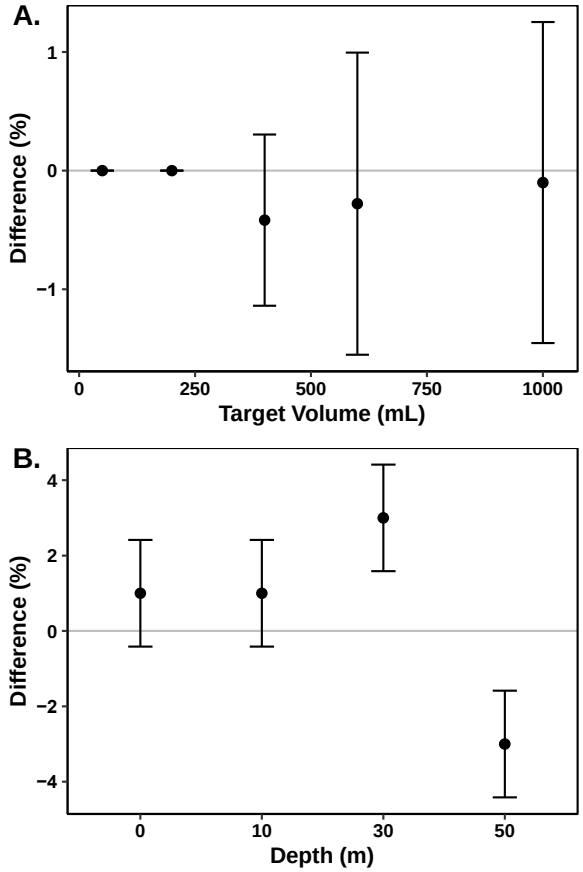

Figure 4: Percent difference in the volume of water programmed to be filtered vs that collected as a function of (A) targeted volume and (B) depth. Error bars are standard deviations.