

SIPs assembly and usage manual

Written by Gitai Yahel and Mariken van Grup

University of Victoria, July 2005

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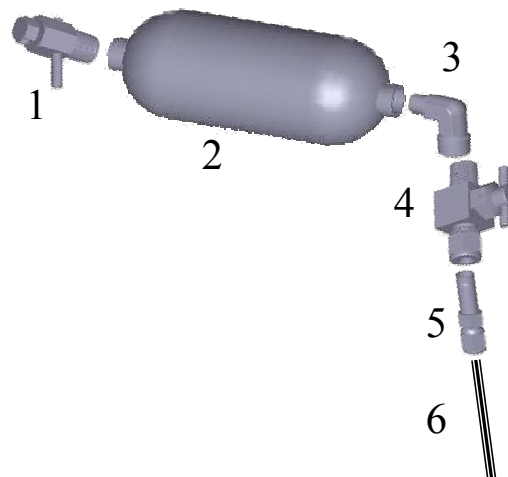
Content:

SIP sampler overview, design and operation	1
Cleaning and Recharging SIPs	3
SIP Assembly	5

SIP sampler overview, design and operation

The SIP is an ROV compatible, simple and inexpensive point sampler (Yahel et al. in press) built from off-the-shelf parts. SIP pairs comprised two pressure sample cylinders (internal volume of 150 mL each) that can be attached to a common handle. Each of the cylinders is equipped with a quarter turn valve and the two valves are joined by a mutual trigger that can be kept normally closed by an elastic cord. An additional bleed valve is installed at the rear end of each sample cylinder to facilitate sample drainage. Pending of the nature of the water analysis required, cylinders can be either vacuum, charged with inert gas at atmospheric pressure, or used as is. The Sampler is positioned in place with one of the ROV manipulators. Sampling is initiated by triggering one of the quarter turn valves valve (or both valves simultaneously) using the second ROV manipulator. Opening the valves allowed the external pressure difference to force the sampled water into the cylinder.

Figure 1. The basic components of the SIP water sampler: (1) Bleed Valve (Swagelok PN SS-BVM4, Stainless steel, 1/4" male NPT thread); (2) Double-ended Sample Cylinder (Swagelok PN 316L-HDF4-150), 316 Stainless steel with internal and external Silcosteel®-CR Surface Treatment coating¹ and internal pressure rating of 125 bars); (3) Street Elbow (optional, Swagelok PN SS-4-SE, 316 Stainless steel, 1/4" mail NPT to 1/4" mail NPT threads); (4) Quarter-turn Plug valve (Swagelok PN SS-4P4T1, 1/4" male NPT thread - 1/4" Tube Fitting); (5) Reducer (Swagelok PN SS-100-R-4, 316 Stainless steel, 1/16" OD - 1/4" OD); (6) Intake PEEK tube. These tubes are available in a variety of internal diameters that allow a simple control of the suction rate.



¹ see appendix 1. Silcosteel®-CR Surface Treatment

To eliminate to the risk of internal pressure buildup when working at greater depth (>750 m), a pressure relief valve can be installed along with the bleed valve at the rear end of the SIP.

The suction rate can be controlled by adjusting the length and internal diameter of the intake tubing according to the equation $F = \frac{\Delta P \cdot \pi \cdot r^4}{8 \cdot K \cdot L \cdot V}$ where F = flow rate ($\text{cm}^3 \text{min}^{-1}$), ΔP = Differential pressure (bar), r =inlet tubing internal radius (cm), $K= 2.417 \cdot 10^{-9} \text{ (s}^{-2}\text{)}$, L = tube length (cm), V = water viscosity ($\text{g cm}^{-1} \text{s}^{-1}$). The orange 10 cm inlet tubing (508 μm internal diameter) deliver an initial suction rate of $\sim 1 \text{ mL s}^{-1}$ at 150 m depth (8°C, 33‰, see Figure 2). Since ΔP and sampling rate decreased exponentially with sampling time, a minimal sampling time should be calculated according to the tube used (5 min at 150 m depth).

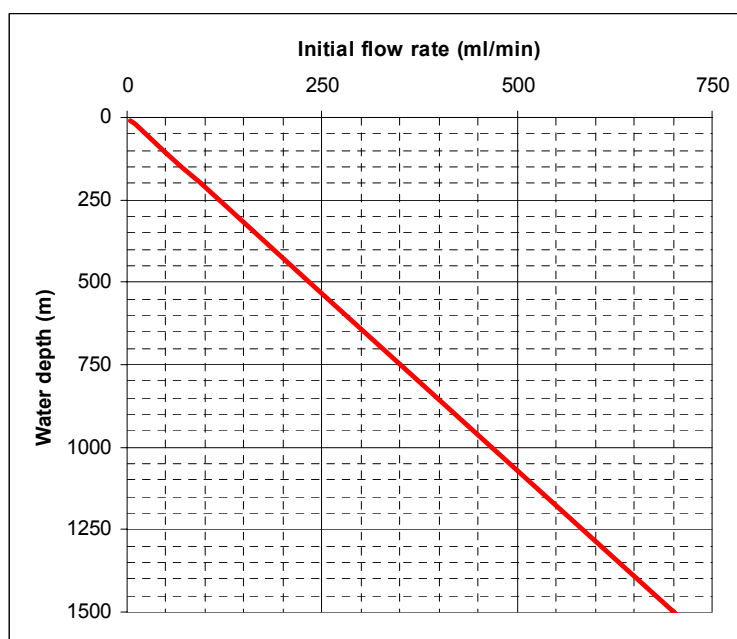


Figure 2. Predicted initial flow rates for the orange intake tube [Upchurch Scientific 1532, external diameter of $\sim 1.59 \text{ mm}$ ($1/16''$) and internal diameter of $\sim 510 \mu\text{m}$ ($0.02''$)] at various water depths.

In addition SIPs can be equipped with an inline pre-filtration set (e.g., Upchurch A-330, high-pressure Semi-Prep inline filters with a A-332X Stainless steel Frit and a PEEK Ring, $0.5\text{-}5 \mu\text{m}$). The inline $0.25 \mu\text{m}$ stainless steel frit filter provided by Swagelok (SS-400-6-1LV-S8) is not reliable and not recommended at this stage. To test the pre-filters used with every second SIP sampler, both filtered and unfiltered samples were analyzed by microscopy and flow cytometry (see below).

Our experience with the SIP samplers suggests that evacuating all gases from the sample cylinders by means of an ultra vacuum pump allows the entire cylinder volume to be used and circumvents the need to handle gas pressurized containers onboard. Pre vacuumed SIPs are also less prone to contamination with ambient water in cases where a valve is accidentally open after sampling (in fact, there is no real need to close vacuumed SIPs after sampling).

Cleaning and Recharging SIPs

Overview:

This procedure is to be used for quick rotation of the SIPs between dives.

Each SIP is initially rinsed with DDW, than briefly by diluted HCl, and three additional rinses with DDW. The DDW are flushed and replaced by high purity helium (or any other inert gas). Finally the gas is evacuated by an ultra high vacuum, the nozzles are installed and the valves are secured (remember to nick the tie-wraps!).

① Wear gloves for the whole procedure, and for the acid wash wear a lab coat and safety glasses.

1.1. Cleaning the nozzles.

- Put disassembled nozzles directly into a DDW bath, allow soaking.
- Use a syringe to backwash nozzles with 1% HCl solution and then 2x with DDW
- Leave cleaned nozzles in DDW until recharging.

1.2. Cleaning the SIPs

1.2.1. Initial water rinse

- Attach DDW hosing to the bleed valves intake of both SIPs.
- Open the bleed valve and the DDW water tank valve.
- Position the SIP with the bleed valves pointing down.
- Pull the handle to open the intake valves.
- Fill the SIPs full and close intake valves.
- Close the DDW water tank valve.
- Disconnect the hosing from the bleed valve of the SIPs.
- Shake well. Discharge the water by turning the SIP upside down (so bleed valves are pointing up) and opening the intake.

1.2.2. Acid wash

- Fill a 60mL syringe with dilute acid (1% HCl)
- Attach the syringe to one of the SIPs.
- While holding intake valve open inject 30ml into the SIP
- Close the bleed valve
- Repeat the steps above for the second SIP
- Shake well and discharge the water (as above).

1.2.3. Final water rinsing

Repeat step 1.2.1 above 3x but DO NOT discharge water after third rinse. SIP must be full to recharge with helium.

1.3. Flushing the water and charging the SIPs with helium

- Connect the helium ‘Y’ hosing to the bleed valve intakes of the SIP sampler.
- Open the helium tank valve and then the bleed valve. Position SIP so that the bleed valves are pointing upwards.
- Open intake valves. As the helium fills the sampler, water is forced out.
- Once SIP sampler is full with helium, no more water exits out of the nozzles, let the helium run through the SIPs for 30 more seconds.
- Close intake valves, close bleed valves. Close helium tank valve and detach hosing from bleed valve intakes.
- Wrench tighten the bleed valves and tape them shut to prevent loosening.

1.4. Vacuuming SIPs

- Inspect the trap to insure it is not full with water.
- Start the vacuum pump and attach the 1/4 turn valve to the vacuum inlet hose.
- Open the valve, vacuum for >3min, close the valve and move to the next SIP

1.5. Nozzles assembly

- Flush each nozzle with helium.
- Mount on the 1/4 turn valve, hand tight fully
- Wrench tight 1/4 turn to the alignment mark.

1.6. Securing the valves

- Make sure the bleed valves are wrench tight and taped three times with electrical tape
- Secure the intake valves with small tie-wraps
REMEMBER TO NICK THE TIE WRAPS!

👁 Before and after the cruise the samplers need to be disassembled, sock in 2% Contrad 70 (Decon laboratories cat. 1002), and rinsed thoroughly with water, 1% HCl and water.

SIP Assembly

(by Mariken van Grup)



Things you need for 1 SIP pair.

Note: You need two lengths of $\frac{1}{16}$ " stainless steel cable, each 54cm long and 6 clamps. The large bolts must be square neck bolts (with a square shape under the head) and are 4" long. The narrow threaded rod is thick and 5" long. You'll also need some Swagelok PTFE tape.

Step 1: Assemble cylinders

Once the Swagelok valves and cylinders are clean, wrap the male end of all the valves with the $\frac{1}{4}$ " PTFE tape. Wrap in the direction of the thread, being careful not to have any tape extend beyond the first thread, so that it would be inside the cylinder once the valve is attached (and potentially come off into the sample). Wrap until the treads are covered by 2-3 layers of tape. Be sure the ends are well secured so they don't enter the cylinder either.

Attach a bleed valve to one end of the cylinder and a quarter turn valve to the other. Tighten as much as you can by hand and then used the vice and a wrench to tighten the valve a further quarter turn. **DO NOT** place the cylinder in the vice as this may crush it partially and make it less resistant to external pressure; instead, secure one valve in the vice and use the wrench to tighten the other valve.



Coat half the cylinders with red duct tape and the other half with green.

Step 2: Assemble and put cylinders in bracket



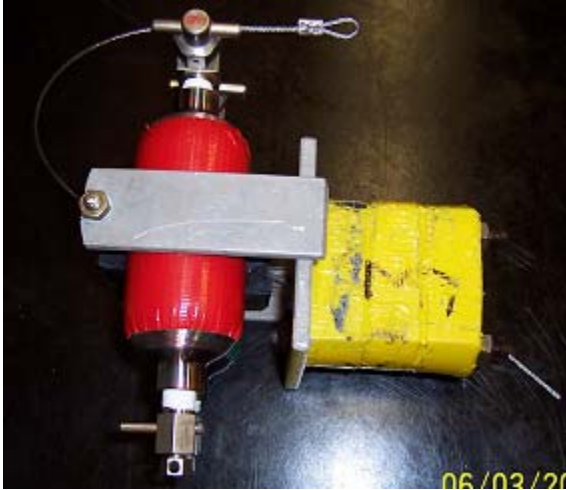
Use the square neck bolts, washers and nuts to attach the hockey-puck handle to the metal bracket as shown. If the bracket is missing the yellow tape along its grooved edge, replace this.

Place assembled cylinders in the bracket with plastic dividers between the two cylinders. Make sure the ends of the nozzles of the quarter turn valves are lined up and that the cylinders are oriented so that the quarter turn valve handle faces horizontally when open and vertically when closed. If you were to hold the bracket by the handle with the bleed valves facing you, the quarter

turn valve handles should be on the right of each cylinder and the red cylinder should be on the right of the bracket. Secure the cylinders in place with the 1/4" threaded rod and nuts, which should fit in the groove of the plastic dividers.



Step 3: Attaching the cable



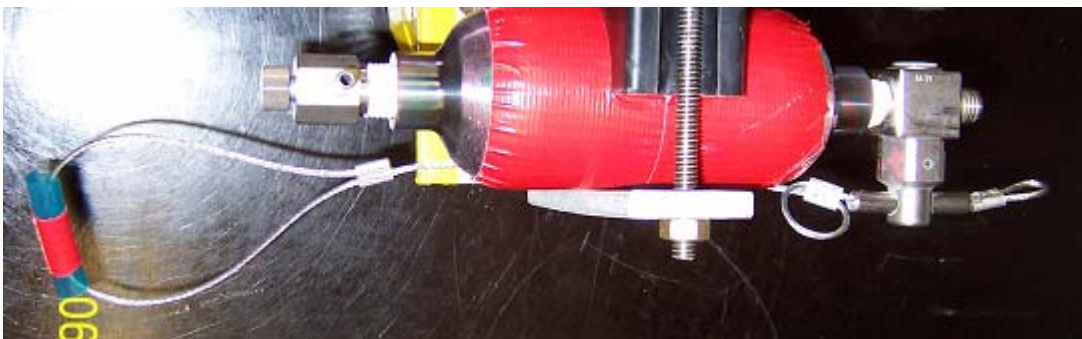
Take one length of the $\frac{1}{16}$ " steel cable (54cm) and use a clamp to make a small loop at one end. Secure the clamp using the vice. Then, thread the cable through the handle of one quarter turn valve as shown (left).

Next, make another loop in the cable on the other side of the quarter turn valve handle (below).



Thread the cable through one of the holes in the bracket, alongside the cylinder. At this point, if you pull back on the cable, the valve should open and if you pull up on your second loop, the valve should close.

Place one of the bits of plastic tubing with colour coded-duct tape over the cable and make one last loop as shown. Place the bracket so that it passes over the edge of the yellow tape as the valve is fully opened. This is so that we can make sure that ROPOS was able to fully open the valve when it pulls on the loop.



Do the same for the second cylinder.

Step 4: Finishing

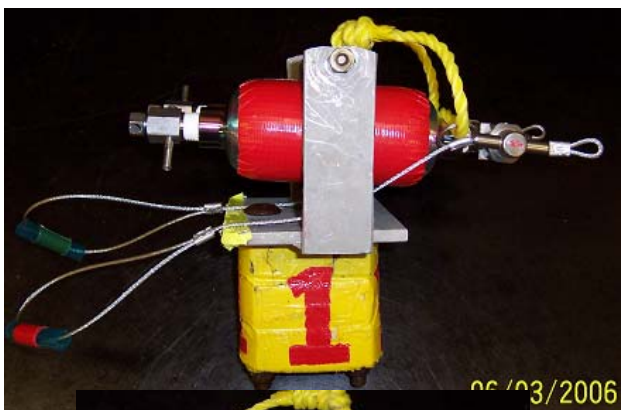
Tie a loop of floating yellow rope through the second loops of both cylinders as shown, using a double fishermen's knot. Tape or burn the ends of the rope.



Label the handle with the SIP pair number, using duct tape or Sharpie (other permanent markers that aren't Sharpie tend to rub off very quickly).

Before using the SIP's each time, secure the quarter turn valves closed by

attaching a tie wrap (zap strap) through the first loop and one of the holes in the bracket of each cylinder. BE SURE to make small nicks in the tie wrap (3-4 in each) with scissors or wire cutters: otherwise, ROPOS may not be able to open the valve, or it may break the cable before it breaks the tie wrap.



Once SIPs are cleaned, purged and vacuumed, it is also a good idea to cover the bleed valve with electrical tape so that no crud gets into the bleed valve opening. (It's also a good way to keep track of which SIP's have been cleaned.)



Reference

Yahel G, Whitney F, Reiswig HM, Eerkes-Medrano DI, Leys SP (in press) In situ feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. *Limnology and Oceanography*