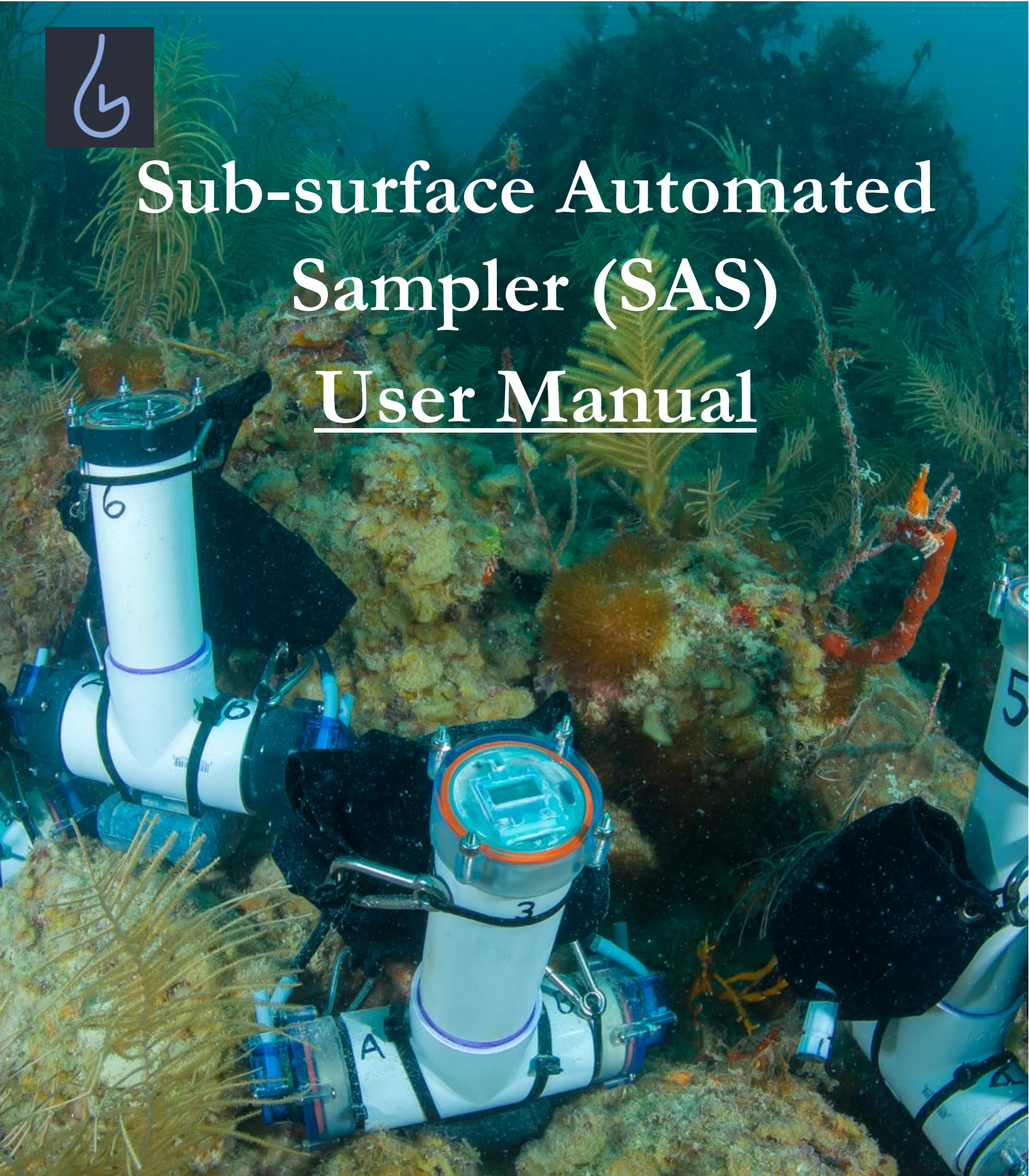




Sub-surface Automated Sampler (SAS) User Manual



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User Manual



Written By Nathan Formel

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This sub-surface automated dual water sampler (SAS) was designed, created, and tested by researchers at NOAA and the University of Miami to enable researchers to study water chemistry on shallow reef habitats and further understanding of the conditions present in coral reef ecosystems. The project was intended to enable not just research institutions, but other interested parties as well by acting as a low cost alternative to other existing automated water samplers. The open-source design will continue to be developed and improved on by NOAA and University of Miami researchers, as well as the science community at large. This user manual is meant to serve as an exhaustive resource for any group to program, deploy, and troubleshoot their own water samplers. Further resources and future updates to the SAS design are available on the NOAA website (<http://www.coral.noaa.gov/accrete/SAS>).

Cover photo taken by Kevin Davidson of the ANGARI Foundation during field deployment of the SAS in Dry Tortugas National Park.

Table of Contents

1. Turning on the sampler
2. Calibrating the sampler
3. Setting the time
4. Choosing mode and sample volume
5. Setting pump sample times
6. Initiating sampling mode
7. Waking up from sampling mode
8. Sampler deployment
9. Collecting the samples and transferring samples for analysis
10. Safely Shipping the SAS
11. Troubleshooting the sampler

1. Turning on the sampler:

Load a set of batteries into two of the four-AA battery packs and plug the packs into the sampler. Please note, the battery pack below the power connection to the circuit board (labeled “Brain” on the internal housing) should be plugged in first (See Image 1). If the battery packs are plugged in out of order the momentary lack of grounding will cause the real-time clock in the Teensy to reset losing any date and time settings. Also note that the battery packs will only connect to the 9-volt connectors in one direction. Be sure to fully insert the packs; they should connect with a click and sit evenly with each other. Once the battery packs are connected the screen on the sampler will turn on and you will see the <STATUS MENU> (See Image2). The <STATUS MENU> includes all the settings and information being recorded by the microSD card during deployment.

The sampler interface operates using the four cursor keys and the enter button of an infrared (IR) remote (See Image 3). The left and right cursors will navigate between menus from the top of the screen. On any screen the up and down cursors in combination with the left and right cursors will allow the user to navigate through the menus, change options and number settings, and save by highlighting “ENTER SET” and pressing enter.

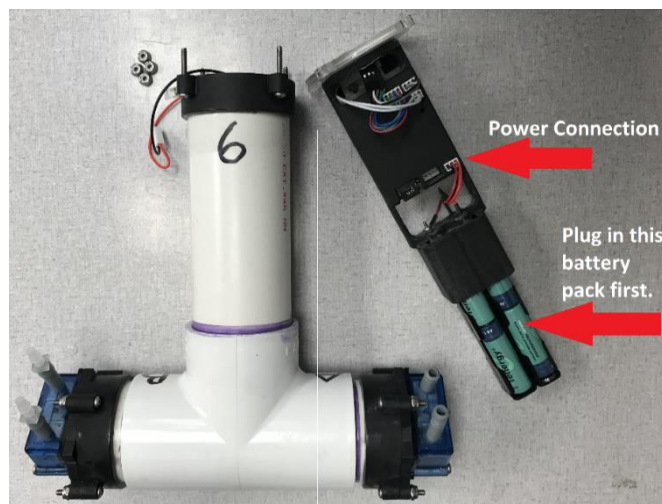


Image 1: Plug in the battery packs in the correct order to power on the sampler.



Image 2: The <STATUS MENU> gives a summary of the sampler settings and date and time for quick reference.

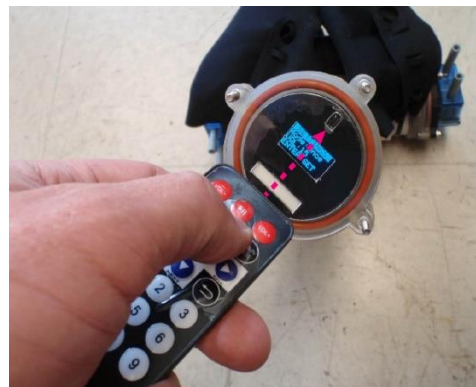


Image 3: Point the IR remote at the IR sensor on the face of the SAS and use the cursors and enter key to navigate through the menus.

2. Calibrating the sampler:

The first step in setting up a new sampler is calibrating the pumps. Despite the pumps and motors all being the same type and model, there is still some variability in their pumping rates. The inter-pump/motor variability is dealt with by calibrating both pumps on every sampler prior to using them for the first time. To calibrate the samplers first load a fully charged set of batteries into two of the 4 AA battery packs and plug the packs into the sampler. The sampler screen will turn on, then press the left cursor on the IR remote to navigate to the <RUNCAL> screen (See Image 4). Connect a longer piece of tubing to each of the pump's tubes. Insert the inlet tubing into a beaker of water and place the outlet tubing into a 100mL graduated cylinder (See Image 5). Use the IR remote to navigate the cursor to the row labeled PA (Pump A), then select START on the sampler screen and hit enter on the remote. Pump A will turn on for 10 seconds. Measure the amount of water transferred by the pump in that time. That volume will give you your calibration number. So, for example, if your pump transfers 15mL of water then you'll have a calibration number of 15.



Image 4: The <RUNCAL> menu allows the pumps to be run manually for 1 minute.



Image 5: Attach longer tubing to the inlet and outlet of the pump tubing for calibrating the SAS pumps.

****To get the most accurate calibration volume the pump should be run twice, first to prime the tubing with water, and the second time to take the calibration measurement described above. ****

Repeat this test for Pump B (PB) as well. Once finished the proper calibrations can be entered into the sampler settings under the <PUMPCAL> menu (See Image 6). Navigate to the <PUMPCAL> menu using the left cursor. Select PA and update the calibration number to what was found in the test for that matching pump. Then select PB and update the calibration number there. Choose “ENTER SET” and hit enter to save the calibration settings. It’s a good idea to check the success of the calibration setting by running a sample volume on both pumps and seeing if the volume set matches the pumped volume. If further adjustment is needed to improve the pump accuracy the calibration settings can be changed. A larger calibration number will decrease the sampling time and volume, and a smaller number will increase the sampling time and volume.

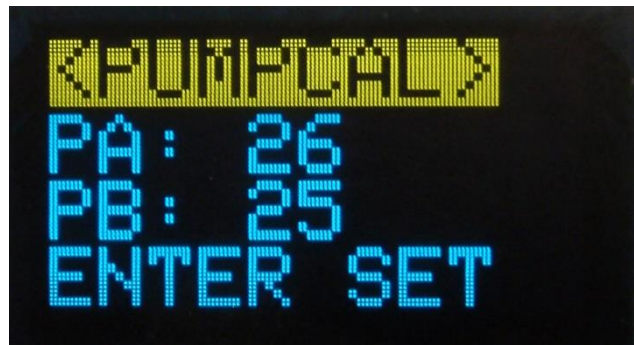


Image 6: The <PUMPCAL> menu is used to calibrate the individual pumps to standardize the sample volumes.

3. Setting the Time:

Once the calibrations are set, the time on the sampler should be set. From the top line of the <PUMPCAL> screen press the left cursor on the IR remote to navigate to the <TIME SET> screen (See Image 7). 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. Be sure to give yourself time on the seconds setting so that you can select “ENTER SET” and press enter at the appropriate time to synchronize the real-time clock (RTC) on the sampler with real time. If you return to the top of the <TIME SET> screen and navigate to the left or right to get back to the <STATUS MENU> you will see the newly set time and date shown.



Image 7: The <TIME SET> menu is used to set the RTC on the sampler to keep track of real-time even in sleep mode and when the battery pack is removed.

4. Choosing Mode and Sample Volume:

The sampler can operate in two different modes: Once and Daily. Once mode will run Pump A and Pump B at a single given time and date and then wait until a new time and date have been set to run again. Daily mode will run Pump A and Pump B at a given time every day until turned off. From the <STATUS MENU> use the right cursor to navigate to the <SETTINGS> menu (See Image 8). Under “Mode” you can use the up and down cursor to switch between “Once” and “Daily”. The next line lets the sample volume be adjusted (both pumps will be set for the same volume). The up and down cursors can be used to increase or decrease the volume in increments of 10mL with an upper limit of 900mL. Once the mode and volume have been chosen select “ENTER SET” and press enter to save the settings. If you return to the <STATUS MENU> you will see the newly set sample mode and volume settings shown.

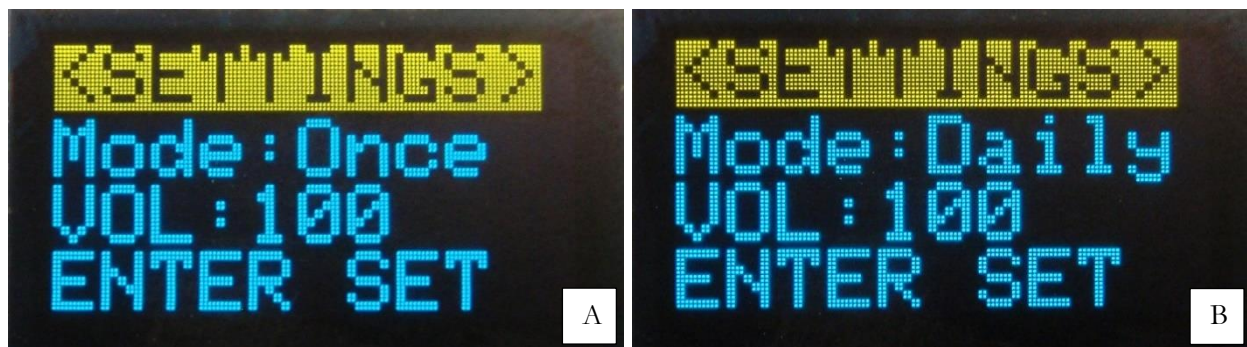


Image 8: The <SETTINGS> menu allows two different modes to be chosen, (A) Once mode for sampling once per pump at a set time and date, and (B) Daily mode for sampling daily on each pump at a set time and starting date.

5. Setting pump sample times:

From the top of the <SETTINGS> menu use the right cursor to navigate to the <PUMP A> menu (See Image 9A). The first line (T) is the time setting for an alarm to go off and activate Pump A while the second line (D) is the date. These options will always both be available, but the date will only be displayed on the <STATUS MENU> menu for Once Mode. Regardless, in Daily mode the date should still be set for the first day that samples are to be taken.

Use the up and down cursors to adjust the time (HH:MM) and the date (DD/MM/YY) and then select “ENTER SET” and press enter to save the Pump A alarm time.

At the top of the <PUMP A> menu use the right cursor to navigate to the <PUMP B> menu (See Image 9B). Following the same steps as above set the alarm time for Pump B and save by selecting and pressing enter on “ENTER SET”. If you return to the <STATUS MENU> you will see the newly set pump times and dates listed if in Once mode, and just the newly set times if in Daily mode.

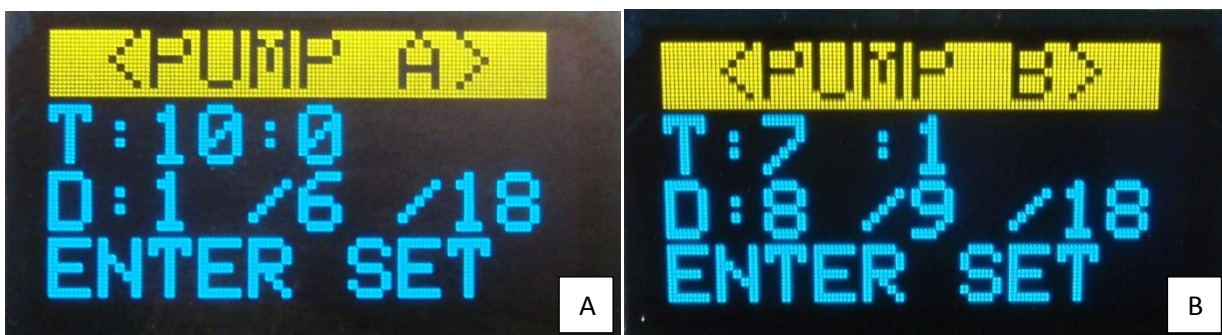


Image 9: The SAS is a dual sampler, and so (A) <PUMP A> menu is used to set a sampling time and date for Pump A, and (B) <PUMP B> menu is used to set a sampling time and date for Pump B.

6. Initiating Sampling Mode:

After calibrating the pumps and inputting all of the settings the pump needs to be in a sleep mode to save battery life and initiate sampling and temperature logging tasks. From the top of any of the menus use the left or right cursor to navigate to the <INITIATE> menu (See Image 10). Select “Press Enter”, press enter on the IR remote, and the sampler will go into sleep mode. The time, date, temperature, battery voltage, and sample code will be logged on the microSD card when the pumps turn on. If either of the pumps are set to go off within 60 seconds of sampling mode being initiated "Wait..." will be displayed on the screen until the pump(s) start. When the pumps are activated the screen will read “Pump A” or “Pump B” and the pump will run to collect the sample. If the timing for the two samples to be taken overlap, the screen will read "Both Pumps" while both pumps are running (See Image 11).



Image 10: The <INITIATE> menu is where the sampling mode is started, putting the sampler to sleep and saving power until the pumps are set to go off to collect a sample.

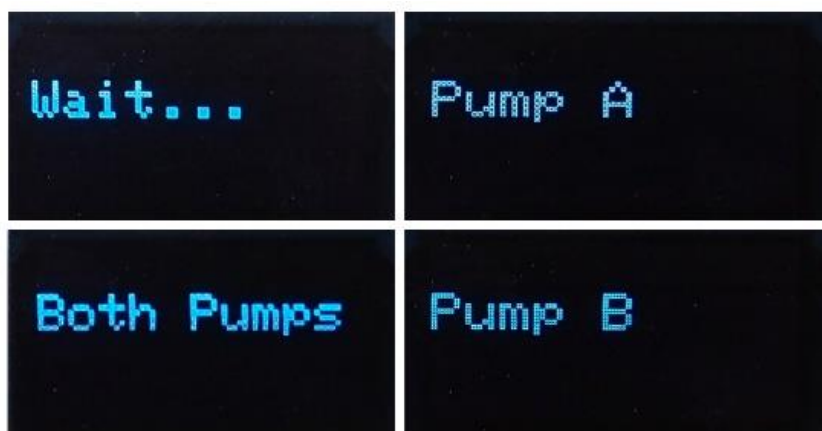


Image 11: Once the sampling program is initiated the four messages above are the only things that should appear on the screen until the sampling mode is interrupted.

7. Waking up from sampling mode:

While the SAS is in sampling mode no changes can be made to the settings. To wake the sampler up from sampling 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 white reed switch (See Image 12). After 2-3 seconds the sampler should come out of sleep mode and display "Interrupt" on the OLED before displaying the STATUS MENU. The reed switch can be activated in the lab, field, or underwater. If the reed switch doesn't seem to be working, reorient the magnet and try again. Be sure to move the magnet across the face of the reed switch, since a static signal will not wake it up.

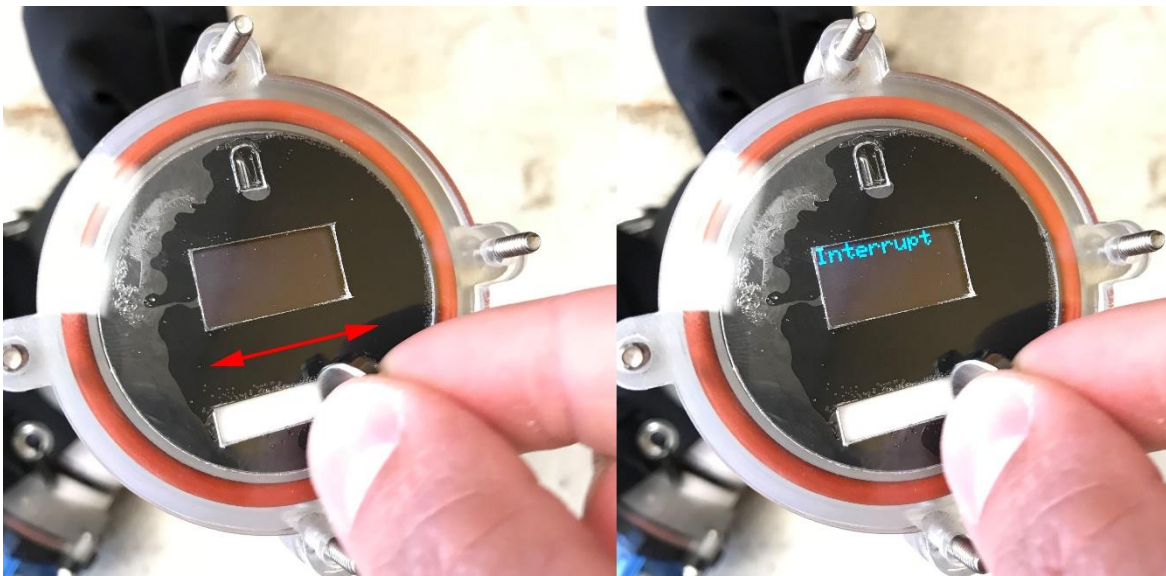


Image 12: Move a magnet back-and-forth in front of the reed switch to wake up the sampler.

8. Sampler Deployment:

Samplers to be deployed should have calibration settings for the two pumps completed, all O-rings lubricated with synthetic lube, fully charged batteries installed, and all securing screws and nuts tightened (no more than hand-tight) to ensure a waterproof seal. Take care when lubricating the O-rings to be sure that they are clean and there is no dirt, hair, or other debris fouling them that might break the waterproof seal.

To preserve the samples and prevent any change to the carbonate chemistry the Tedlar sample bags should be dosed in advance with a saturated mercuric chloride (HgCl_2) solution. The volume of the HgCl_2 used should be 0.05 to 0.02 percent of the total sample volume (Dickson et al., 2007). A hypodermic needle can be used to inject the HgCl_2 through the membrane in the top of the Tedlar bag valve (See Image 13).

****Be careful not to puncture the Tedlar bag by pushing the needle too far into the valve membrane. ****



Image 13: Use a hypodermic needle to inject mercuric chloride into the Tedlar bag through the valve membrane prior to collecting any samples.

The sampler can be deployed at any depth up to 180 feet of seawater. It is possible the sampler may be able to be deployed deeper, but 180 feet represents the maximum depth that the design has been tested to. The settings for time and sampling can be made with an IR remote in the lab, in the field at the surface, or underwater. If an IR remote is to be used underwater it should be kept in a waterproof bag to prevent failure of the remote. Once all settings are completed and the sampler has been initiated the Tedlar bag valves should be opened one half turn to be ready to sample.

The natural buoyancy of the sampler should be offset by attaching two pounds of weight to make it slightly negatively buoyant. The sampler can be secured on the substrate by attaching extra weights to the bottom which will maintain an upright orientation, or by securing it to underwater structure (See Image 14).

The intake tubing for the sampler pumps should be unobstructed when deployed. For that reason it's important to make sure that the sampler is oriented upright when placed on the substrate to avoid sediment clogging the intake tubes.



Image 14: The SAS should be deployed upright on the bottom to avoid obstructing the pump intake, or it can be attached in any orientation to underwater structures in the water column.

9. Collecting the samples and transferring samples for analysis:

If the samplers are set to DAILY mode the Tedlar bags will have to be replaced in the interim between samples. To replace the bags, close the Tedlar valve on the completed sample. Remove the Tygon tubing from the valve stem. Carefully take the neoprene cover off of the Tedlar bag.

****Be careful handling the full sample bag and avoid handling the bag by the valve as that bag/valve interface is likely the weakest seal on the Tedlar bag. ****

Once the filled Tedlar bag is safely removed place a new closed Tedlar bag dosed with mercuric chloride in the protective neoprene bag. The Tedlar sample bag should be placed in the folded contours of the protective bag with as few creases as possible. The valve of the Tedlar bag will come out of both holes in the neoprene to secure the neoprene bag flap closed. Reroute the Tygon tubing through the slits in the neoprene cover and attach to the stem of the Tedlar bag valve (See Image 15). Open the valve a half turn and leave the sampler to take its next daily sample. **This procedure must be repeated for every extra sample taken on Daily mode.**



Image 15: The SAS with Tedlar sample bags inserted into the protective neoprene cover and connected to the sampling pumps.

Once the samples have been taken and no others are needed the sample bag valves should be closed and the samplers returned to the surface. Care should be taken to store the filled sample bags away from sharp objects and direct sunlight. Assessment of the length of time the Tedlar bags can be stored or left in the field without affecting the carbonate chemistry of the seawater sample

is still underway. Preliminary results comparing seawater samples in Tedlar bags to the industry standard borosilicate glass bottles (Dickson et al., 2007) show samples to be stable in Tedlar bags in salt water or air for up to two weeks without altering the chemistry significantly

Analysis of the carbonate chemistry parameters of dissolved inorganic carbon (DIC) and total alkalinity (TA) can be done directly from the sample bag. If the sample must be transferred to another storage container use caution to minimize gas exchange and splashing in emptying the bag into another container. If this is necessary, be sure to collect more than the necessary sample volume to allow for overflow of the sample into the new container, following normal sampling guidelines laid out by Dickson et al. (2007). One way to carry this out is to attach a length of tubing to the tedlar bag valve, place the tubing in the bottom of a clean borosilicate glass bottle, and gently squeeze the bag until all of the sample has been transferred into the bottle, avoiding splashing and bubbling by keeping the end of the tube at the bottom of the bottle. The bottle can then be sealed and analysis can be run from the bottle when convenient.

Repeated deployment of the samplers without exchanging or charging the main batteries is possible but not recommended. The optimal working voltage is between 10 and 12 volts. It's not recommended that the samplers be used with less than a 10 volt charge as the sample volume can become more variable at lower voltages and eventually will cease to take samples at all as battery charge is further reduced.

To retrieve the sampling data from the microSD card first remove the battery packs, then remove the microSD card. Download the two text files, dataLog.txt and sampleParam.txt. The **dataLog** file has all of the sampling time and date settings, the battery charge and temperature at the time of sampling, and a sample code for each of the pumps to identify their status (0 = Waiting to pump,

1 = Initiating pump, 2 = Pump running, 3 = Pumping finished) with a line for each sample taken in the following format:

Time(HH:MM:SS),Date(DD/MM/YYYY),Temp °C,Battery Pack Voltage,Pump A Code, Pump B Code

Example: 12:10:10,1/15/2018,24.3,10.21,1,0

The **sampleParam** file has the settings for the sampler including mode, calibrations, pump volume, and time and date for scheduled samples in the following format:

Mode(0=Daily, 1=Once),PA Calib.,PB Calib.,Pump Volume in mL,1 (Temperature Constant)
PA settings for Time (HH,MM),Date (DD,MM,YY)
PB settings for Time (HH,MM),Date (DD,MM,YY)

Example: 1,24,21,700,1
12,30,5,10,18
10,5,5,11,18

The sampling parameters on the microSD card (sampleParam.txt) are constantly referenced by the sampler program. If the sampler is on when the card is removed the programming on the Teensy microcontroller may need to be reset. Be sure to remove the battery packs before removing the microSD card from the SAS. For the same reason, after downloading the text files from the microSD card, be sure to plug the card back into the microSD slot on the circuit board before plugging the batteries back in. If the batteries are plugged in without the microSD an error message will be displayed saying "MicroSD not detected!" and the sampler STATUS MENU will not be

displayed. If, after putting the microSD card back in and reconnecting the power, the sampler settings are different than what was programmed, the Teensy should be reset.

To reset the Teensy remove the batteries, remove the circuit board cover, and press the small tan pushbutton next to the microSD card slot on the Teensy microcontroller. Plug the battery packs back in and the parameters set on the microSD card should be restored on the sampler.

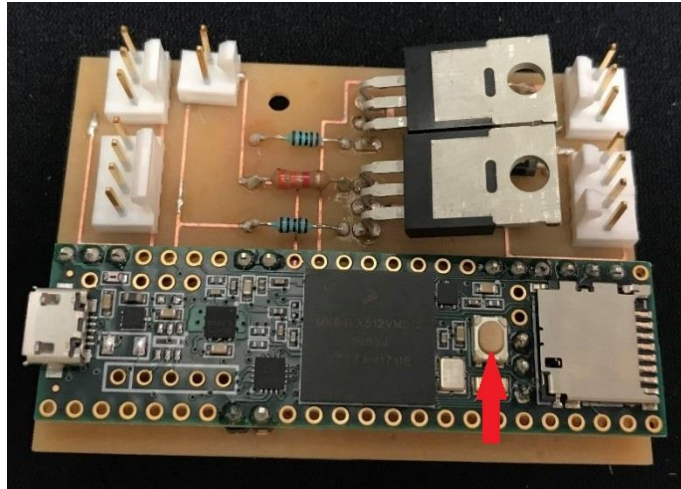


Image 16: The SAS circuit board with the red arrow indicating the Teensy reboot button, for resetting the programming.

10. Safely Shipping the SAS

The SAS is built to withstand deployment on reefs, but the jolts and rough behavior experienced when mailing the SAS or even in having it stowed as luggage in a plane's cargo hold can prove too much for some of the components. To prevent SAS breaking in transit, as with other scientific equipment, some precautions are recommended. To prevent over pressurizing the sealed SAS housing and compromising its watertight seals, be sure to ship the SAS without the top/main O-ring. Even with the O-ring removed the acrylic face of the internal armature can still create an airtight seal with the housing endcap, so make sure the locknuts on the face are not tightened down all the way. Putting packing peanuts, styrofoam, or even cloth stuffing in the body of the SAS and then installing the armature on top will both pad the base of the SAS, and also add cushioning for the armature to be installed nearly all the way, and immobilized with the locknuts, but not closed completely, allowing for unpressurized travel.

11. Troubleshooting Sampler:

The motor is running in the wrong direction.

The motor shaft will turn clockwise or counterclockwise according to the order its positive and negative wires are connected to it. If the motor is spinning in the wrong direction switch the order of the wires on the KK connector that connects the motor to the circuit board and that will make it turn in the opposite direction.

The motor isn't running.

The OLED can turn on with only one battery pack attached, but the motors cannot. If a motor is not firing at the time the alarm is set for but the OLED is displaying the "<SAMPLING> Pump..." message, look at the battery packs and make sure they are both fully inserted and clicked into place.

The IR sensor (or remote) isn't working to move through the menu.

The IR remote is powered by a single coin cell battery and requires very little energy. If this is the first time using the remote make sure there is a battery inserted and that there's no plastic covering on the battery preventing it from powering the remote. Look at the wires connecting the IR sensor to the board. Since the connectors on the IR sensor are a simple slide on Dupont connector it's possible they aren't fully pushed on and may be preventing the IR sensor from working. Check both the DuPont connectors and the KK connectors to make sure they're full attached and then try the IR remote again.

What if the Auto Sampler time and date reset every time I remove the batteries?

The real-time clock (RTC) on the Auto Sampler is powered by the coin cell battery on the circuit board when a power source (i.e. battery pack) is not attached. Normally once the time is set the RTC will continue counting down at all times and keep track of the time regardless of having a battery pack attached to the sampler. When the battery packs are plugged into the sampler they must be plugged in one at a time, with the battery pack below the power connection to the circuit board being plugged in first. If the battery packs are plugged back in out of order the momentary lack of grounding will cause the RTC to reset. Try resetting the time and removing and replacing the battery packs in the proper order and see if the time still defaults to an alternate setting. Another possibility is when the voltage of the coin cell battery on the circuit board falls below 3 volts the RTC is no longer powered and the sampler will be unable to retain the set time and date, requiring the time and date to be reset every time the power source is reconnected. Replacing the depleted coin cell with a fresh battery will solve that issue.

The sample bag didn't fill up all the way.

The Tedlar sample bags can hold a 1 liter sample. If the bag is less full than expected, make sure that the Tedlar bag valve is only opened one half turn. Opening the valve too far can allow the water to be pumped out of the valve head and not into the bag once resistance starts to build up.

Also make sure the calibration step was done to adjust for variances in the pumphead pumping rates, if not, complete the pump calibration process and set the new calibration number for the pump. Other reasons that could cause the sample to be incomplete is issues with a clogged pumphead or bad tubing. If the deployment site is particularly silty or the SAS is surrounded by sand the pumpheads can get clogged up and stop working. For this reason it is a good idea to examine the SAS after each deployment to see if the pumpheads need cleaning. Some of the tubing provided with the pumpheads by the manufacturer has been found to be faulty and can sometimes seal or weld shut after sitting in the pumphead for an extended period of time. If the tubing has welded shut it should be replaced with similarly soft silicone peristaltic tubing.

Both pumps are firing at the same time.

If anything is bridging the gap between the power pins for the motor a misfire can occur. To avoid this check and clean off the circuit board with a soft brush or compressed air. This will help remove any hair, dust, or debris that might create a connection to bridge that gap. Also check the MOSFETs on the circuit board and make sure they're not pressed together (See Image 17). If they are, pull them apart slightly to establish a gap and avoid connecting the two pump circuits. Test the sampler using the RUNCAL menu to see if both pumps still activate at the same time.

The reed switch isn't waking up the sampler from sampling mode.

If the sampler pumps are running, the reed switch will not work until the pumps are finished sampling. Otherwise the reed switch works with a redundant signal, meaning you have to activate the switch two times in succession. Make sure you're actively moving the magnet across the face of the reed switch. Try reorienting the magnet. If the "Interrupt" message is displayed followed by "Wait..." it means the redundant signal was not completed, so keep trying. The SAS will go from "Interrupt" to programmable mode with no other messages when done properly. If all of the above is tried and the SAS isn't waking up there might be something wrong with the reed switch connection or the SAS batteries may be dead. Removing and replacing the power source will allow the SAS to turn back on and the wiring for the reed switch can be checked to make sure it's connected as described in the build manual.

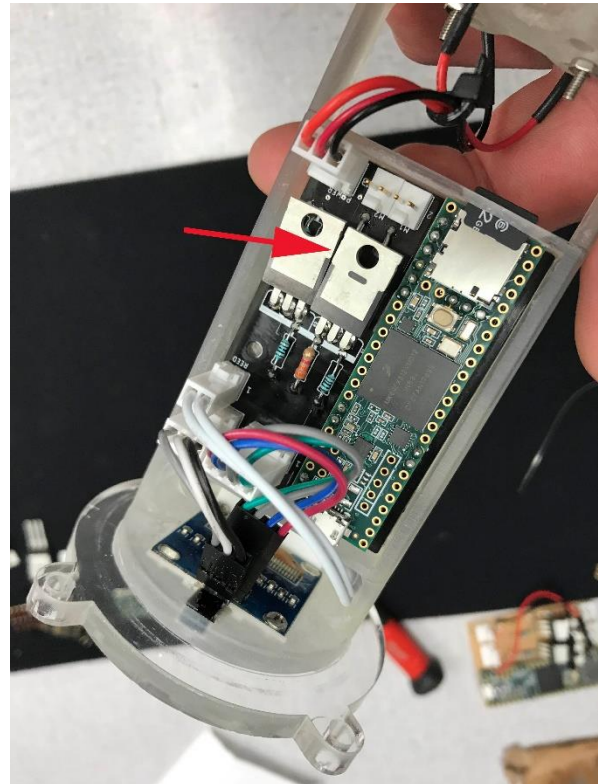


Image 17: The red arrow is pointing at the gap that should be present between the two MOSFETs to prevent both motors from firing simultaneously.

As the SAS continues to be used and further developed please send any feedback or questions about the sampler and its design or function, or the user and build manuals, to Nathan.formel@noaa.gov

References:

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to Best Practices for Ocean CO₂ Measurements. PICES Special Publication 3, 191 pp.

