

The autonomous clean environmental (ACE) sampler: A trace-metal clean seawater sampler suitable for open-ocean time-series applications

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Abstract

The fundamental role of the micronutrient Fe in controlling phytoplankton growth in large parts of Earth's oceans and a lack of information on seasonal transitions in remote regions motivated us to create an autonomous water sampler capable of collecting uncontaminated open-ocean seawater samples with monthly resolution over a full annual cycle. Phytoplankton are at the base of the food chain, which take up carbon dioxide through photosynthesis. Assessing Fe availability is essential to understanding both ocean productivity and our climate. This need is particularly important in the Southern Ocean, where Fe limitation is wide spread and access is difficult especially in winter. To address this need, we have developed an autonomous system capable of observing iron concentrations over a full seasonal cycle, at the subnanomolar concentrations that are important in the open ocean. The automated clean environmental sampler has been developed initially for 1-yr deployments on oceanographic moorings. Twelve samples per unit can be programmed to collect 65 mL of seawater through a noncontaminating, primarily Teflon sample path. The system is vanned to maintain its intake tubes in upstream water and has been tested to 100 m depth. The system was deployed during a GEOTRACES section (SR3 line, GS01) in 2018 alongside an industry standard, trace metal clean rosette, and no significant difference was found in Fe concentrations in the 50 pmol L⁻¹ range, proving its capability at collecting uncontaminated seawater samples in the open ocean at temperatures above 0°C.

Despite a wealth of macronutrients, phytoplankton growth is limited by the availability of the important micronutrient iron (Fe) in the Southern Ocean and the north and equatorial Pacific Oceans. This has major implications for the carbon cycle and the associated ecology of these regions. This lack of Fe is not ubiquitous, either in time or space (Tagliabue et al. 2014). Theoretically, we expect Fe concentrations in shallow sunlit zones to follow a specific seasonal cycle, but detailed observations of the full seasonal cycle in the high nutrient, low chlorophyll (HNLC) Southern Ocean have never been made due to the technical, logistical, and financial challenges of maintaining a year-round presence in remote regions such as the Southern Ocean. We expect that deep winter mixing drives entrainment of higher concentrations of trace metals from depth into surface waters (Cloete et al. 2019). Spring sees a rapid decrease in surface dFe concentration as the mixed

layer shoals and both biological uptake and abiotic particle scavenging accelerates (Mtshali et al. 2019). We expect that Fe concentrations should remain low through summer and drive species succession from microphytoplankton with rapid growth rates and high Fe demand in spring toward nanophytoplankton and picophytoplankton with lower Fe demand later in the season. When deep winter mixing and entrainment of higher Fe waters from depth begins again in autumn, the cycle is expected to be complete (e.g., Tagliabue et al. 2014; Fig. 1). However, an understanding of this complex sequence is based on scarce measurements, often from different cruises and various times of the year with limited temporal resolution, and in addition, portions of the Southern Ocean receive sporadic aerosol inputs that can decouple iron availability from seasonal ocean stratification (Bowie et al. 2009). Due to the critical role of Fe in Southern Ocean carbon cycling and ecology, we need to better constrain the seasonal cycle of Fe supply. The lack of seasonal observations led Grand et al. (2019) to specifically call for the design and implementation of in situ sensors and autonomous samplers for bioactive trace metals within the next decade. We propose that a moored water sampler which can collect a time series of

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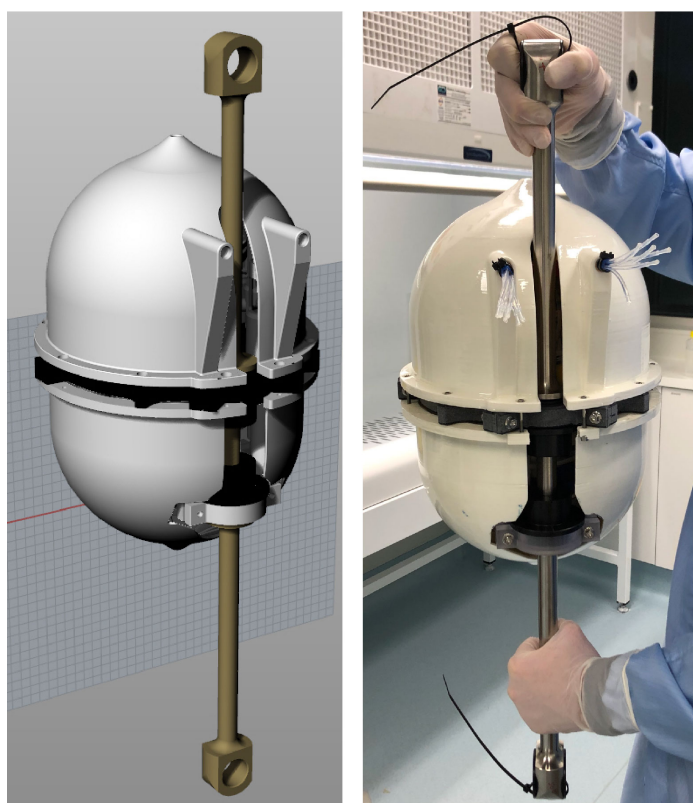


Fig. 1. The third revision of the ACE sampler as deployed in March 2019 at the SOFS site. The 12 Teflon intake tubes can be seen protruding from the upper domed shroud, with their position being forward of the remainder of the ACE sampler. The domed shrouds are 3D printed in acrylonitrile butadiene styrene (ABS; five perimeter walls, 80% infill, white TitanX by FormFutura) and then coated in clear epoxy (Smooth-On, XTC-3D®) to achieve a smooth glossy finish to resist biofouling and strengthen the structures. The tension rod meets the SOFS mooring requirement for up to 2 tonne loading for more than 8 million wave cycles over an annual deployment. The tension rod and all external fasteners are made of titanium.

uncontaminated water samples over a yearly seasonal cycle will provide unprecedented quantification of this fundamental process.

Groups have produced moored trace metal samplers in the past, with the most notable example being the MITESS (Bell et al. 2002). Unfortunately, the system is highly complicated to reproduce and so large to be cumbersome for many applications. The system works by automatically opening a sample bottle lid and then closing it again while submerged and fixed to a mooring, which inherently requires the entire sampler body itself to have the same extremely high requirements for trace metal cleanliness as the inside of the sample containers. Biofouling of the mooring would therefore impact the samples, as the intake is not physically removed from the sampler body. This problem is overcome, as in the current design, if the sample is pumped into a sample container, allowing the Teflon (small surface area and low biofouling potential) intake tubes to be physically removed from the main body of the

sampler. Another available option is retrofitting and modifying a Remote Access Samplers (RAS) made by McLane Research Laboratories. However, after months of cleaning and modifications of the system, including changing the sampling containers and various components, we could not achieve low trace metal blanks as it was not designed from the ground-up to be suitable for this application. Japanese colleagues produced the ANEMONE sampler (Okamura et al. 2014), and we attempted to adapt it for trace metal clean sampling, and although the system is elegant in its design and successful at sampling concentrations characteristic of hydrothermal vents, Fe blanks were too high for open-ocean sampling even after careful cleaning and reversing the flow path to place the pump downstream of the sample containers.

Recently, a system named PRISM has been described by Mueller et al. (2018) that has blank levels “typically less than 0.1 nmol kg^{-1} Fe, with sporadic exceptions as high as 2 nmol kg^{-1} .” This is clearly a system capable of short-term coastal sampling, where concentrations are relatively high, and the ability to preserve samples with acidification is advantageous, but “sporadic contamination,” being the hallmark of routine contamination of trace metal sampling, is problematic for open-ocean sampling. PRISM also uses a single filter for all filtration, which after extended deployments at sea, would no longer be suitable for filtration of the remaining samples due to active particle leaching and bacterial remineralization within the filter itself. This remineralized dissolved Fe would then be supplied to the subsequent samples. Multiple one-way valves in the upstream flow path of PRISM also represent multiple dead space locations for trapped contaminants, making reliable cleaning difficult. The exterior of PRISM is not constructed of entirely low trace metal components (notably the burnable lock wires), making handling within a clean room a contamination risk.

The Autonomous Clean Environmental (ACE) sampler presented here overcomes these challenges by utilizing individual $0.2 \mu\text{m}$ filters for each sample that removes bacteria and phytoplankton and isolate this from the sample with a 40 cm buffer of Teflon intake tube. By excluding all bacteria and phytoplankton from the sample, no further preservation is required until acidification in the laboratory after recovery to mobilize any components adhered to vial walls. The exterior of the ACE sampler is made exclusively of low-contamination risk materials, namely plastic or titanium, with a rubber Sub-Conn communication port, making handling within a clean room for preparation and recovery much less likely to lead to contamination of critical samples via cross contamination. The whole flow path is simplified with no dead space other than the filter which is individually acid washed. The simplification of the flow path also reduces components required, keeping costs as low as possible.

After considerable effort to make existing systems fit for purpose, the decision was made to design and fabricate a water sampler specifically suited to trace metal clean time-series

sampling in the Southern Ocean. The design brief for the ACE sampler was as follows: (i) the system must collect contamination-free filtered seawater samples, suitable for quantification of dissolved trace metals in the HNLC open Southern Ocean where concentrations can be persistently in the subnanomolar range. (ii) The system must be light weight (~ 10 kg) and compact to minimize stress on the mooring (and thus its likelihood of failure!). (iii) It must be self-powered for 1-yr deployments. (iv) It must be pressure tolerant to approximately 100 m to sample within the euphotic surface ocean, where Fe limitation of phytoplankton productivity is important and may respond to Fe inputs across the base of the surface mixed layer. (v) It must collect as many samples as possible within the size and weight restrictions. (vi) Sample size must be no less than 60 mL to allow a 66.7 times preconcentration factor using a seaFAST preconcentration system and inductively coupled plasma, mass spectrometer (ICP-MS) detection to give the necessary precision required for the HNLC Southern Ocean (Wuttig et al. 2019). (vii) All surfaces must resist biofouling by techniques such as utilizing smooth finishes, as using classical biocides which contain copper, zinc, or mercury compounds represent severe contamination risks. (viii) It must also be relatively low cost (< AUD\$1000 per sample to be in line with the RAS system manufactured by McLane Research). As such, the design represents a significant challenge and the current version of the ACE sampler represents 4 yr of research, design, and prototype testing.

Three major design revisions have been completed on the ACE sampler, with each revision having been deployed for approximately 1 yr within the Sub-Antarctic Zone on the Southern Flux Study mooring at the Southern Ocean Time Series site (SOFS; ~ 142°E, 47°S). SOTS is an Australian Integrating Marine Observing System contribution (<http://imos.org.au/facilities/deepwatermoorings/sots/>) to the global OceanSITES network (<http://www.OceanSITES.org>). The third version of the ACE sampler (Fig. 1), the focus of this publication, was unique in being mounted in-line at 50 m depth as a vaned, freely rotating system mounted on a titanium (Ti) tension rod using Delrin bushes to place its intake tubes perpetually in upstream, uncontaminated water. Accordingly, it is highly compact to minimize mass and drag, which can deleteriously impact mooring fatigue life. Two earlier versions were mounted within cylindrical compression tubes in the SOFS surface float, which determined their cylindrical shape. These versions are also shown here for completeness, because they were used for some of the testing. An additional version has been developed for use on an autonomous underwater vehicle (details of its implementation are beyond the scope of this article).

Materials and procedure

Materials

All acid washing of plastic ware was conducted following the recommendations of the GEOTRACES program (Cutter

et al. 2010) with some custom modifications due to the unique system outlined below. Ultra-high-purity (UHP) water was supplied from a Milli-Q Advantage A10® and Q-Pod Element® (Merck Millipore). Distilled HCl acid was produced on a Savillex DST-1000 sub-boiling still using IQ grade HCl (Seastar Chemicals). All cleaning and handling of the flow path components was performed under an ISO class 5, Smoothflow ducted laminar flow hood (Lab Systems Group) housed within an ISO class 7 clean room at the University of Tasmania's Institute of Marine and Antarctic Science.

Our experience collecting uncontaminated seawater samples from coastal to the HNLC open ocean led us to realize that the only way to make a reliable contamination-free sampling system was to keep the sampling path as simple as possible, both mechanically and in terms of materials used. We specifically avoided the use of valves, as these can be a consistent source of contamination, either directly or via trapping small volumes of liquid within dead space which are difficult to adequately and reliably clean. Therefore, the ACE sampler works by drawing seawater through the individual intake tubes via the individual filters, through the 65 mL sample containers and out to waste, via individual microperistaltic pumps fitted with Pharmed (Cole-Parmer Masterflex) pump tubing (Fig. 2) and, in doing so, displaces the UHP water prime fluid. In this way, the entire flow path, not just the intake side, represents a very low contamination risk. This keeps the intake side of the sampling as simple as possible, with the fewest possible convolutions or dead spaces, which could trap contamination. Peristaltic pumps were chosen due to their proven compatibility with trace metal research, for instance, in flow injection, chemiluminescence detection (FIA-CL) Fe analyzers (Bowie et al. 2002, 1998; van der Merwe et al. 2009). Furthermore, this style of pump also acts as a pinch valve when deactivated, restricting sample exchange along the flow path at all times except during sampling which was required for this simplified valve-free sampling system. The design targeted replacing the need for valves by using relatively long inlet and outlet tubes on the sample containers, through which slow diffusion effectively isolates the sample (see Assessment section below for more detail, including Fick's first law calculations of diffusive transfer). Therefore, to limit migration of contamination along the flow path, 40 cm lengths of 3.18 mm (1/8 inch) diameter tubing were placed between the filter and the sample container and the sample container and the microperistaltic pump. This restricts ingress of contamination from the exhaust port and from dissolved trace metals leached from particles off the filter.

Due to the selection of peristaltic pumps for sampling, the main housing and sampling containers must be pressure compensated so that external ambient pressure is not expressed across the internal flexible pump tubing or along the flow path into the sample containers (Fig. 3). Due to the small size of the printed circuit board (PCB) and batteries, we chose to pressure compensate the entire main housing to keep the system compact and avoid the added complexity of a separate

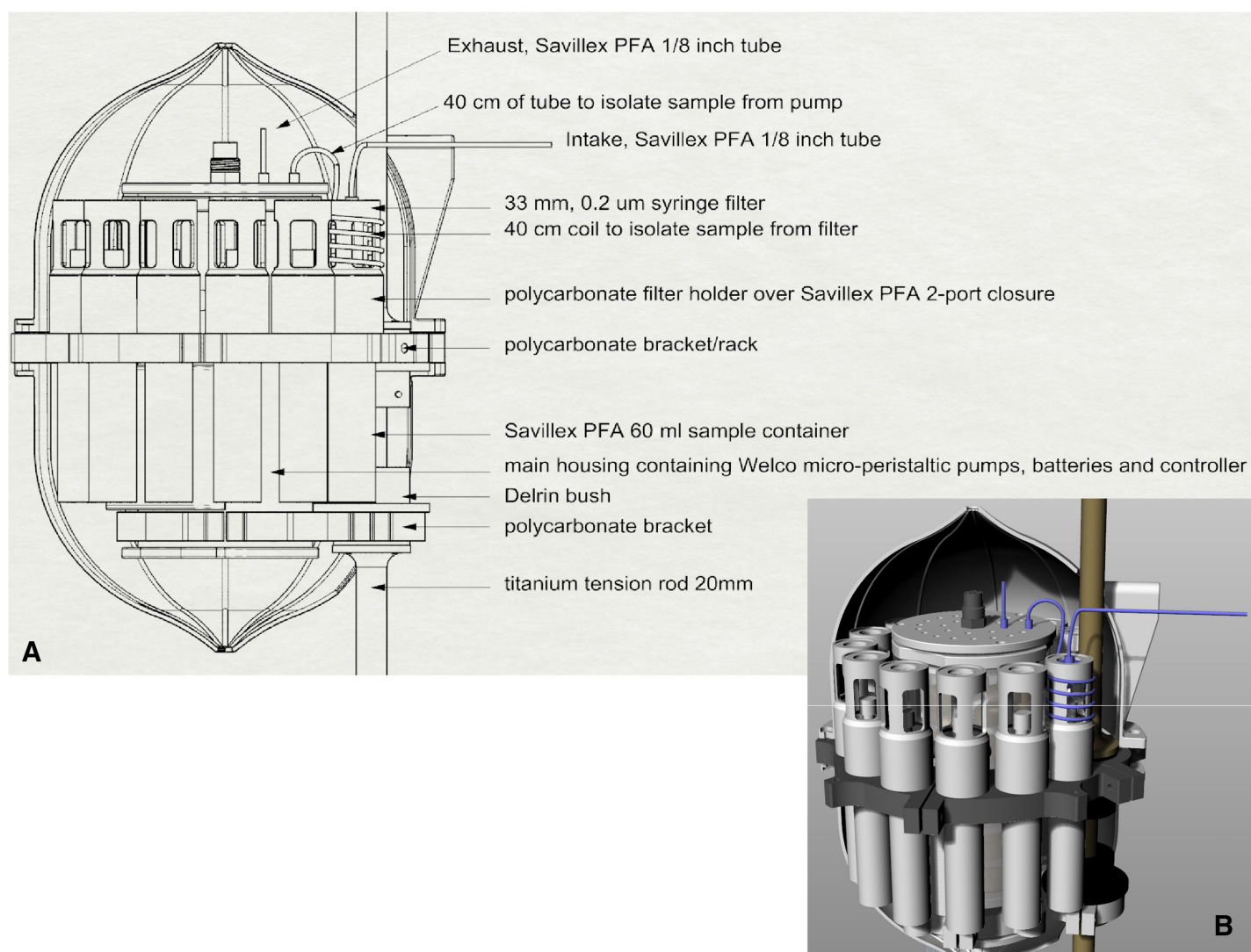


Fig. 2. Main components of the ACE sampler (A), and the general flow path illustrated in blue (B). Water enters via the intake, then filter, isolation coil, sample container, second isolation length, into the main housing and microperistaltic pump and finally leaves via the exhaust tubes displacing the UHP water prime fluid in the process.

pressure housing with associated pressure rated connectors. Likewise, the flow path is completely filled with UHP water prior to deployment to allow pressure compensation of the sample containers. To run the pumps reliably in pressure compensation fluid, a brushless DC motor is required, and the microperistaltic pumps (WPM model, Welco) used here are of high quality, low cost, compact, and light weight and can be ordered with a brushless DC motor and reduction gearbox. The pressure compensation fluid, as used on the MITESS sampler (Bell et al. 2002), is Fluorinert (FC-40 by 3M) as it represents a lower contamination risk than other pressure compensation fluids available, will evaporate completely leaving no residue, is inert, is nonconductive, is thermally and chemically stable, and is also compatible with silicone-based peristaltic pump tubing (which silicone oil is not).

To achieve contamination-free sampling, materials selection was critical. The ACE sampler's outer surfaces are made entirely out of plastic, Ti, or rubber and as such represent a low contamination risk for trace metals (Table 1). This is important for two reasons: first, while handling the sampler within a clean room, it represents a lower contamination risk to the critical samples via cross contamination on surfaces, gloves, etc. Second, when the sampler is deployed, it avoids producing dissolved contamination within the vicinity of the sampler's intakes, which could be swept into the intakes with unfavorable water currents. Furthermore, the intake tubes on this design are physically removed from the body of the sampler and maintain an upstream position at all times relative to it. This is achieved by mounting the whole sampler on Delrin spacers (known as bushes or bushings), which are free to

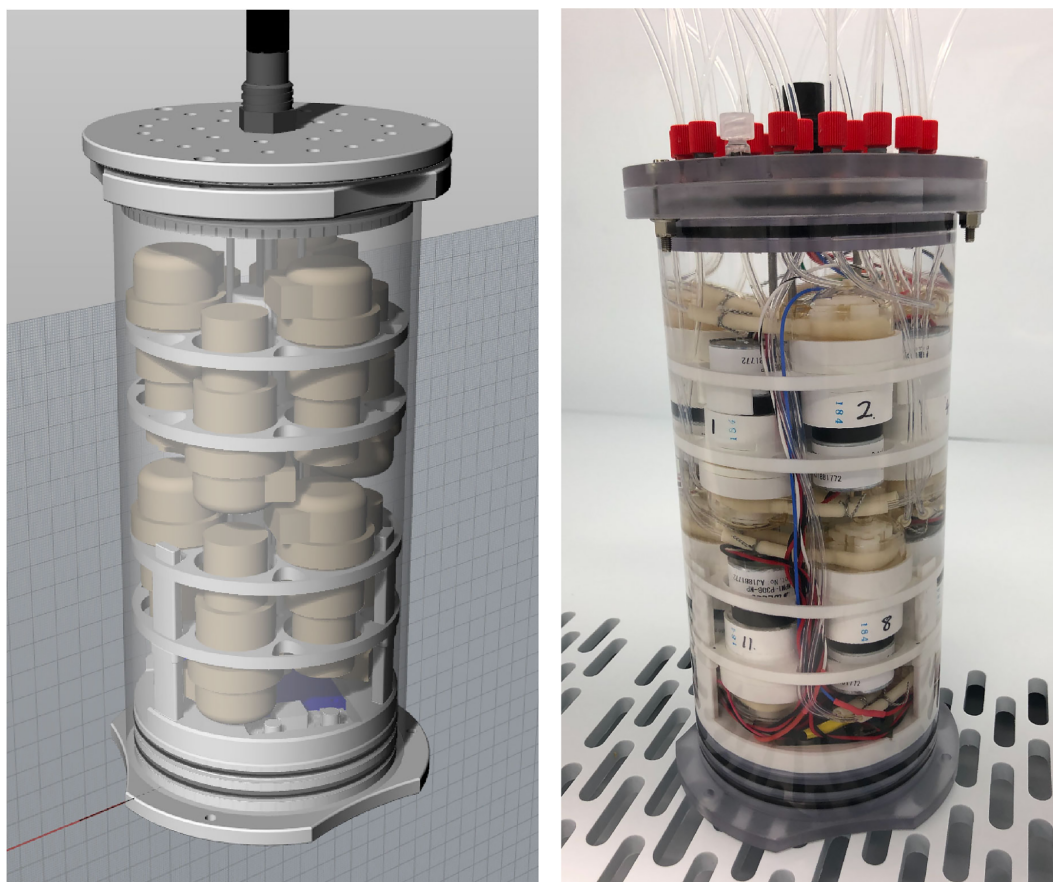


Fig. 3. Main housing of the ACE sampler containing pumps, controller, and battery submerged in Fluorinert (FC-40, 3M) as pressure compensation fluid. The PCB and DC–DC converter are mounted at the base of the main housing on a 3D-printed ABS (100% infill) mount. Likewise, the pumps are mounted on 3D-printed ABS brackets. The full assembly is attached to the top cap, making disassembly a simple case of removing the top cap with the full assembly lifting out of the FC-40 pressure compensation fluid.

rotate around the Ti tension rod. The drag on the bulk of the sampler body keeps the system vaned into the water current and the location of the intake ports and tubes will remain upstream at all times except perhaps during particularly quiescent periods when water currents are not sufficient to overcome the drag on the Delrin bushes. In the Southern Ocean, this would be a very rare occurrence but may be more of a problem in other ocean basins. The upstream intake ports are also made of PFA Teflon, which prior deployments have shown to be resistant to biofouling. This orientation, material selection, and small surface area of the intake ports reduces biofouling build-up in their vicinity, avoiding sampling artifacts and contamination.

The most critical material of course is the material in direct contact with the seawater samples. Here, we use a per-fluoroalkoxy copolymer (PFA) Teflon (Saville) intake path and sample containers (Fig. 4). The only exceptions to the PFA sample path are the 33 mm and 0.22 μm nominal pore diameter syringe filters (Millex by Merck Millipore) that use a modified acrylic housing and polyethersulfone (PES) filter

membrane, and polycarbonate Luer-lock barb fittings (Cole-Parmer) used to connect 2 cm lengths of clear Tygon (Masterflex) flexible tube to the hard 1/8 inch fluorinated ethylene propylene (FEP) tubes to the filters. Tygon tubing has been used routinely in FIA-CL Fe analyzers with no evidence of contamination, evidenced by extremely low manifold blanks in our lab et al around the world (de Jong et al. 1998; Measures et al. 1999). PES filter membranes are used routinely during filtration for open-ocean trace metal research, such as the Pall Acropak (0.8/0.2 μm cartridge type) recommended for GEOTRACES work (Cutter et al. 2010), and we found the Merck Millex PES membranes to be similarly suitable for small volume filtrations.

Electronics

The electronics were designed for ultralow-power standby currents for efficient use of batteries during long-term deployments to keep the overall size and weight at a minimum (Fig. 5). The ACE sampler uses an Arduino Pro Mini (made by Sparkfun, 3.3 V/8 MHz) and a DS3231 real-time clock (RTC;

Table 1. Components exposed to seawater, the material of construction, and the method of manufacture.

Component	Material	Method of manufacture
Main housing tube	Polycarbonate	Off-the-shelf component
Main housing upper cap	Polycarbonate	CNC milled
Main housing lower cap	Polycarbonate	CNC milled
Main housing lock ring	Polycarbonate	CNC milled
Lower bracket	Polycarbonate	CNC milled
Upper bracket/rack	Polycarbonate	CNC milled
Fasteners	Titanium	Off-the-shelf component
Filter holder	Polycarbonate and acrylic	CNC milled and laser cut
Filter housing	Modified acrylic	Off-the-shelf component
Tension rod	Titanium	Milled and lathe
Tension rod bushes	Delrin	Milled and lathe
Sample containers	PFA Teflon	Off-the-shelf component
Tubing	PFA Teflon	Off-the-shelf component
Tube compression fittings	Delrin	Off-the-shelf component
Tube barb fittings	Polycarbonate	Off-the-shelf component
Communication connector	Chloroprene rubber and titanium	Off-the-shelf component
Domed shroud	ABS and epoxy	3D printed and coated in epoxy

Maxim Integrated) to allow programming of specific sampling dates and durations for the 12 pumps. The RTC is powered from a digital line on the Arduino during updates and sampling and runs during standby on its 3 V, 140 mAh lithium manganese dioxide coin cell battery (1632). Interrupts are programmed on the RTC to wake the Arduino out of low-power sleep. The Arduino also records (to electrically erasable programmable read-only memory (EEPROM)) the battery voltage, temperature (from the RTC), pump duration, and a date stamp after each sampling event. Power is supplied to the pumps from two nonrechargeable 7700 mAh, 3.6 V, C size lithium thionyl chloride batteries (Saft LS26500) wired in series, via a high efficiency 12 V step-up DC–DC regulator (Pololu S18V20F12) enabled via a digital line on the Arduino only during sampling. MOSFETs controlled via the Arduino allow high current switching of the pumps. Unregulated

power is supplied to the Arduino (and RTC) via a separate 7700 mAh, 3.6 V, C-size lithium thionyl chloride battery (Saft LS26500). Separating the three C-size batteries this way eliminates the risk of controller malfunction due to voltage drops during high current discharge during sampling. Standby power consumption of the Arduino is 0.1 uA, and the RTC running on battery backup is 3 and 2.7 uA for the DC–DC converter with its 100 k pull-down resistor on the enable line removed (controlled via the Arduino digital lines).

At this standby power consumption, the C-size battery for the controller provides decades of capacity, whereas the coin cell battery for the RTC will last for approximately 5 yr. The capacity is also ample for the 22-min pumping duration of the 12 pumps at 140 mA system current draw, when considering reduced capacity due to low ambient temperatures (~5°C). Factoring in the 85% efficiency of the DC–DC converter, the batteries could run the system with pumps running for approximately 46 h. Each deployment of 22 min per pump is 4.4 h total pumping time, so the system could easily be deployed multiple times on a single-battery set.

To avoid unnecessarily drawing upon the batteries during cleaning and priming steps performed in the laboratory prior to deployment and to enable programming once the controller and batteries are sealed within the pressure compensated housing, the 3.6 and 7.2 V lines, together with the Arduino serial communication, are brought up to the SubConn Micro Circular (MCBH8F) bulkhead connector on the top cap of the main housing. By using a modified connector that supplies 3 V (via USB) and 3–18 V via an external battery, the system can be run, tested, and modified without using the internal batteries. During deployment, the system is enabled by connecting a SubConn cable that has several conductors shorted that acts as an on-switch when mated.

Code

The controller on the ACE sampler runs C++-based Arduino code. It comprises eight case structures that allow the event times to be programmed in a [yyyy-mm-dd HH:MM:SS] format that are then saved to EEPROM (Table 2). These event times can be called back by entering “e” in the serial interface. Likewise, the next chronological sampling event can be called by typing “a” in the serial interface. The RTC stores the next chronological sampling event and then the system is put to low-power sleep, and the voltage common collector (VCC) (3.6 V) is cut to the RTC to enforce running on battery backup. If this step is not performed and the RTC remains powered with VCC (3.6 V) from the Arduino, the standby power consumption would be significantly higher. When the programmed sampling event occurs, the RTC sends an interrupt to the Arduino which then wakes from low-power sleep, runs the pump for the duration specified, records system information to the EEPROM, and finally sets the next sampling event from the Arduino EEPROM to the RTC before finally setting the system to low-power sleep again.

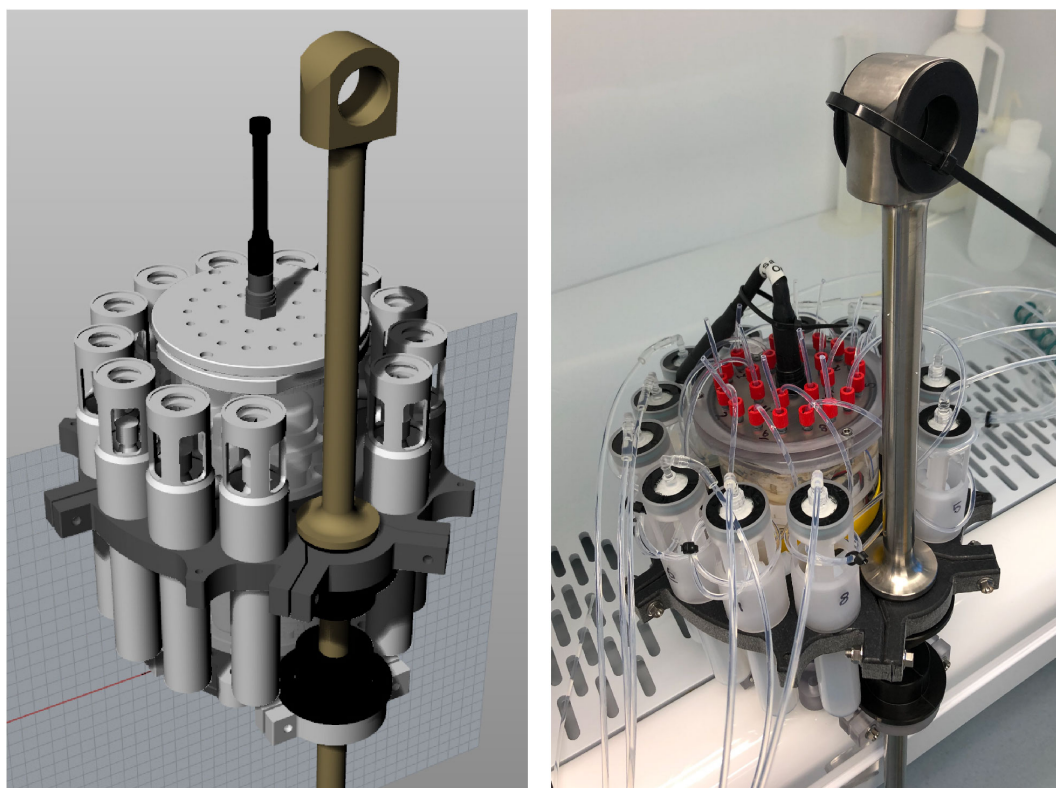


Fig. 4. The ACE sampler with outer shroud removed to reveal the sample containers, filter holders, intake tubes, communication port, and main housing. Note the bushes (a.k.a. bushings) shown in the photo at right that electrically isolate the Ti tension rod from the plastic-jacketed steel mooring line.

Via the SubConn bulkhead connector on the top cap of the ACE sampler and the serial interface, the pump duration can be set, critical system information such as battery voltage and

temperature can be displayed, and the internal RTC time can also be set. A cyclic redundancy check (CRC) is built in to check for correct writing of data to the EEPROM. After

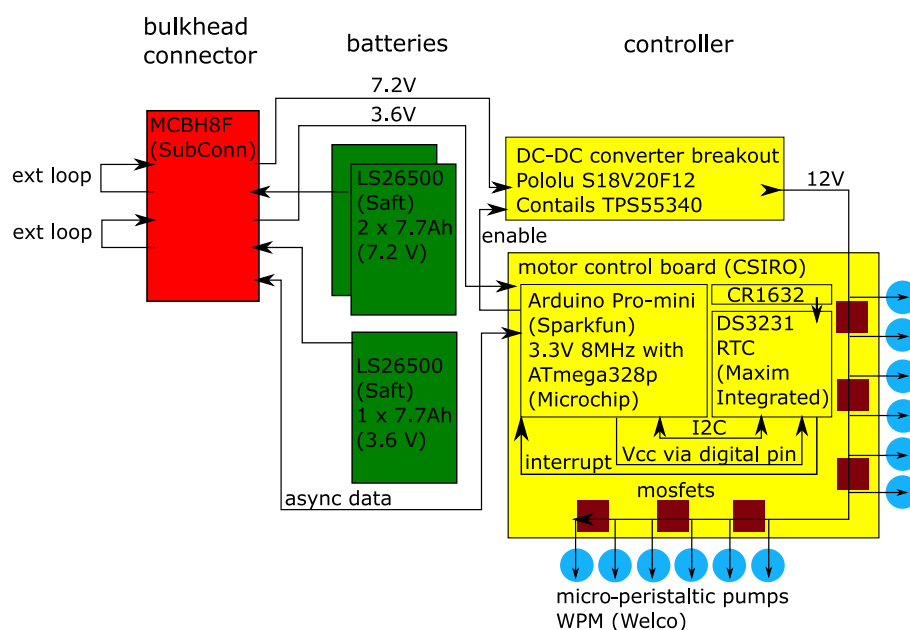


Fig. 5. Flow diagram of the electronics used on the ACE sampler.

Table 2. The ACE sampler serial interface allows the following commands to be entered to perform various programming functions and to allow manual operation of the pumps during predeployment cleaning and priming.

Serial commands	Output
a	Display current RTC alarm
e [pin] [yyyy-mm-dd HH:MM:SS]	Print or set event time
l	Print logged events
m [pin] [0 1]	Turn pump on or off
p [seconds]	Set or display pump time
t [yyyy-mm-dd HH:MM:SS]	Display or set time
crc [write]	Display/set EEPROM CRC
i	Display info, battery, temperature

sampling, a log can be displayed indicating for each sampling event a time stamp, the pumping duration, which pump fired, battery voltage, and ambient temperature of the RTC, which should be equilibrated with the external ambient temperature via the pressure compensation fluid.

Procedures

Before deployment, the ACE sampler is always bled of all air pockets to enable pressure compensation. The main housing is filled with Fluorinert (FC-40, 3M), and the flow path including the sample containers is filled with UHP water (prime fluid). When a pump runs, seawater displaces the UHP prime fluid via internal density stratification that limits mixing (see Complete sample exchange within sample containers section). Tests were carried out to determine the best prime fluid (acidified or unacidified UHP), and the results are detailed below (see Southern Ocean field testing section). Pressure equilibration of the main housing is achieved via an external flexible bag, also filled with Fluorinert and connected to the main housing via Teflon tubing. Sample containers are equilibrated with external pressure through the flow path. The process to prepare an ACE sampler for deployment is detailed below.

Sampler preparation

After the main housing containing the pumps, PCB and batteries are assembled, the housing is flooded with FC-40 Fluorinert (3M) and all air bubbles are bled from the housing using the two ports on the top cap. After this, the pressure compensation membrane (250 mL Tedlar PVDF gas sampling bag) containing 125 mL of FC-40 can also be bled and attached to one of the two available top cap ports. The volume in the pressure compensation bag allows expansion and contraction due to temperature fluctuations as well as pressure compensation. The pressure compensation membrane is secured in a three-dimensional (3D)-printed and epoxy-coated housing fixed between the sample containers and main housing to protect the membrane. After this step, the main

housing is sealed, and the exterior of the housing can be thoroughly washed to lower cross contamination to successive steps of the preparation. We use 2% Decon® 90 (Decon Laboratories) solution with UHP water to remove oils and grease first, followed by copious UHP rinses.

All of the manifold components including tubing are thoroughly washed using a 2% (v:v) Decon® 90 (Decon Laboratories) UHP solution for 1 week, UHP rinsed, and then acid washed in 6 mol L⁻¹ HCl (Emsure grade, Merck) for a month and UHP rinsed copiously again prior to assembly. All tubing is cut to length using a precleaned ceramic kitchen knife (Kyocera). During assembly, the intake lines are attached to a 2 cm length of Tygon tube (Masterflex) followed by a 40 cm length of tube into the sample containers, which passes through the compression fittings in the cap and terminates at the bottom of the sample container. This subassembly can then be acid washed carefully as a separate unit and only joined to the lines entering the main housing at a later point, to minimize cross contamination as a precaution. After connection of the sample container subassemblies with the main housing, the system, excluding the fragile filters, is again filled with 1.2 mol L⁻¹ UHP distilled HCL for 1 week as a further acid wash step and then rinsed copiously with UHP water. The syringe filters are cleaned offline with a gentler acid wash by rinsing in UHP and then filled with 0.5 mol L⁻¹ distilled HCl for 48 h and then rinsed thoroughly with UHP water again and stored in UHP until assembly (~ 2 weeks).

Prior to deployment, the intake tubes and sample containers are rinsed thoroughly and then filled with UHP water and carefully bled of any air bubbles by running the pumps, with intake tubes placed into a container of UHP water while visually inspecting the flow path for bubbles. After the system is bled of air bubbles, the intake tubes are plugged with acid-washed HDPE bungs to avoid losing prime, and the filters are again rinsed with UHP and then installed inline within the intake stream. The bungs are then removed, and the intake tubes placed into a container of UHP water and the pumps run again to verify that all syringe filters are flowing well and are not blocked due to air bubbles. The intake tubes are then plugged again until deployment to keep the flow path sealed and contamination free.

The system can then be programmed for sampling at any date and time within the sampling duration. At this stage, the Ti tension rod is secured to the brackets using Ti M6 fasteners (Probolt Australia) via the Delrin bushes and the shrouds fitted to the sampler, although feeding the intake tubes through the intake tube guides on the top shroud. The shrouds are then securely fastened to the main rack/bracket of the sampler using M4 Ti fasteners. The intake tubes are secured inside the intake guide with all-plastic, washed zip-ties. The sampler can now be bagged prior to deployment. At the last moment before deployment, the outer bag and intake bungs are removed, before the sampler is deployed overboard (Fig. 6).



Fig. 6. The ACE sampler being deployed in March 2019 at the SOFS observatory (140E, 42S) as part of the air-sea flux mooring. D-shackles immediately adjacent to the ACE sampler are coated in a flexible rubber coating (Plasti Dip), and the mooring wire is sheathed in a plastic cover. The ACE sampler is free to rotate around the Ti tension rod to maintain intakes oriented into the uncontaminated upstream water.

The advantage of this design is that the entire sampler can be prepared in a clean room, sealed, bagged, and then sent out on a research vessel for deployment without any other handling required other than the final steps before deployment.

Sample recovery

Upon recovery, the systems intake and exhaust ports are sealed with low-density polyethylene (LDPE) bungs to prevent ingress of contaminants. The biofouling must then be removed to a point where cross contamination via handling and surfaces within the clean laboratory is minimized. This is done by first removing large organisms such as goose neck barnacles with a blunt plastic instrument. After this, the surface film of biofouling can be removed with a 2% (v:v) Decon mix and sponges and plastic brushes/tooth brushes. The shrouds, Ti tension rod, and Delrin bushes are removed and a thorough rinse with UHP water is performed, and it can be transferred into the clean room for further cleaning. While

keeping the whole system sealed, the filter holders are removed from the top of the sample containers and a new toothbrush and UHP water used to further clean thoroughly around the sample container cap and fittings. A final 5% (v : v) distilled HCl acid rinse from a squeeze bottle around the tube fittings and sample container thread followed by a UHP rinse is performed. The sample containers are then allowed to dry under HEPA-filtered air in a clean room. The exhaust compression fitting on the sample container can now be loosened and the exhaust tube removed. At this point, there are two ways to proceed. Either the sample can be acidified through the exhaust port and subsequently drawn out via an all-plastic, acid-washed syringe and 1/16 in PFA tube or the cap can be unthreaded, the sample acidified with distilled HCl and a new acid-cleaned Savillex FEP cap (without tube ports) fitted. The samples are normally stored acidified for at least 24 h to mobilize components adsorbed to the vial walls. The samples are then ready for analysis, and the sample containers chosen fit directly into a 7×3 autosampler rack as used, for instance, for seaFAST S2 Pico (Elemental Scientific) preconcentration before sector-field ICP-MS detection.

Assessment

Southern Ocean field testing

To test the ability of the ACE sampler to collect uncontaminated seawater samples in the HNLC open ocean, the ACE sampler was deployed during a SR3 repeat oceanographic transect in February 2018 (GEOTRACES section GS01). The ACE sampler was deployed at trace metal rosette (TMR) Sta. 49 (64.5°S, 132.07°E) with a bottom depth of 1497 m and surface seawater temperature of approximately -1°C . The ACE sampler was deployed at 30 m on a 7 mm Dyneema line, and coincident samples were taken at 30 m using the industry standard and proven noncontaminating, TMR.

Sample handling

All sample handling was conducted in a containerized clean room and directly below the flow of HEPA laminar flow hoods. Due to the relatively short deployment, no significant biofouling developed. Therefore, the system only required thorough rinsing with UHP water over the exterior sampler body, followed by drying under a flow of HEPA filtered air within the containerized clean room. The samples were decanted from the ACE sampler into acid-washed LDPE 125 mL sample bottles. All samples that were not filtered inline by the ACE sampler were filtered at this stage using the same 33 mm Millipore Millex 0.2 μm SUPOR (PES) syringe filters and acid-washed all-plastic (nonrubber) syringes. As we wanted immediate feedback on the results while at sea, the samples were analyzed by FIA-CL onboard the RV *Investigator*.

Dissolved Fe analysis: Flow injection chemiluminescence detection

Dissolved Fe samples were analyzed using FIA-CL utilizing in-line preconcentration onto an 8-hydroxyquinoline (8-HQ)

resin (Fe[III] method), adapted from de Jong et al. (1998) and Obata et al. (1993). All sample analysis was performed within a containerized clean room. The mean detection limit (3X analytical blank standard deviation) of the FIA-CL method with 3-min preconcentration was $0.05 \pm 0.02 \text{ nmol L}^{-1}$ ($n = 3$). Reanalysis of calibration standards throughout each analytical run was used to check for analytical drift. Repeated analysis ($n = 3$) of a well-characterized in-house standard validated the accuracy of the daily calibration.

Results of a comparison between the ACE sampler and TMR

Due to the added components in contact with the sample during inline filtration, we tested whether this added step or the pH of the prime fluid added any significant contamination (Fig. 7). To do this, we took three replicate seawater samples for three treatments: unacidified UHP prime fluid and no inline filtration, unacidified UHP prime fluid and inline filtration, and acidified UHP prime fluid and no inline filtration. The potential effect of filtration alone was tested in the lab by filtration of unacidified UHP, which was subsequently acidified and analyzed together with the other samples (filtered UHP). Finally, UHP prime fluid that was deployed in the sampler and recovered was analyzed (blank UHP). Although the results were very close to the detection limit of the FIA-CL system on the day, contamination, if present would have been clearly identified, as all samples collected were less than 100 pmol L^{-1} with an average of $60 \pm 20 \text{ pmol L}^{-1}$. A one-way analysis of variance (ANOVA) was used to test the null

hypothesis that the means of all groups were equal. The test results were $p = 0.557$ ($F = 0.740$), and therefore, we accept the null hypothesis that the means are statistically indistinguishable. Therefore, no significant difference was observed between any of the ACE sampler treatments and the mean concentration of the seawater collected using the TMR in this test. We therefore conclude from this test that the ACE sampler can collect contamination-free seawater samples over a short-term deployment, irrespective of the pH of prime fluid or whether inline filtration is utilized or not. The caveat being that the system was deployed for a relatively short period in this test, this experiment does not quantify the potential issues associated with long-term deployments including biofouling cross contamination and leaching contaminants from materials used to store samples. Full analysis and interpretation of long-term deployments at the SOTS observatory will be published in a companion article.

Lab testing

To test the efficiency of sample exchange and for contamination testing over the full range of trace metals, we collected samples using the ACE sampler in the lab from a bulk container of low-Fe seawater. Samples were collected from a 2-liter LDPE sample container and filtered in-line with the pumps set to run for 22 min per sample. The same bulk seawater was subsampled for analysis and called "blank SW." The samples were stored in the ACE sampler for 2 weeks, prior to acidification and analysis to assess contamination potential over the

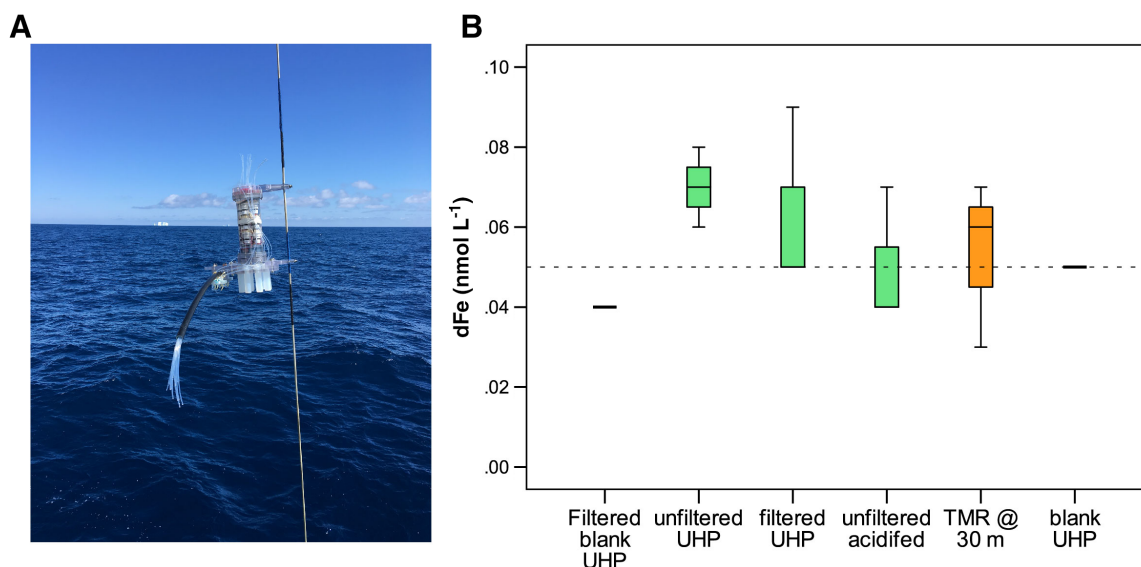


Fig. 7. ACE sampler in its surface float form factor, clamped to Dyneema for deployment during the SR3 voyage at 64.5S, 132.07E (A) and the results of three treatments compared with blanks and the TMR (B). The three treatments tested were: sample containers filled with UHP water prior to deployment and unfiltered by the ACE sampler but subsequently filtered in the lab after sample collection and before analysis (unfiltered UHP, $n = 3$), sample containers filled with UHP water prior to deployment and filtered by the ACE sampler inline (filtered UHP, $n = 3$), and sample containers filled with acidified UHP water (pH 1.8) prior to deployment and unfiltered by the ACE sampler but subsequently filtered in the lab after sample collection and before analysis (unfiltered acidified, $n = 3$). Finally, blank UHP water from unsampled but deployed sample containers (blank UHP, $n = 1$) and UHP filtered in the lab and analyzed (filtered blank UHP, $n = 1$). The dFe concentration from samples collected at the same depth with the TMR is also shown (orange, $n = 3$). The detection limit of the FIA-CL system during this run is indicated with a dotted line (0.05 nmol L^{-1}).

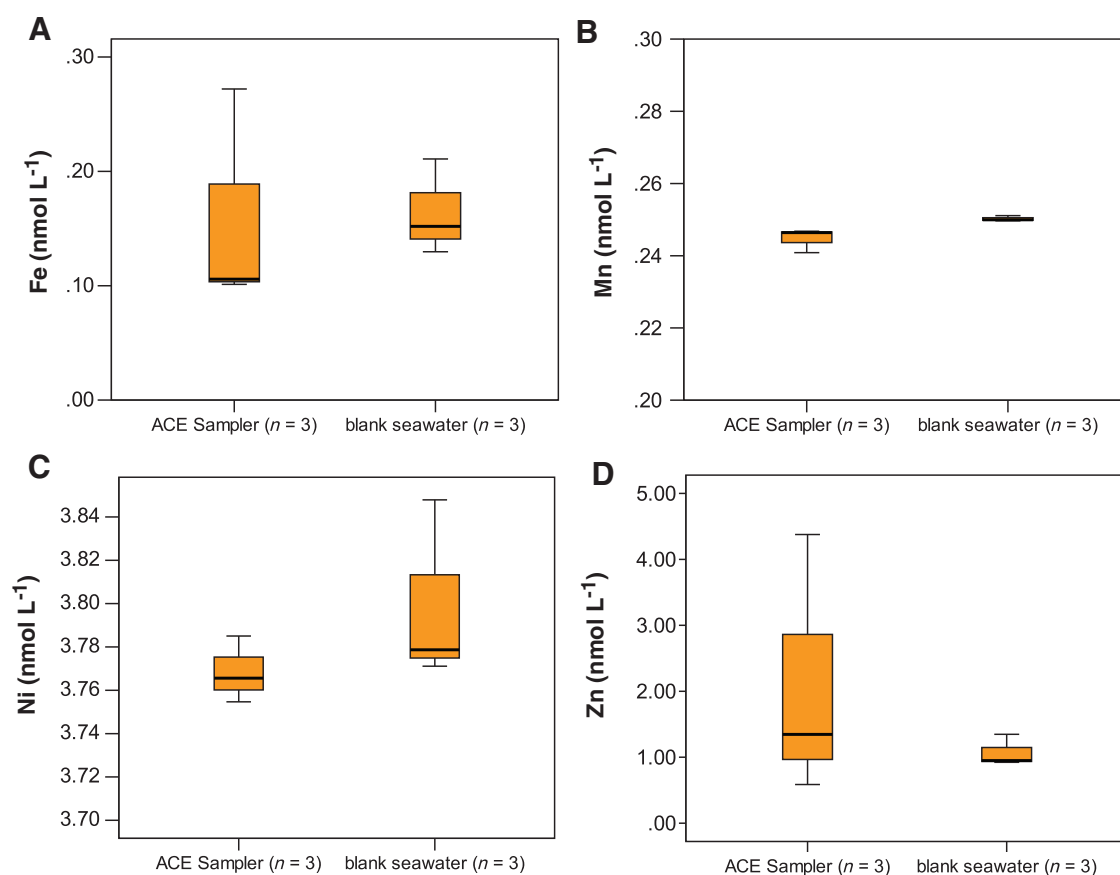


Fig. 8. Results of a lab test where the ACE sampler collected seawater from a bulk container, stored it for 2 weeks, before being analyzed by seaFAST preconcentration and ICP-MS analysis.

medium term. The samples collected using the ACE sampler and the blank SW was preconcentrated (40-fold) using a seaFAST S2 Pico (Elemental Scientific) and analyzed via a Sector Field, Inductively Coupled Plasma Mass Spectrometer (SF-ICP-MS; Element 2, Thermo Scientific). All data processing was performed using a modified version of the R script (<https://www.R-project.org>) written by M. Rijkenberg and documented in Wuttig et al. (2019). The results of a thorough array of quality control experiments for the method used in our lab were published in Wuttig et al. (2019). Briefly, on the day of the analysis, the full method seaFAST blank (acidified MQ) was 0.049 ± 0.002 , 0.003 ± 0.001 , 0.24 ± 0.06 , and 0.105 ± 0.145 nmol kg⁻¹ for dFe, dMn, dZn, and dNi, respectively. This resulted in detection limits ($3 \times \text{SD}$ of the blank) of 0.01, 0.001, 0.17, and 0.43 nmol kg⁻¹ for dFe, dMn, dZn, and dNi, respectively, which generally agree well with our 3-yr average with the exception of dNi which, on this day of analysis, had a detection limit approximately four-fold higher than our 3-yr mean (Wuttig et al. 2019). No data for the presented elements were below the detection limits on the day of analysis. Precision was checked by repeat analysis ($n = 4$) of a well-characterized in-house standard, which agreed well with our 3-yr mean. Analytical drift on the ICP-MS was monitored via

repeat analysis of a 1 ppb standard and no correction was necessary.

The trace element results are shown in Fig. 8A–D. Dissolved Zn, Ni, and Fe were not significantly different between the treatments (one-way ANOVA, $p = 0.43$, $F = 0.78$, $n = 3$; $p = 0.30$, $F = 1.40$, $n = 3$; 0.95 , $F = 0.01$, $n = 3$, respectively). Dissolved Mn was significantly different between the treatments (one-way ANOVA $p < 0.05$, $F = 7.95$, $n = 3$), with the ACE sampler being slightly lower in concentration (ACE sampler mean 0.245 vs. 0.250 nmol kg⁻¹ for the blank seawater). No statistically significant difference in dissolved Zn was observed, however, more variability was observed for this element in the ACE sampler treatment than the blank seawater due to one replicate that was higher in concentration for the ACE sampler.

Pressure testing

The complete system was depth tested to 30 m (with sporadic excursions to 40 m) during contamination testing on the SR3 oceanographic transect. Leaks into the main housing, if present, are clearly visible as beading on the surface of the FC-40 pressure compensation fluid due to its extremely

hydrophobic properties. No leaks were detected in the FC-40 pressure compensation fluid during the 4-h test at 30 m. Furthermore, the system was tested in a modular fashion within a fluid-filled pressure chamber within the CSIRO laboratories. In this test, a miniaturized, two-sample prototype version of the system was tested to 100 m utilizing a membrane for pressure compensation. It successfully operated at this depth for 1 h and collected two samples, verified by adding dye to the pressure housing fluid. Upon recovery, the UHP prime water was exchanged with the external dyed solution. Due to the all-metal construction of most pressure chambers, they represent a significant contamination risk for a newly constructed ACE sampler, so the decision was made that no further lab pressure testing would be conducted. Due to the pressure compensated nature of the system and the selection of pressure tolerant batteries and electronic components, which were tested individu-

where J is the diffusion flow rate, D is the diffusion coefficient, A is the cross-sectional area of the tubing, C_1 and C_2 are the concentrations at either end of the tubing, and L is the length of the tubing. Fe^{2+} has a diffusion coefficient of $9.05 \text{ cm}^2 \text{ s}^{-1}$ at 25°C , 34 psu (Yuan-Hui and Gregory 1974).

The concentration at the “contaminated” side of the tube (C_1) was set to 2 nmol L^{-1} and at the sample container end (C_2) to 0.05 nmol L^{-1} . This represents a highly conservative upper estimate of dissolved Fe mobilized by bacterial remineralization on the filter vs. a typical HNLC Southern Ocean surface seawater concentration. Typically, HNLC Southern Ocean surface particulate Fe is less than 1 nmol L^{-1} (van der Merwe et al. 2015) and a large portion of this will not be mobilized, and only 220 mL is passed across the filter, so normally the concentration gradient would be much lower. The Teflon tubing has an internal diameter of 1.56 mm.

$$J = \frac{(9.05 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}) \cdot (1.986 \times 10^{-6} \text{ m}^2) \cdot (2 \times 10^{-6} \text{ mol m}^{-3} - 5 \times 10^{-8} \text{ mol m}^{-3})}{0.4 \text{ m}}$$

$$J = 8.76 \times 10^{-21} \text{ mol s}^{-1}$$

$$J = 0.3 \text{ pmol a}^{-1}$$

ally to 100 m in the above test, we do not expect any problem using the system at or less than 100 m. After this depth, the limiting factor will be the pressure tolerance of the batteries. Lithium polymer pouch-type batteries have minimal voids in their construction and once pressure compensated, should be capable of increasing the depth rating further, but this remains to be tested.

Diffusive isolation along the flow path

Large concentration gradients can develop along the flow path of the ACE sampler due to bacterially mediated dissolution of particulate trace metals on the filter after sampling and biofouling development in the vicinity of the exhaust tubes. To limit ingress of these contaminants into the sample containers during long-term deployments, we added 40 cm of tubing between the filter and sample container on the intake side and another 40 cm of tubing between the sample containers and the pump. To determine if diffusion alone could transfer contaminants along the 40 cm of tubing intended to isolate the sample, we used Fick's first law to calculate the diffusion rate as follows:

$$J = \frac{DA[C_1 - C_2]}{L}$$

We can see from this calculation that during 1 yr of deployment, 40 cm of 1.56 mm internal diameter tubing effectively isolates a potentially contaminated end of the tubing from the critical sample as 0.3 pmol a^{-1} (0.6% of a typical sample) is well within the error of analytical uncertainty.

Complete sample exchange within sample containers

Due to the relatively slow sample flow of the micro-peristaltic pumps of 10 mL min^{-1} , the sample containers would mix rather than completely exchange with the external seawater unless density driven sample exchange was used. Therefore, we deploy the system fully primed with UHP water, and the intake tube terminates at the very bottom of the sample container. Due to the large density differential between UHP water and the inflowing seawater, the sample container fills from the bottom and remains stratified until all the UHP water has been expelled from the top of the sample container. This stratification can be clearly observed in the lab during benchtop tests either with or without dye. Due to the lack of air in the sample containers, the stratification is not easily broken down by movement during sampling. Although the sample containers exchange in approximately 6.5 min, we continue to run the pumps for a further 15.5 min or 22 min in total to ensure complete

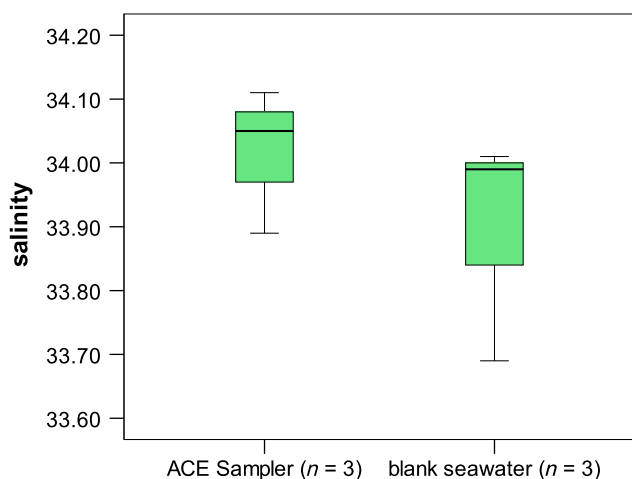


Fig. 9. Salinity of the samples collected by the ACE sampler during the lab testing documented above vs. the blank seawater used in the test. No significant difference was observed between the treatments (one-way ANOVA, $p = 0.38$, $F = 0.96$, $n = 3$).

sample exchange and minimal mixing. This results in 3.4 times the volume of the sample containers passed through each container for each sampling event. Pumping duration was chosen after measuring the salinity of homogenized samples at various time intervals. After approximately 10 min, the salinity stabilizes and the remaining pumping duration aids to flush the sample container. The salinity of the samples collected by the ACE sampler during the lab testing vs. the blank seawater used in the test is shown in Fig. 9. No significant difference was observed between the treatments (one-way ANOVA, $p = 0.38$, $F = 0.96$, $n = 3$), and therefore,

we accept the null hypothesis that the means are statistically indistinguishable.

Discussion

There is currently no other system available that can collect uncontaminated filtered samples over long-term deployments in the HNLC open ocean. Although the PRISM sampler (Mueller et al. 2018) shows promise, the issues outlined in the introduction and, in particular, the shared filter between all samples cannot avoid bacterially mediated leaching of trace metals from particles into successive samples. Our laboratory and field experiments demonstrate the capability of the ACE sampler to collect uncontaminated filtered or unfiltered seawater samples in the HNLC open ocean where dissolved trace metals are often found in the picomolar range. This has been achieved by using pumps and materials with a proven track record and simplifying the flow path as much as possible to avoid convolutions and dead space. Although short term, high temporal resolution sampling is significantly less challenging, with careful cleaning to remove biofouling to avoid cross contamination during sample recovery or a secondary pressure compensated enclosure, longer term time-series sample sets will be achievable.

Obtaining data sets from remote and logistically challenging regions using autonomous trace metal clean samplers has been identified as a target within the next decade for the community (Grand et al. 2019). This system will help achieve that goal and be particularly useful for researchers interested in capturing the trace metal cycling over diel—yearly cycles. Researchers interested in capturing processes and events will also be able to quantify the fluxes associated with many of sources of trace metals to the ocean.



Fig. 10. Biofouling after 8 months of deployment on the mounting brackets of the first generation of ACE sampler, mounted in a surface float. Gooseneck barnacles (order Pedunculata) are a common biofouling organism which must be removed with a blunt plastic spatula or blade prior to sample recovery. Likewise, a thin biofilm will also develop over the sampler, which can be removed with a 2% Decon® 90 (Decon Laboratories) solution and sponges or brushes.

Manufacturing costs and availability

Although the significant cost of this system is in design and testing, the manufacturing costs are relatively low. The cost of manufacture will vary widely based on geographical location, exchange rates, and whether fabrication of components can be completed in-house or not. At the time of writing, the ACE sampler can be manufactured for approximately AUD\$10,000 (USD\$7000) with costs approximately evenly divided between four main categories: Savillex components, electronics including the 12 Welco pumps and Fluorinert, fabrication of the CNC milled components, and finally the Ti tension rod, fasteners, and associated bushings. Although the system is not currently available for purchase, commercialization opportunities are under development, and therefore, currently, the source CAD files are not available for distribution. However, interested labs or organizations are encouraged to get in contact on a collaborative basis for future research opportunities.

Comments and recommendations

After months at sea, the biofouling can be severe, even at open-ocean locations (Fig. 10) but especially at coastal locations. Due to the bio-essential nature of Fe, biology actively concentrates this micronutrient (Lannuzel et al. 2010; Nicol et al. 2010; van der Merwe et al. 2011), and as a result, biofouling can be a significant source of cross contamination during sample collection. Therefore, long-term (e.g., 12 months) deployments represent an appreciable challenge for trace metal research. The development of antibiofouling coatings that are compatible with trace metal research would reduce the likelihood of contamination during sample recovery, but workable prototypes may be many years away (Grand et al. 2019). In the near future, production of a larger portion of the ACE sampler out of Teflon may reduce build-up of biofouling. Likewise, shorter deployments in coastal areas or longer deployments in HNLC oceans may limit biofouling to manageable levels. Future iterations of the ACE sampler could include a second pressure compensated enclosure (using UHP water as the compensation fluid) surrounding the sampling containers to provide a physical barrier between biofouling and the super critical sample containers. However, this would add extra complexity, cost, size, and weight to the system but may be necessary for biofouling-prone environments.

Deployment in environments where temperatures are expected to be lower than the freezing point of freshwater present another challenge as the prime fluid is prone to freezing and the battery capacity would be reduced. We have experimented with using trace metal clean brine solution (~ 70 psu) as the prime fluid, which lowers the freezing point to around -4°C . This requires reversing the intake and exhaust positions on the sampling containers to maintain the density-driven sample exchange. This modification shows potential but adds an extra layer of complexity and will require more work to ensure the capability for deployments

below 0°C . It is a functional improvement that we will continue to explore as capturing sea ice and glacial melt fluxes, for instance, is an area of active research in our lab. Resolving these cycles will shed new light on important processes with direct impact on carbon cycling and ecology.

This system has been designed from the ground-up to be suitable for the highly demanding task of collecting uncontaminated trace metal clean seawater samples during time-series studies. As a result, it requires specialist handling and is generally more labor-intensive to use than a typical water sampler. For instance, multiple intake tubes can be difficult to work with but are required on this system to remove valves or manifold parts from the flow path that may trap dead space and contaminants. Changing the batteries or servicing the pumps requires opening the main housing, which is flooded with FC-40 pressure compensation fluid. The batteries chosen are nonrechargeable due to their extremely low self discharge rating, and although they have capacity for multiple deployments, rechargeable batteries have been used previously for shorter deployments and charged through the bulkhead connector to make the system easier to redeploy rapidly.

Currently, sample collection takes 22 min per sample to ensure complete sample exchange, which is no problem for long-term deployments but may be limiting if rapid deployments, such as onboard autonomous underwater vehicles or other mobile platforms, are considered. Running the pump continuously until a sample is desired would enable event driven sampling, but the interval between samples would remain at 22 min. The sampling time could be shortened dramatically by selecting larger, more powerful peristaltic pumps, but the overall system size and weight will also be increased.

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Conflict of Interest

None declared.

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