VacuSIP usage and makeup

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The "VacuSIP" is a simplification of the SIP system developed by Yahel et al. (2007), and it is considerably cheaper and adapted for SCUBA base work. The system was originally designed after Wright and Stephens (1978) and Riisgård (1991) for direct *in situ* sampling of the rates and efficiencies by which suspension feeders remove (or discharge) substances from (to) the water they filter. Nevertheless, VacuSIPs can be used wherever a controlled and clean, point-source water sample is required. A simple valve operation or needle piercing by a SCUBA diver allows the external water pressure to force the sampled water into an evacuated sample container. The small ID of the tubing (usually 20-50 µm PEEK) insures controlled suction rate during the sampling (see below) and a negligible dead volume.

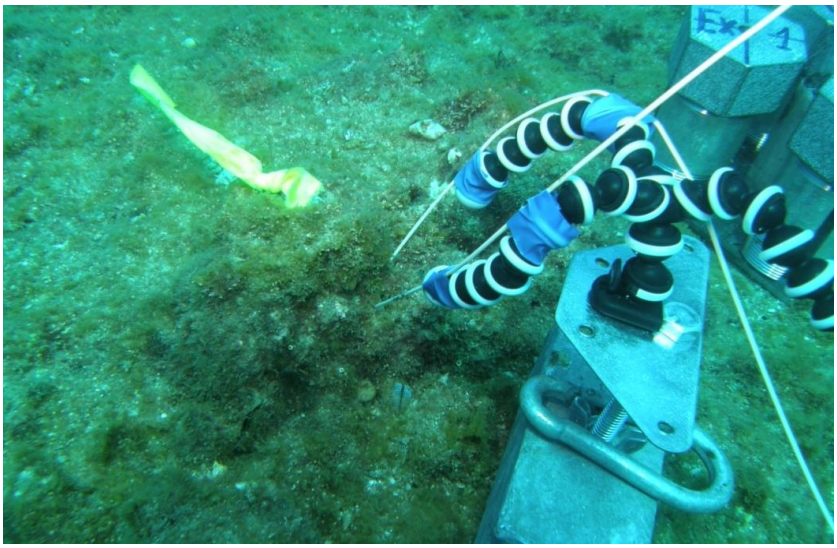

Fig.1. A pair of VacuSIPs is used for simultaneous sampling of the water inhaled and exhaled by a solitary ascidian (*Micrcosmus exasperatus*). East Mediterranean, 10 m depth.

For *in situ* sampling of suspension feeders metabolism, the pumping activity of each specimen sampled is first visualized by releasing fluorescein dyed seawater next to the inhalant orifice(s) and then observing it flow from the excurrent (e.g. Fig. 2B in Yahel et al. 2007); then the water inhaled and exhaled by the studied specimen (incurrent and excurrent) are sampled simultaneously using a pair of VacuSIP water samplers (for makeup, see below). The difference in concentrations of a substance (e.g., bacteria) between a pair of samples provides a measure of the retention (or production) of the substance by the animal (Yahel et al. 2005). Collection of the water inhaled and exhaled by pumping suspension feeders with exhalant orifice as small as 2 mm is made with a PEEK tubing (UpChurche Scientific) with OD of 1.6 mm (1/16") placed inside the excurrent orifice (avoiding contact or disturbance of the

sampled organism), for SCUBA depths we use tubes with internal diameters of 25 µm (UpChurche Scientific, cat no. 1567) or 50 µm (UpChurche Scientific, cat no. 1570). At the distal (sample container) end, the tube is fitted with a Male Luer connector (IDEX Health and Science, P-655 1/4-28) attached to a syringe needle. For small volume samples (3-20 mL) we use commercial Vacutainers (same tubes used for standard blood tests in human, make sure you use Vacutainers with no additives!, e.g., Grenier Bio-One GmBH, 45501, Vacuette, 9 ml Z no additive). For larger water samples we use 100 mL penicillin bottles or EPA vials (e.g., Cole-Parmer, KH-99536-12).

Fig. 2. VacuSIP with male Luer connector intended for large collecting containers such as penicillin or EPA bottles.

Fig 3. The entire setup with 100 mL penicillin bottles in a costume made carrier. The Peek tubing is attached to a weighted Joby Gorillapod GP1(http://joby.com/gorillapod/original) that allow excellent manoeuvrability and exact positioning of the tube inlet

As discussed by Yahel et al. 2005, to insure clean collection of exhaled water and avoid accidental suction of ambient water, the suction rate should be considerably slower than the excurrent rate. 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 *the F= flow rate* ($cm^3\ min^{-1}$), ΔP= Differential pressure (bar), *r*=inlet tubing internal radius (cm), *K*= $2.417^{\bullet}10^{-9}$ (s^{-2}), *L*= tube length (cm), *V*= water viscosity (g $cm^{-1}\ s^{-1}$). However, the above formula should be considered as a first order approximation since ΔP and sampling rate decreased with sampling time. Using evacuated containers, sometimes with unknown vacuum poses additional complications. Therefore, field test is highly recommended. At 10 m depth and ~22°C seawater (40 PSU), a 50 cm inlet tubing with an internal diameter of 25 µm delivers an average suction rate of ~26 µL s^{-1} (1.56 mL min^{-1}). If required, VacuSIPs can be equipped with an inline pre-filtration set.

Our experience suggests that evacuating all gases from the sample container by means of an ultra-vacuum pump allows the entire volume to be used and minimize the risk of plugs popping upon descent.

Parts list:

1. Fittings:
   IDEX Health and Science:
   - P-200X Flangeless Ferrule 1/16in Blue/pk, WO# 0563239
   - P205X Male Nut1/16in Green/10pk, WO#0558631
   - P-658 1/4-28 Female to Female Luer, Qty 6

   OR

   - P-655 1/4-28 Female - Male Luer / 10pk
2. Peek Tubing:
   UpChurch Scientific

Please note that we and colleagues (R. Coma and M. Ribes) had recently problems with the Peek tubing sizes and some of the supplied tubing was clearly below the stated ID. Coma and Ribes are now using much larger inner diameter tubes (180-250 µm) for suction rates of ~ 1mL min^{-1} at 5-6 m depth using vaccumed 40 mL EPA vials. We found that the 63 µm ID works well for us using Vacutainers at 10-12 m depth (19°C, 60 cm tube length) with initial flow rates of ~0.8 mL per min. It is thus recommended to verify the cat number at your local supplier and physically test the tubing underwater at the designiated depth.

   - 1559 1/16" x 25 µm (5ft)
   - 1560 1/16" x 63 µm (5ft)

3. Vacutainers:
   Grenier Bio-One GmBH
   - 45501, Vacuette, 9 ml Z no additive, 16 x 50 Pcs
   - 367290 - BD Vacutainer® multiple sample luer adapter
4. Gorillapod (original, (http://joby.com/gorillapod/original)

References:

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Wright, S. H., and G. C. Stephens. 1978. Removal of amino acid during a single passage of water across the gill of marine mussels. Journal of Experimental Zoology **205:** 337-352.

Yahel, G., D. Marie, and A. Genin. 2005. InEx - a direct in situ method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnol. Oceanogr. Meth. **3:** 46-58.

Yahel, G., F. Whitney, H. M. Reiswig, D. I. Eerkes-Medrano, and S. P. Leys. 2007. In situ feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. Limnology and Oceanography **52:** 428-440.