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Update of the PERISCOP System for Isobaric Sampling of Deep-Sea Fauna

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ABSTRACT

We here present an update of the PERISCOP pressurised recovery device (PRD), which has allowed the recovery of live deep-sea fauna, following active sampling by manned or remotely-operated submersibles. It was designed in 2006, has since been deployed almost a hundred times (93), and its operation has now become almost routine. Various hydrothermal vent megafaunal organisms (shrimp, crabs, annelids, mussels, fish,..) were successfully targeted and recovered, allowing access to samples in excellent physiological condition. Gradually, the system was modified, aiming at improved reliability, depth of operation, and simplicity of use, especially regarding its compensator, i.e. the system that compensates for pressure loss experienced during ascent through the water column. Two types of compensators are presented, called “active” and “passive” (with so-called “water-filled” or “oil-filled” modes regarding the latter). Their respective uses are reported and discussed here, and while the active system proves more efficient (recovering at minimum pressure in the range 93.8-98.2 % of *in situ* pressure, as opposed to 80.9-86.6% for the water-filled passive system, while no compensation leads to a range of 63.5-73.0%), the simplicity of the passive compensator greatly improves reliability and ease of use and maintenance. Finally, the monitoring of pressure and temperature of the PRD and the surrounding water column permits to discuss various technical aspects of pressurised recovery, and to propose further improvements.

29 **KEY WORDS** : Isobaric sampling, Hydrostatic pressure, pressure compensation, physiology

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42 reported in this paper.

43

44 **Contributions of authors**: BS LA GH designed the PERISCOP and its successive upgrades,
45 and participated to the deployments at sea. BS administrated the financial supports which
46 contributed to the PERISCOP, and wrote the manuscript.

47

48

49 INTRODUCTION

50 The deep sea is by far the largest ecosystem on the planet, but it remains the least explored
51 and understood ([Ramirez-Llodra et al, 2010](#), [Fang et al, 2010](#)), especially regarding the
52 ecology and biology of the organisms inhabiting this environment. One evidence is emerging
53 though: the deep-sea biome is threatened by climate change and other anthropogenic stressors
54 ([Ramirez-Llodra et al, 2011](#), [Sweetman et al, 2017](#)). Providing significant advances in
55 fundamental knowledge on the ecology, biology, and physiology of deep-sea organisms is
56 therefore an urgent matter, but this goal is challenged by the difficulties in accessing their
57 habitat. Accessing deep-sea habitats often requires distant and costly high-sea expeditions,
58 and the use of equipments specifically adapted to the environmental parameters prevailing at
59 depth. Among those parameters, the major obstacles to investigations are low temperatures
60 and high hydrostatic pressures, especially for physiological studies, as witnessed by the
61 apparent trauma inflicted to deep fauna recovered with neither temperature nor pressure
62 control. As recently reviewed in [MacDonald \(2021\)](#), it is quite clear today that isobaric and
63 isothermal sampling are needed prior to undertaking physiological studies of deep biota.

64 The PERISCOP pressurised recovery device (PRD) was designed in our laboratory, and first
65 operated at sea in 2006. It aimed at recovering mobile hydrothermal vent fauna in good
66 physiological conditions, from depths as great as 3000 m ([Shillito et al, 2008](#)), by maintaining
67 the pressure and temperature prevailing in the environment of these organisms. It has since
68 been operated several times (93), allowing for example the deepest recovery of a live fish
69 (*Pachycara saldanhai*, [Shillito et al, 2008](#)), or the first *in vivo* determination of thermal
70 resistance, in the case of the thermophilic vent tubeworm (*Alvinella pompejana*, [Ravaux et al,](#)
71 [2013](#)). The PERISCOP was gradually modified, aiming at improved reliability, and simplicity
72 of use. One specific focus was the compensator of the PRD, i.e. the system that compensates
73 for pressure loss experienced during ascent through the water column ([Shillito et al, 2008](#)).

74 We here present an update of the PERISCOP PRD, with improvements based on data and
75 practical experience we acquired at sea. We hope this work provides help to other
76 investigators, in the design and operation of future PRDs.

77

78 **METHODS**

79 **The PERISCOP Pressurised Recovery Device (PRD) (Figure 1)**

80 PERISCOP is a PRD of internal volume of 6.6 L, and is constructed with 316 or APX4
81 stainless-steel type components (Figure 1). It is also described in [Shillito et al \(2008\)](#), and
82 main modifications are indicated below. Pressure inside the PRD is recorded by an
83 autonomous probe (SP2T 4000, NKE instruments, Hennebont, France), while an optional
84 temperature logger may be inserted inside the PRD (S2T 6000, NKE instruments, Hennebont,
85 France). All the probes were pre-set to record data once every minute throughout each
86 deployment. The PRD's main aperture is a quarter-turn globe-valve allowing a 10 cm
87 diameter free passage, whereas the bulk of the PRD is a cylinder of 10.6 cm internal diameter
88 and 50 cm length. The PRD is enclosed in a rectangular-shaped syntactic foam casing, which
89 contributes to both thermal insulation and floatability. Compared to the first PERISCOP
90 described in [Shillito et al \(2008\)](#), the major modifications consisted in re-designing the collar-
91 shaped clamps that maintained the globe-valve and the sampling cylinder together (see figure
92 3 in cited reference), in order to obtain a more rigid behaviour under pressure, and to increase
93 its maximum depth of operation from 3000 to more than 3600 m. In order to improve both
94 thermal insulation and floatability, the casing was redesigned with a syntactic foam of lower
95 density (from 0.54 to 0.42, same manufacturer, BMTI Toulon France), and its side
96 dimensions were slightly enlarged (from 32 to 34 cm). As a result, the weight of the entire
97 device is 113 kg, appearing at 36 kg under water (104 kg and 43 kg respectively for the PRD
98 described in [Shillito et al , 2008](#)).

99

100 **The two types of pressure compensators (Figure 1C, 2 and 3)**

101 During ascent through the water column, changes in environmental pressure and temperature
102 can modify the pressure prevailing inside the PRD, because of metal expansion and seal

103 movements, thereby justifying the use of a pressure compensation unit (or so-called
104 compensator). Unlike most other PRDs, our compensators do not rely on pressurized gas, and
105 two systems are presented: one called active, as opposed to the other, named passive. The
106 principles are described in figures 2 and 3 respectively. Briefly, the active compensator
107 requires that a high-pressure reservoir is loaded with seawater at a pressure of about 45-50
108 MPa before each deployment (on board ship), and that a reference container is closed *in situ*
109 by the submersible's hydraulic arm, just before the PRD begins the ascent to the surface, in
110 addition to closing the PRD's main globe-valve (Figure 2). From then on, the compensator,
111 by means of two differential valves (Tescom, Elk River, Minnesota, USA), compares the
112 pressures prevailing in the reference container and the PRD, and consequently compensates
113 for either pressure loss or gain in the PRD (see Figure 2). It is this active compensator that
114 was first designed and used (Shillito et al, 2008), and major improvements consisted in 1 -
115 integrating the differential valves inside the main container, at atmospheric pressure (they
116 were previously exposed to surrounding pressure, see Figure 2 in Shillito et al, 2008), and 2 -
117 increasing the volume of the reference container, from 90 mL to 300 mL (stainless steel
118 container, HOKE-LAA, Asnières, France). The weight of the active compensator is 27.85 kg.

119

120 Alternatively, the passive compensator does not require pre-conditioning prior to each
121 deployment, and the only manipulation required *in situ* is the closing of the main globe-valve
122 of the PRD. Unlike the active system, which integrates off-the-shelf components, this
123 compensator was entirely designed in our laboratory, with the help of computer-assisted
124 design facilities (SolidWorks, Dassault systems, France). Its principle relies on the elasticity
125 of its internal container (of 4 L internal volume, made of Titanium TA6V alloy), and the
126 compressibility of the liquid it contains (either water or oil). This container is stored in an
127 external container (made of 316 stainless steel alloy), and is therefore always surrounded by

128 atmospheric pressure during deployments. Upon mooring at depth, the environmental
129 pressure “loads” the compensator with seawater by pushing a piston (see Figure 3). During
130 recovery (ascent through the water column) this seawater is provided to the PRD as its
131 internal volume expands due to the decreasing surrounding pressure. The weight of the
132 passive compensator is 26.7 kg.

133

134 **Operation of the PERISCOP PRD by a submersible and associated shuttle device** 135 **(Figure 4)**

136 The PERISCOP system is attached to a shuttle device, which allows mooring and recovery of
137 various instruments and tools related to the submersible’s activity during scientific dives. The
138 shuttle presented in Figure 4 belongs to the Nautilie team (Ifremer’s manned submersible, so-
139 called Human Occupied Vehicle, or H.O.V.), however the PERISCOP has also been
140 successfully used with two other shuttle devices belonging to the Victor 6000 team (Ifremer’s
141 Remotely Operated Vehicle, or R.O.V., see [Shillito et al, 2008](#)). As described in [Shillito et al ,](#)
142 [2008](#), the PERISCOP system allows active sampling of fauna by means of a deep
143 submersible, i.e. it allows choosing for samples, as opposed to most other PRDs which rely on
144 attraction of fauna to bait. In order to track the pressure history of samples with respect to the
145 recovery sequence, an additional autonomous probe (SP2T 4000, NKE Instruments,
146 Hennebont, France) is mounted on the shuttle, and records the temperature and pressure of the
147 surrounding water column.

148

149 **In situ sampling process (Figure 5)**

150 As previously described ([Shillito et al, 2008](#)), the sampling cell is made from PVC and
151 transparent Polycarbonate. It mainly consists of two interlocked cylinders, an outer cylinder
152 connecting to the submersible’s suction device, and an inner cylinder for confinement of

153 fauna, with an internal diameter of 95 mm and length of 350 mm. Fauna are sampled using
154 the suction power of the submersible, through a 50 mm diameter flexible nozzle. The suction
155 device of the submersible is connected to the sampling cell *in situ* immediately prior to
156 sampling, and disconnected afterwards. Once this has been done, the submersible separates
157 the two cylinders composing the sampling cell. The inner cylinder contains the samples, and
158 will be stored further inside the PRD, before allowing the shuttle to ascend towards the
159 surface.

160 In the case of sessile fauna, which can be sampled directly with the submersible's hydraulic
161 claw, another sampling cell made of 316 stainless steel was designed (and named "Croco"),
162 which allows direct positioning inside the PRD once the sampling cell is closed (see Figure 6
163 E-F, and [Ravaux et al, 2013](#)).

164

165

166 RESULTS

167 Overview

168 Overall, the PERISCOP PRD was operated 93 times, at East Pacific Rise (EPR) vents (14
169 deployments), Mid Atlantic Ridge (MAR) vents (67 deployments), and in the Mediterranean
170 Sea (a few nautical miles off Toulon, on the French coast, 12 technical deployments), at
171 depths ranging from 750 to 3650 m. 49 of these deployments were achieved with the manned
172 submersible Nautilie, and 44 with the R.O.V. Victor 6000. Various hydrothermal vent
173 organisms were sampled and further studied *in vivo* on board the oceanographic ship, using
174 dedicated pressure aquaria (Shillito et al, 2014): alvinellid tubeworm colonies (*Alvinella*
175 *pompejana* and *Alvinella caudata*) and associated fauna (not shown, see Ravaux et al 2013,
176 Papot et al, 2017), bythograeid crabs *Bythograea thermhydron* (not shown), vent mussels
177 *Bathymodiolus azoricus* (Figure 6 E-F, see also Szafranski et al, 2015), *Bathymodiolus*
178 *puteoserpentis* and associated fauna (not shown, see Duperron et al, 2016, Piquet et al 2019,
179 2020). MAR hydrothermal vent shrimps *Rimicaris exoculata*, *Rimicaris chacei*, and
180 *Mirocaris fortunata*, were the main targets, totalising 51 deployments (see Figure 5A, and
181 Figure 6 A-B, see also Auguste et al 2016, Methou et al 2019, Le Bloa et al, 2020, Ravaux et
182 al 2009, 2019, 2021, for recent biological studies). Finally, 3 zoarcid fishes, 1 *Thermarces*
183 *Cerberus* (not shown), and 2 *Pachycara saldanhai* (Figure 6 C-D, see also Shillito et al, 2008)
184 were sampled and recovered in good physiological condition, as witnessed by their
185 behavioural activity.

186

187 Regarding the use of different pressure compensation systems, 48 deployments used the
188 active compensator, 32 used the passive compensator, and 8 deployments occurred without
189 any compensator. Finally, 5 deployments did not retain pressure, resulting either from a
190 deliberate choice, or from important leakage during recovery.

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In terms of reliability, all 8 deployments without compensation behaved as expected (100 % reliability), i.e. recovering at a minimum of 60% of *in situ* pressure, with only one minor leak detected (EssNaut 2021 # P3 deployment). For the 32 deployments using the passive compensator, 30 behaved as expected, i.e. recovering at pressures above 80 % of *in situ* values (~ 94 % reliability), despite minor leaks detected in two of these deployments (see Table S2). The two remaining deployments experienced a failure of the autonomous pressure probe (no data), or a leak during ascent resulting in 71 % retention of *in situ* pressure (Table S2). For the 48 deployments using the active compensator, reliability was lower, with 15 deployments (~31 %) behaving as expected, i.e. recovering at pressures above 90 % of *in situ* values. Within the remaining deployments, 27 (~56 %) still remained above 75 % retention of *in situ* pressure, and only 3 (~ 6 %) failed to retain more than 50 % of *in situ* pressure values.

The following results focus on the pressure history inside the PERISCOP PRD during its operation, from the moment it leaves the bottom, ascends through the water column, further reaches the surface, is hauled on board ship, and finally opened after releasing the pressure. For clarity, only 50 deployments are presented here, and while all the deployments using passive compensation are presented, only some of the successful deployments are shown regarding the use of active compensation. The data shown correspond either to technical deployments in Mediterranean waters in winter, i.e. with a temperature gradient of less than 2°C between bottom and surface waters (almost isothermal water column, Table S1), or to scientific deployments at MAR vent sites, with a temperature gradient of about 20 °C between bottom and surface waters (non-isothermal water column, Table S2).

216 **Operating in an isothermal water column (Mediterranean Sea, Figure 7-8, Table S1)**

217 All 12 deployments in this area were technical tests, in order to train the submersible crews to
218 manipulating the PERISCOP PRD *in situ*, and to evaluate the pressure-retaining performance
219 of different compensators, in the absence of significant heat transfer, due to an almost
220 isothermal water column (less than 2°C gradient between sampling site and surface water, see
221 temperature data in Figures 7-8). The autonomous pressure/temperature probe fixed on the
222 shuttle (see Figure 4E) provides the pressure prevailing around the PRD, and the temperature
223 of the surrounding water column, and therefore allows tracking of the different steps during
224 recovery (Figure 7). Figure 7 shows pressure and temperature data throughout a deployment
225 using the active compensator (Deployment # P6, EssNaut 2016 cruise, see Table S1).
226 Throughout the process, the pressure inside the PRD remained above 97.6 % of *in situ*
227 pressure (bold blue line), and reached 98.3 % just before pressure was released on ship deck
228 (indicated by arrow 3). Interestingly, when the shuttle was launched, meaning that the PRD
229 began its ascent towards the surface (indicated by arrow 1), the pressure prevailing inside the
230 PRD followed that of the surrounding water column, until about 1 MPa was lost. After this
231 loss, corresponding to an ascent of about 100 m through the water column, the pressure in the
232 PRD stabilized.

233
234 Figure 8 shows pressure and temperature data throughout a deployment with no pressure
235 compensation (Deployment # P5, EssNaut 2021 cruise, see Table S1). Throughout the
236 process, the pressure inside the PRD remained above 65.2 % of *in situ* pressure (bold blue
237 line), and reached 66.6 % just before pressure was released on ship deck (indicated by arrow
238 3). As for all the recoveries presented in this work, when the PRD began its ascent towards
239 the surface (indicated by arrow 1), the pressure inside the PRD first followed that of the
240 surrounding water column, until about 1 MPa was lost. After this loss corresponding to an

241 ascent of about 100 m through the water column, the pressure continued to decrease, but
242 following a lower rate. When the shuttle (and PRD) reached the surface (indicated by arrow
243 2), the pressure inside the PRD reached its minimum value, before slightly increasing until
244 pressure release (indicated by arrow 3), 36 minutes after the shuttle had reached the surface.

245

246 Generally, for the 7 deployments using the active compensator (Table S1), minimum pressure
247 retention ranged from 93.8 to 97.6 %, upon reaching surface waters, and final pressures upon
248 release ranged from 93.9 to 98.3 %. When the passive compensator was used, and loaded with
249 oil (3 deployments, Table S1), minimum pressure retention ranged from 83.3 to 84.4 %, upon
250 reaching surface waters, and final pressures upon release ranged from 86.7 to 89.1 %. When
251 no compensation was used (two deployments, see Table S1), the minimum pressure retention
252 was 63.5 and 65.2 % of *in situ* values, and final pressures upon release 60.1 and 66.6 %
253 respectively.

254

255 **Sampling in a non-isothermal water column (MAR sites, Table S2, Figures 9 to 12)**

256 Most of the deployments presented here involved sampling of fauna, mainly hydrothermal
257 vent shrimps (see Figure 5A, Figure 6 A, B). Which ever compensation system was employed
258 (including absence of compensation), all deployments resulted in samples in apparently very
259 good physiological condition, as suggested by the highly active behavioural response just
260 after decompression of the PRD. This opposes to the weak activity of shrimps collected
261 without pressure retention, with only slow and uncoordinated movements for shrimps
262 originating from the Rainbow site at about 2300 m depth, and almost total absence of motion,
263 suggesting moribund or dead organisms, in the case of deeper sites beyond 3000 m depth
264 (TAG, Snake Pit, Broken Spur, see Table S2).

265

266 The thermal conditions encountered during these MAR cruises corresponded to cold bottom
267 waters, and warmer surface waters: 3.5 / 24 °C for the Rainbow site (2300 m depth), for
268 bottom and surface water temperatures respectively, 2.6 / 23 °C for the TAG site (3600 m
269 depth), 2.5 / 23 °C for the Snake Pit site (3400 m depth), and 3 / 26 °C for the Broken Spur
270 site (3100 m depth). Temperature gradients between bottom and surface waters therefore
271 ranged from + 20.4 to + 23 °C.

272

273 Figure 9 shows pressure and temperature data throughout a deployment using the active
274 compensator (Deployment # D5, MomarDream cruise, see Table S2). Throughout the
275 process, the pressure inside the PRD remained above 97.6 % of *in situ* pressure (bold blue
276 line), and reached 103.5 % just before pressure was released on ship deck (indicated by arrow
277 3). As for all recoveries presented in this work, when the PRD began its ascent towards the
278 surface (indicated by arrow 1), the pressure prevailing inside the PRD first followed that of
279 the surrounding water column, until about 1 MPa was lost. After this loss corresponding to an
280 ascent of about 100 m through the water column, the pressure in the PRD stabilized. When
281 the shuttle (and PRD) reached the surface (indicated by arrow 2), the pressure inside the PRD
282 increased by approximately 1 MPa in a few minutes, before it stabilised again. It can be seen
283 here that the water column was gradually warmer as depth decreased (thin red line). For this
284 deployment, an additional autonomous temperature probe was inserted inside the sampling
285 cell (where collected organisms are stored), and allowed to observe gradual warming of the
286 sample, reaching about 17 °C upon final pressure release (indicated by arrow 3), almost 50
287 minutes after the shuttle had reached the surface.

288

289 Figure 10 shows pressure and temperature data throughout a deployment using the passive
290 compensator, filled with water (Deployment # P6, Bicoose 2 cruise, see Table S2). Throughout

291 the process, the pressure inside the PRD remained above 84.5 % of *in situ* pressure (bold blue
292 line), and reached 94 % just before pressure was released on ship deck (indicated by arrow 3).
293 As for all recoveries presented in this work, the ~1 MPa pressure loss is observed at the
294 beginning of the shuttle ascent, followed by pressure decrease at lower rate. When the shuttle
295 (and PRD) reached the surface (indicated by arrow 2), the pressure inside the PRD reached its
296 minimum value, before gradually increasing until pressure release (indicated by arrow 3), 38
297 minutes after the shuttle had reached the surface.

298

299 Figure 11 shows pressure and temperature data throughout a deployment with no pressure
300 compensation (Deployment # P16, Bicoso 1 cruise, see Table S2). Throughout the process,
301 the pressure inside the PRD remained above 72.4 % of *in situ* pressure (bold blue line), and
302 reached 79.3 % just before pressure was released on ship deck (indicated by arrow 3). As for
303 all recoveries presented in this work, the ~1 MPa pressure loss is observed at the beginning of
304 the shuttle ascent, followed by pressure decrease at lower rate. When the shuttle (and PRD)
305 reached the surface (indicated by arrow 2), the pressure inside the PRD reached its minimum
306 value, before gradually increasing until final pressure release (indicated by arrow 3), 16
307 minutes after the shuttle had reached the surface.

308

309 Finally, Figure 12 displays the final pressure values inside the PRD just before pressure was
310 released on the deck of the ship (i.e. for access to samples, see Table S2), as a function of
311 time since the shuttle had reached the warm surface waters (also in Table S2). These values
312 are compared to the mean of minimum pressures (red points), which occurred either when the
313 shuttle reached the surface (passive or no compensation) or before (active compensation), and
314 are therefore positioned on the vertical axis of the graph (Time is 0 minutes when reaching the
315 surface). These minimum values (mean % +/- SD) are 72.3 +/- 0.5 (n = 5) for no

316 compensation, 84.2 +/-1.3 (n = 22) for water-filled passive compensation, 91.0 +/- 0.3 for oil-
317 filled compensation (n = 3), and 97.9 +/-0.3 for active compensation (n = 4).

318 For all types of compensation, pressure generally increased after the PRD had reached the
319 surface. The lowest variations were observed for active compensation (triangles), while the
320 highest were those occurring in the case of oil-filled compensation, which may have exceeded
321 116 % of *in situ* pressure (squares). In the case of water-filled compensation or in the absence
322 of compensation (plain and empty circles respectively), final pressure always remained below
323 100 % of *in situ* pressure, even after almost one hour of delay after reaching the surface.

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327

328 **DISCUSSION**

329 **Overview**

330 Although most of the following discussion focuses on pressure compensation issues, other
331 aspects of PERISCOP deployments should first be addressed: the sea-going experience
332 presented here confirms the efficiency of the general principle, i.e. the use of a submersible-
333 assisted sampling (either R.O.V. or H.O.V., [Shillito et al, 2008](#)). This has allowed scientists to
334 target species, as initially experienced by pioneering work of [Koyama et al \(2002\)](#), a clear
335 advantage compared to using baited traps, the latter method being uncertain regarding
336 effective capture, and strictly restricted to mobile fauna. Our choice to achieve collection by
337 using sub-sampling cells (Periscopette or Croco sampling cells) prior to recovery (in the
338 PRD) proved its efficiency. The small size and weight of these cells clearly increases
339 manoeuvrability during the sampling action *in situ*. Moreover, because the submersible does
340 not have to transport the whole PRD (unlike the PRD described in [Koyama et al, 2002](#)), it
341 leaves more possibilities for other experiments to be carried out during dives, which in turns
342 facilitates the regular use of isobaric sampling deployments, within the tight time schedule of
343 an oceanographic cruise. Last, this method probably increased the reliability of an effective
344 sealing of the PRD's globe-valve (see Figures 5 and 6), since the introduction of a calibrated
345 sub-sampling cell into the PRD helped to prevent the samples or associated particles from
346 getting stuck across the closing seals. Deficient sealing of a PRD's main aperture, in relation
347 to sample introduction, is indeed a most likely cause of leakage (see discussion in [Jackson et](#)
348 [al, 2017](#), and [Case et al, 2017](#)).

349

350 Finally, as initially introduced by [Bianchi et al \(1999\)](#), the systematic use of pressure data
351 recording (both inside and outside of the PRD) throughout the entire sampling cycle is to be
352 encouraged (as in [Garel et al, 2019](#), [Wang et al, 2022](#)), because it proved very helpful in

353 detecting malfunction, and also because it revealed that the final pressure (i.e. when the PRD
354 was back on board the ship) was not necessarily the lowest experienced during the process.

355

356 **Analysis of pressure history inside the PRD during recovery, without pressure**
357 **compensation**

358 When attempting to retrieve samples from deep waters inside a sealed container, pressure
359 variations are likely to occur, and depend on several phenomena, which have long been
360 documented (MacDonald, 1975, Yayanos, 1978). During the ascent of the pressure-retaining
361 device (PRD) towards the surface, the pressure prevailing in the surrounding environment
362 decreases. Provided there is no leakage due to seal failure, the hydrostatic pressure inside the
363 PRD drops because of the initial movement and compression of elastomer seals which ensure
364 sealing of the PRD, and because of the following elastic expansion of the metallic PRD walls
365 (see ANNEX 1 for more detail). Both phenomena cause an increase of the internal volume of
366 the PRD, and consequently an expansion of the seawater trapped inside the PRD, resulting in
367 significant pressure drops. On the contrary, a temperature rise expected when reaching warm
368 surface waters may result in increased pressure inside the PRD. Although this may
369 compensate for pressure losses described above, this phenomenon should be avoided as much
370 as possible, on physiological grounds (MacDonald, 1975, Childress et al, 1978)

371

372 The pressure loss due to seals depends mainly on the volume variation due to the positioning
373 of seals within their respective grooves, and at lesser extent on deformation of seals while
374 being compressed, once the final sealing position is reached. In our study, we estimated that
375 the movement of seals would result in a pressure loss of ~1 to 1.5 MPa at 5°C, whatever
376 working pressure was envisaged. The pressure loss due to the expansion of the PRD metallic
377 walls was experimentally estimated at the laboratory, in isothermal conditions, and predicted

378 to be about 26% of working pressure, at 4°C (see ANNEX 1). From there, accounting for seal
379 movement, it would appear that the PERISCOP would theoretically retain about 70 % of *in*
380 *situ* pressure when operated at 36 MPa (3600 m depth), and about 63 % when operated at 12
381 MPa (1200 m depth), in isothermal conditions.

382

383 Figures 8 and 11 allow to observe the pressure-retaining performance of the PRD alone, when
384 no pressure compensation system is employed. The data are in relatively good agreement with
385 predictions, minimum pressure values reaching respectively 65.2 and 72.4 % retention of *in*
386 *situ* pressure, measured for deployments at 1250 and 3620 m depths respectively. In the first
387 seconds of the PRD ascent, pressure drops at the same rate as the surroundings, as if the PRD
388 was still in an open position. In fact, this behaviour likely corresponds to the seals moving
389 across the grooves inside which they are housed, at almost equal pressures on both sides of
390 seals, until they are blocked against the groove edge. It is only then that they reach an
391 effective sealing position, allowing a difference of pressures to build up. Later during the
392 ascent, the PRD walls gradually expand proportionally to the decrease of surrounding
393 pressure, which stabilizes when the PRD reaches the surface. From then on, the pressure
394 inside the PRD may change because of thermal conditions. In the case of an almost isothermal
395 water column (Figure 8, about + 2°C gradient) the pressure increase is small (~ + 1.5 % after
396 almost 40 min in surface waters), while it is more important (~ + 7 % after only 16 min in
397 surface waters) when surface waters are warm (Figure 11, about + 20°C gradient).

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402 **Analysis of pressure history inside the PRD during recovery, using pressure**
403 **compensation**

404 Comparison of deployments with or without pressure compensation can be done for similar
405 thermal conditions (non-isothermal water column, red points in Figure 12, and Table S2). The
406 best pressure-retaining performance is obtained when using the active compensator (about 98
407 % of *in situ* pressure, Figure 12). The use of passive compensation (oil- or water-filled
408 modes) leads to a lower minimum pressure-retaining performance (91 % and 84 %
409 respectively). Both methods (passive and active) however improve the pressure-retaining
410 performance of the PERISCOP PRD, which retains a minimum pressure of about 72 % of *in*
411 *situ* pressure, when no compensation is used. Additionally, the active system seems less
412 sensitive to the impact of warming on pressure, since it displays the smallest pressure increase
413 after reaching warm surface waters. The reference container inside the active compensator is
414 probably less subject to thermal variation and subsequent pressure increase, and therefore
415 drives the release of seawater from the PRD as gradual heating of the latter occurs. On the
416 contrary, the passive compensator is more sensitive to thermal conditions encountered in
417 warm surface waters, particularly when using oil-filled compensation, as discussed below.

418

419 Reliability is an important issue when considering repeated use of pressure-retaining
420 sampling. The initial compensation system (active) presented by [Shillito et al \(2008\)](#)
421 gradually gained in reliability, through improvements described above. Indeed, the integration
422 of the differential valves inside the compensator's main container was a progress: their
423 functioning (opening and/or closing) became more reproducible, probably because they were
424 then submitted to a constant external pressure (atmospheric), and also to more stable thermal
425 conditions, throughout the sampling process. However, such a compensator still involves
426 more complex connections (compare figures 2 and 3), and requires more operations than the

427 passive system (both *in situ* and on board the ship). The active system therefore requires more
428 attention (maintenance) in order to reproduce regular functioning. On the contrary, the passive
429 system may be less efficient in terms of pressure retention, but its more simple design
430 increases reliability and ease of operation.

431

432 **The use of oil for passive pressure compensation**

433 We made a few deployments using an oil-filled passive compensator, which performs better
434 than its water-filled analog in similar conditions (non-isothermal water column), regarding the
435 minimum pressure (see red points in Figure 12). This improvement is due to the higher
436 compressibility coefficient of silicon oil, with respect to water (Kiyama et al, 1953, Fine and
437 Millero, 1973). However, this oil-filled mode seems more sensitive to heating and subsequent
438 pressure rise upon reaching warm surface waters (Figure 12). The pressure increase due to
439 overheating of a trapped liquid is dependant on the ratio between the thermal expansion
440 coefficient of a given liquid, and its isothermal compressibility coefficient (Ramirez et al,
441 2010). The thermal expansion coefficient of commercially-available silicon oils is typically 5
442 to 15-fold that of water, while their compressibility coefficients are about two to four-fold that
443 of water (Kiyama et al, 1953, Fine and Millero, 1973, see also Ramirez et al, 2010).
444 Consequently, the ratio between the thermal expansion and the isothermal compressibility
445 coefficients is likely to be more important for silicon oil than for water, thus leading to
446 increased overpressure upon warming in surface waters. Therefore, although the use of oil as
447 a compensating fluid is promising in terms of pressure compensation, the thermal insulation
448 of the passive compensator should be improved in the future, in order to limit the potential for
449 overpressure. Ultimately, provided efficient thermal insulation is achieved, this tendency for
450 overpressure could be used in the future as a means of compensating pressure losses in the

451 PRD through controlled heating of an oil-filled passive compensator. Our group is currently
452 exploring this possibility.

453

454 **Comparison with other PRDs and their conventional (gas-loaded) compensation systems**

455 Most other PRDs used in the field of geology, microbiology, or studies of larger fauna,
456 involve the use of gaseous-nitrogen-loaded pressure accumulators, in order to compensate for
457 pressure losses during recovery. Such systems are efficient, and help maintain pressures close
458 to *in situ* values. The general principle is explained in [MacDonald \(1975\)](#) and [Yayanos
459 \(1978\)](#). Schematically, the volume increase due to seal movement and container expansion
460 leads to a loss in pressure, which is inversely proportional to the enclosed volume V , and to
461 the isothermal compressibility coefficient B of the enclosed fluid ($(\delta P / \delta V)_T = - 1 / (B \times V)$).
462 The principle is therefore to increase the volume and compressibility of the enclosed medium,
463 by adding a highly compressible gaseous component to the system. Ideally, the gas quantity
464 should be maximised at operating pressure (the pressure encountered at sampling depth),
465 implying an initial loading pressure slightly inferior to the operation pressure (i.e. about 90
466 %). Using a gas accumulator, [Garel et al \(2019\)](#) maintained +/- 5% of *in situ* pressures in
467 microbial samplings from 3000 m depth, as evidenced by constant recording of pressure
468 history throughout the sampling process. Workers in the same group had earlier shown that
469 their compensation system could improve by about 25% the performance of their samplers
470 ([Bianchi et al, 1999](#)). Using a much larger fish sampler (90 l), [Drazen et al \(2005\)](#) retained
471 about 93-95 % of *in situ* pressure, following recoveries from 1350-1500 m depths. Many
472 other examples have been recently reviewed by [MacDonald \(2021\)](#). Overall, in terms of
473 pressure-retaining performance, while the active compensator presented in this work (94%
474 pressure retention at the lowest) compares well with nitrogen accumulators mentioned above,
475 the passive compensator seems somewhat less efficient (around 80% at the lowest, for water-

476 filled compensator). However, unlike the passive compensator, the gaseous accumulators and
477 our active compensator both need to be pressurised prior to deployment. Moreover, in the
478 case of gas-loaded accumulators, such a pressurisation may prove unpractical to achieve when
479 targeting loading pressures above 20-30 MPa, i.e. pressures above those readily available in
480 commercially-used nitrogen cylinders. Additionally, it should be recalled that because of their
481 elevated internal energy (compared to pressurised liquids), pressurised gases may present
482 more safety issues for users.

483

484 The passive compensator, in its water-filled version, involves the elasticity of its internal
485 container (Figure 3), and acts as a loaded mechanical “spring” which sends seawater back to
486 the PRD as the latter expands. When compressible silicon oil is used, a function similar to that
487 of a gas accumulator is added, through the introduction of a fluid more compressible than
488 water. While silicon oil remains significantly less compressible than nitrogen gas, even at
489 high pressures (about 10 to 3 fold lower, between 10 and 110 MPa, [Ramirez et al 2010](#),
490 [Kiyama et al 1953](#), [Priede, 2018](#)), it nevertheless remains a significant contribution to the
491 decrease of the $(\delta P / \delta V)_T$ term mentioned above. In addition, the large volume involved (4 L
492 for the compensator internal container, while the PRD has a volume of 6 L, resulting in a total
493 10 L volume) also participates to reducing this term.

494

495 **Pressurised recovery at hadal depth**

496 The PERISCOP PRD and its compensator systems were not designed for hadal depths of
497 operation (beyond 6000 m), the deepest recoveries occurred from 3650 m depths on the Mid-
498 Atlantic Ridge. Designing a new PRD based on the PERISCOP, but upgraded at higher
499 working pressures, is a current challenge, because large volumes (PERISCOP has a 10 cm
500 diameter opening, for more than 6 L volume) and realistic PRD wall thicknesses (in order to

501 respect maximum weight constraints) will necessarily require that pressure losses are
502 compensated for. It is likely that an active system may prove difficult to design, especially if
503 loading pressures above 110 MPa are required (in the high-pressure reservoir, see methods
504 section). Alternatively, we believe that the general principle of a passive liquid-filled
505 compensator may prove quite efficient for hadal use. Recently, [Wang et al \(2022\)](#) maintained
506 about 80-85% of *in situ* pressure during sampling of amphipods in a relatively small PRD
507 (about 0.7 L volume), at the bottom of the Mariana trench (10900 m depth). They used a
508 hybrid compensation system, by firstly using a liquid injector to push the seals towards their
509 sealing position, and secondly using a nitrogen accumulator pre-loaded at 30 MPa, a pressure
510 which can be reasonably reached by using commercially available pressurised nitrogen
511 bottles. Increasing the loading gas pressure would probably improve the performance of their
512 system, but would prove unpractical, if not hazardous. Moreover, the pressure-retaining
513 performance obtained by these authors, although lower than theoretically expected, could
514 prove sufficient to insure good physiological conditions of the samples (although their trials
515 resulted in 100% mortality of samples, this was likely due to overheating in surface waters,
516 rather than insufficient pressure compensation (see [Yayanos, 1978, 2009](#))). A liquid-filled
517 passive compensator such as presented here would not require pre-loading. Of course, the
518 design of such an instrument would require that sufficient volume and elasticity of its internal
519 container is involved, and that buckling issues are accounted for, regarding its external
520 container. We advocate that future samplers will tend to increase in volume, in order to
521 envisage broader sampling capacities, and propose that passive liquid compensators will
522 prove more appropriate for such uses. As discussed above, and in the following lines, thermal
523 insulation remains a major issue in deep-sea sampling of live fauna, but also for the stability
524 of a liquid-filled compensator, should liquids such as silicon oil be employed (Figure 12).

525

526 **Is full pressure compensation necessary ? What about thermal insulation ?**

527 The PERISCOP PRD has mostly been used at depths greater than 1000 m, and while some of
528 the deployments occurred in Mediterranean waters (technical deployments), most faunal
529 samplings were achieved at hydrothermal vent sites (Mid-Atlantic Ridge, and East Pacific
530 Rise), mainly at depths between 1700 and 3600 m. Several thousand organisms were thus
531 recovered, representing various taxa. Before its reliability was improved, it is the active
532 compensator that was responsible for most misfunctions. However, even when pressure-
533 retaining performance was as low as 65 % (either no compensation or compensation partial
534 misfunction), most organisms were recovered in apparent very good physiological conditions,
535 as suggested by their generally active behaviour responses (see biological studies mentioned
536 in results). This was not always the case for fauna recovered without pressure: while most
537 vent organisms recovered from 1700 m depths (Lucky Strike vent field) possibly survived
538 several weeks, if not months, at atmospheric pressure (Shillito et al, 2015, 2020), deeper-
539 originating fauna were more impacted. *Rimicaris exoculata* vent shrimps from depths around
540 2300 m (Rainbow vent field) still appeared fairly active upon recovery, but sometimes
541 displayed uncoordinated movements, and although they were generally responsive to
542 mechanical stimulation, their activity would gradually fade in a matter of one to two days. On
543 the contrary, shrimps of the same species, but originating from deeper sites (beyond 3000 m
544 depth), were usually recovered in a motionless state, appearing moribund, with weak and
545 uncoordinated responses to stimulation. For these organisms, re-pressurisation in dedicated
546 laboratory pressure aquaria often failed to restore normal behaviour. Low tolerance to full
547 decompression has been reported for other species (Treude et al, 2002, Ravaux et al, 2013),
548 and Pradillon (2012) had already mentioned that 2000 m depth could be a physiological
549 obstacle for survival to decompression. Other authors have also discussed this point (Brown
550 and Thatje, 2014).

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Still, many studies involving isobaric sampling (including ours) report on the fact that full pressure recovery is not always reached, and that recovery at partial pressure is tolerated by many taxa (including fish, [Koyama et al, 2002](#)), even among the deepest occurring ones ([Peoples et al, 2019](#), [Yayanos, 1981, 2009](#)). Additionally, highly performant compensation systems such as the active compensator we present are an example of the increasing complexity (in both design and operation) required to obtain full pressure recovery, compared to the passive compensator. The latter is of much more practical use, significantly lower cost, and its simplicity implies increased reliability on the long term, in addition to the absence of pressurised gas, which significantly increases the safety of pressure-manipulating procedures. Such simple cheap and safe equipments could then become widespread among the community of deep-sea scientists, including for use in hadal environments.

In future developments of PRDs, recovery in isothermal conditions should not be overlooked. The vent fauna reported in this work are mostly quite eurythermal (see [Bates et al, 2010](#), [Van Dover, 2019](#)), and therefore tolerate temperature increases which occur when the PRD reaches surface waters (Figure 9). Such tolerance is unlikely to occur with stenothermal fauna originating from colder environments, since thermal challenge has long been identified as a major cause of trauma during deep-sea sampling ([Childress et al, 1978](#), [Wilson and Smith, 1985](#), [Wang et al, 2022](#)). This underlines the necessity of improving thermal insulation for our equipment. We are in test phase for additional thermal insulation of the main globe-valve of our PRD, which is a major thermal bridge with the surrounding environment.

574 **CONCLUSIONS**

575 We here presented an update of the PERISCOP pressure retaining device (PRD), which was
576 improved since its initial design, and which operation is now close to routine. The data and
577 practical experience we acquired at sea, lead us to propose the following conclusions, which
578 we hope may help other investigators in the design and operation of future PRDs.

579 1) Pressure and temperature history inside the PRD throughout the sampling cycle should be
580 recorded.

581 2) Active sampling with a submersible (as opposed to trapping based on attraction to bait)
582 allows to target species, and therefore significantly widens scientific perspectives.

583 3) Full pressure retention throughout PRD operation is not always reached, however pressure
584 retention as low as 65% may suffice for recovering live samples. This would allow for further
585 physiology experiments in dedicated aquaria to take place, after restoring full *in situ* pressure.

586 4) The use of gas-free pressure compensators should be encouraged, because increased safety
587 leads to more practical and wide use by biologists, and because gas-loaded systems may
588 prove of limited efficiency as sampling depth increases beyond 3000 m depths.

589 5) Passive compensators such as presented here are safe and easy to use, and relatively cheap.
590 Moreover, we advocate that they may be effective tools at hadal depths. The more complex
591 (but more efficient) active compensator may still be envisaged in particular cases of
592 pronounced intolerance to decompression.

593 6) Finally, thermal insulation should not be overlooked, in many cases surface waters are
594 much warmer than deep environments, and this may lead to serious trauma, even on pressure-
595 recovered samples.

596 Pioneering works on the effects of pressure on living organisms started way back in the 19th
597 and 20th centuries ([Draper and Edwards, 1932](#), [Sébert, 2002](#) for review), but physiological
598 studies on live deep-sea animals in laboratory conditions are still in their infancy. One reason

599 for this is that sampling in the deep-sea and further maintaining *in situ* pressure conditions at
600 the laboratory, are challenging issues, on both technical and financial grounds. However,
601 because the biology of deep fauna remains largely unknown, and because the field of
602 investigations is therefore so wide, we believe that undertaking such studies is a very
603 rewarding process, and an urgent matter in the face of growing human impacts on the Deep
604 Biosphere. A main objective of the work presented herein, is to incite a wide community of
605 marine biologists to realize that such studies are feasible, and necessary.

606

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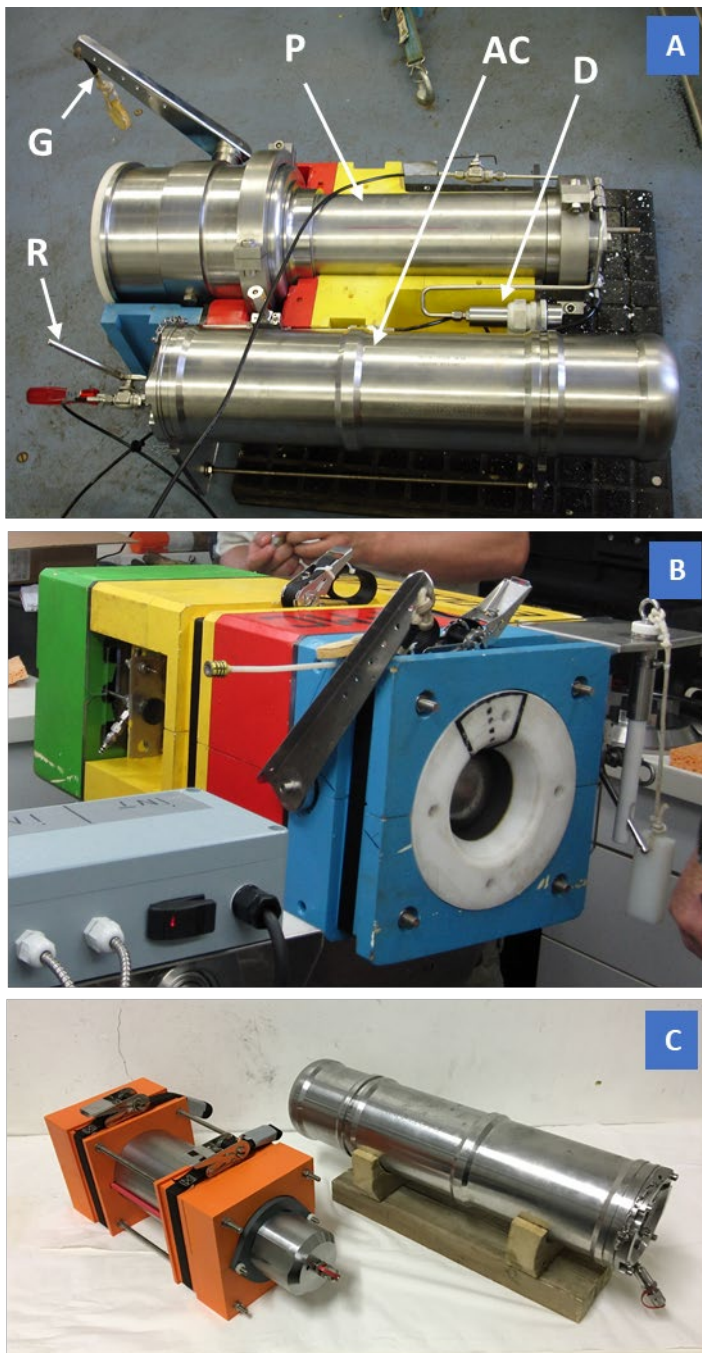
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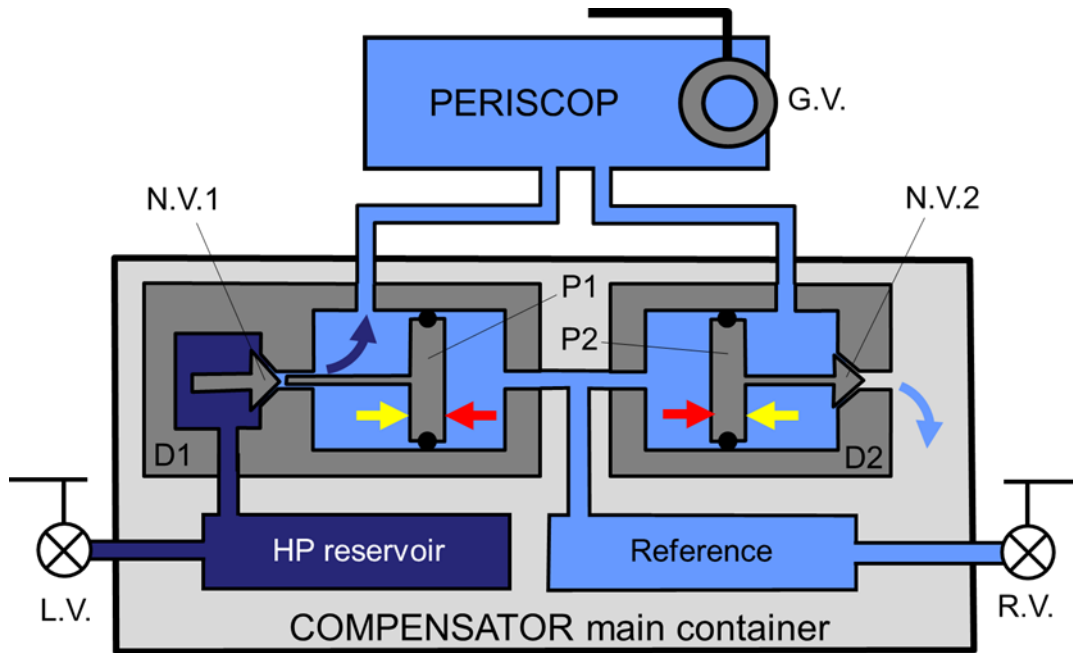
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825 **Figure 1 : The PRD PERISCOP**

826 A, B - the PRD (P) is positioned in syntactic foam casings (here coloured in blue, red, yellow
 827 and green, fully assembled in B), with the active compensator (AC) alongside. A pressure data
 828 logger (D) is connected to the PRD. G and R are the handles of the two valves (GV and RV in
 829 figure 2) which need to be manipulated *in situ* before samples can be recovered.

830 C - the two types of compensators alternatively used in this work : passive (left) or active
 831 (right). Full length of the compensators are approximately 55 and 65 cm, and a diameter of 13
 832 and 15 cm respectively.

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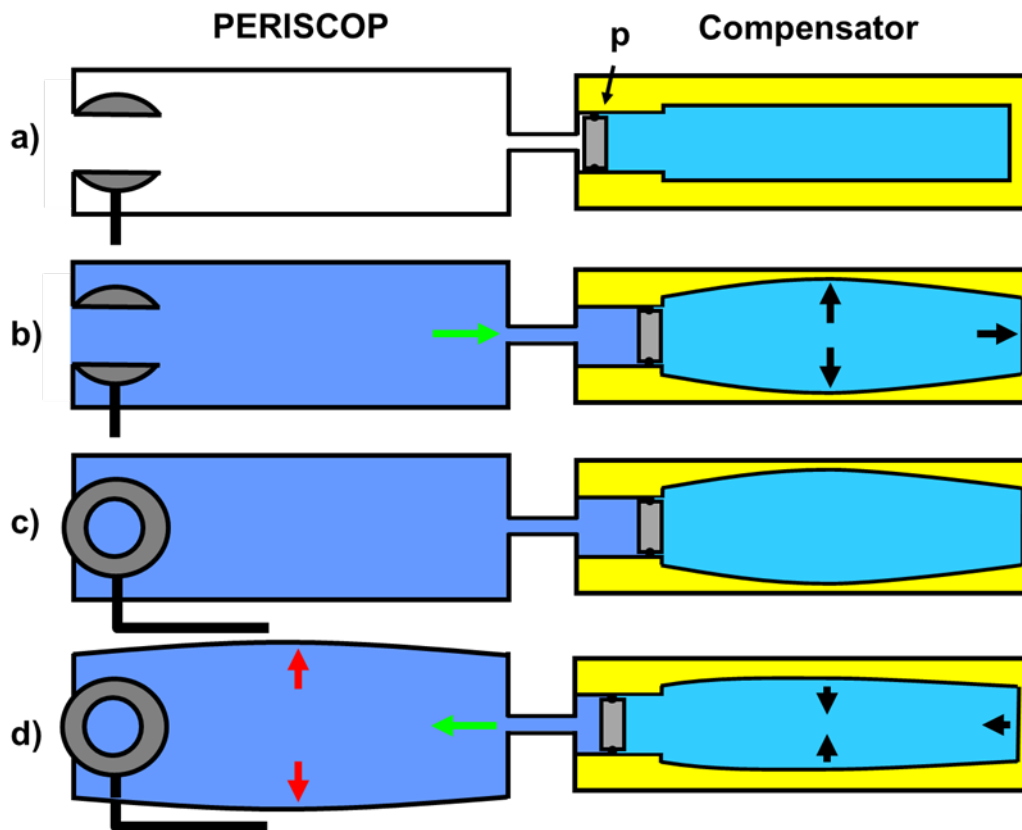


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Figure 2 : Principle for active pressure compensation.

836 The PRD (PERISCOP) is hydraulically connected to the compensator, by means of two
837 differential valves (also called « dome-valves ») D1 and D2 (dark grey). The compensator is
838 composed of three containers, and two differential valves. The inside of the main container
839 (light grey) always remains at atmospheric pressure. Inside this container, one high-pressure
840 container (HP reservoir) is connected to D1 and to a loading valve (LV), the other one
841 (Reference) is connected to both D1 and D2, and another valve (RV) allows connection to the
842 surrounding environment.
843

844 Prior to sampling, HP reservoir is loaded with seawater at about 45 MPa pressure, by using
845 LV, which further remains closed throughout the sampling operation. Upon mooring, the PRD
846 and the reference container remain open, thus remaining at identical pressure, that of the
847 surrounding environment. In situ, and once samples have been stored inside the PRD, its main
848 globe-valve (GV) is closed by the submersible's hydraulic arm, before closing RV. From then
849 on, during recovery (ascent in the water column), the reference container is not submitted to
850 variations in surrounding pressure (because it is stored inside the main container, at
851 atmospheric pressure). Therefore it maintains a stable internal pressure. During recovery, the
852 pressure in the water column surrounding PRD decreases, thereby allowing expansion of the
853 PRD walls, and consequent internal pressure loss. Therefore, the pressure inside the reference
854 is higher (red arrows), and consequently actuates the piston (P1) and the needle-valve (NV1)
855 of D1, resulting in extra seawater (dark blue arrow) injected from the HP reservoir towards
856 the PRD, until pressures in PRD and Reference equilibrate. Conversely, if the pressure in
857 PRD increases above the reference pressure (yellow arrows), the piston (P2) and needle-valve
858 (NV2) of D2 are actuated, thereby releasing seawater inside the Compensator main container
859 (light blue arrow), until pressures in PRD and Reference equilibrate. Note that, for clarity, the
860 size of D1 and D2 is exaggerated on this scheme, however the water volumes enclosed in
861 these valves is negligible compared to the volumes of PERISCOP and Reference containers.
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Figure 3 : Principle for passive pressure compensation.

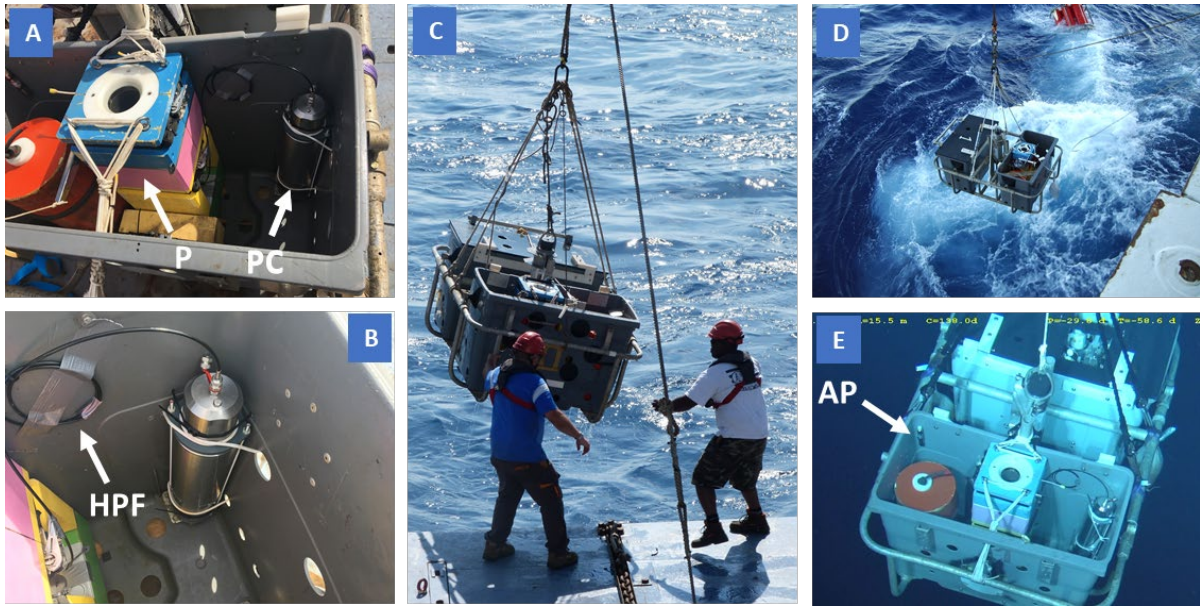
The PRD (PERISCOP) is hydraulically connected to the compensator, by means of a piston (p). The compensator consists of a double-wall container filled with water or oil (light blue). The interspace (yellow) between container walls remains at atmospheric pressure throughout the entire sampling process.

a) Prior to mooring at depth, all compartments are at atmospheric pressure.

b) At depth, seawater (dark blue) at *in situ* pressure forces the piston back in the compensator (green arrow), thereby expanding the inner container (black arrows).

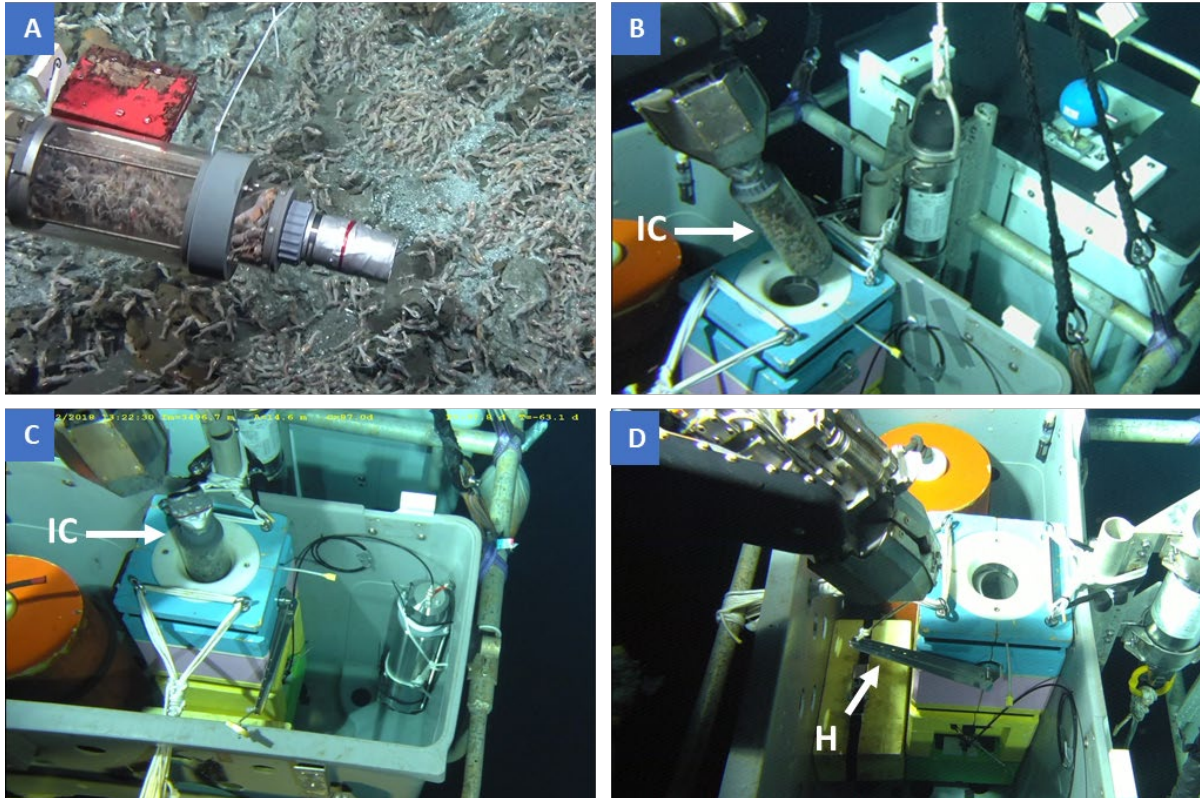
c) At depth, the globe-valve of the PRD is closed.

d) Upon recovery, the surrounding pressure decreases, thereby allowing expansion of the PRD walls (red arrows), and consequent internal pressure loss, which is partially compensated by the compensator piston forcing seawater back inside the PRD (green arrow), due to the contraction of the inner container (black arrows).



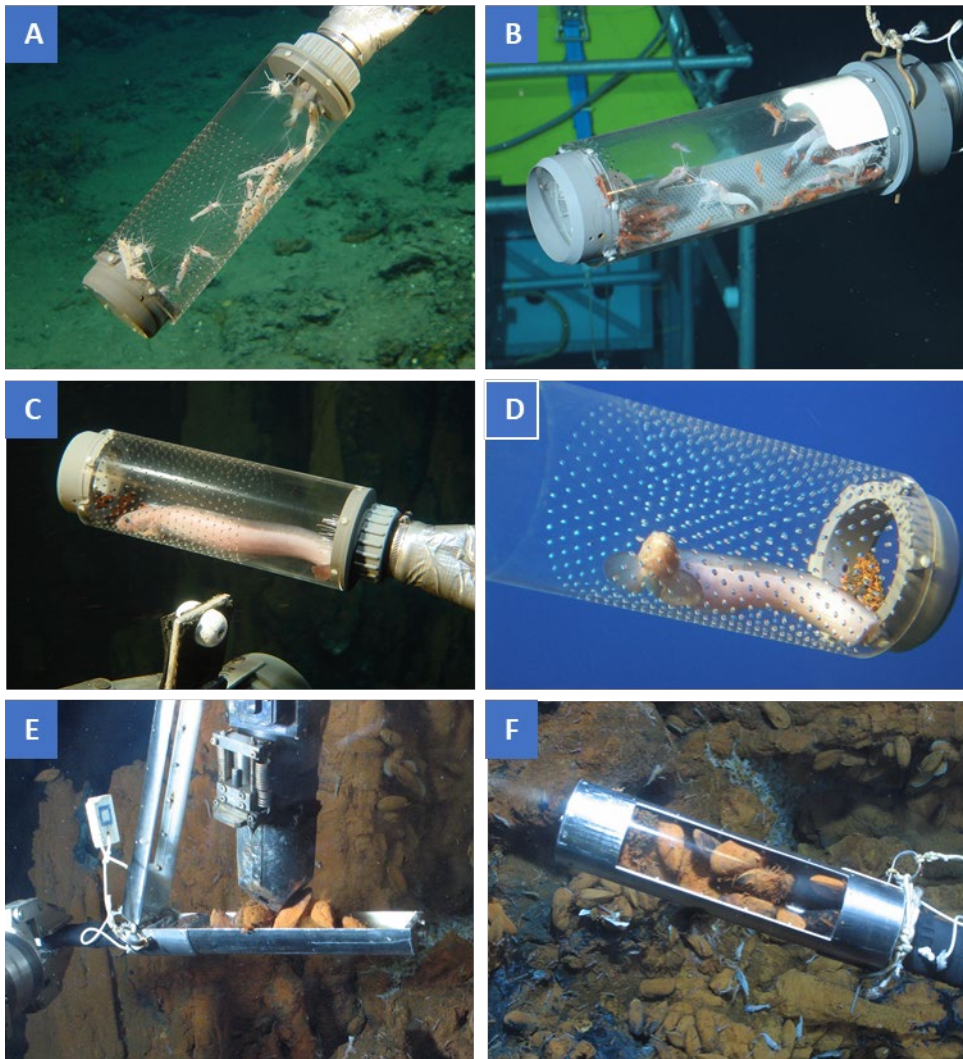
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Figure 4 : Operation of the PERISCOP PRD, using a shuttle device. The shuttle presented here belongs to the Ifremer Nautilie submersible team.
 A : PERISCOP (P) and its passive compensator (PC) is fixed inside one of the 2 containers of the shuttle device.
 B : Close-up view of the passive compensator, which is linked to the PRD through a high-pressure flexible hose (HPF).
 C and D : mooring operation of the shuttle.
 E : The shuttle with the PERISCOP PRD at depth (photographic credit Ifremer). An autonomous pressure/temperature probe (AP) is mounted on the shuttle's container.



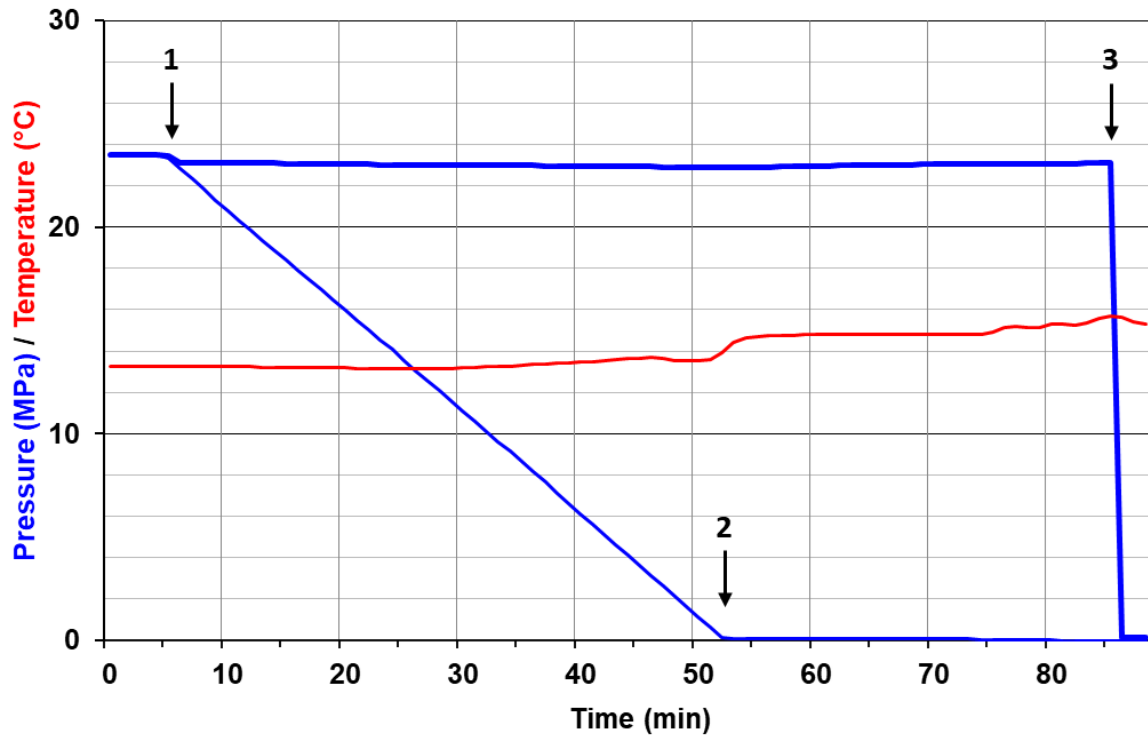
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Figure 5 : *In situ* sampling with the deep-sea manned submersible Nautilie at the TAG hydrothermal vent field, during the Bicos2 cruise (photographic credits Ifremer).
 A : by connecting the submersible's suction device to the sampling cell (see also Shillito et al, 2008), the Nautilie operators may aim for specific fauna, here vent shrimps (*Rimicaris exoculata*).
 B and C : The sampling cell is disassembled, and its inner cylinder (IC) is held by the submersible's hydraulic arm, for introduction inside the PERISCOP PRD.
 D : The hydraulic arm closes the PERISCOP PRD by pulling the handle (H) of the quarter-turn globe-valve.



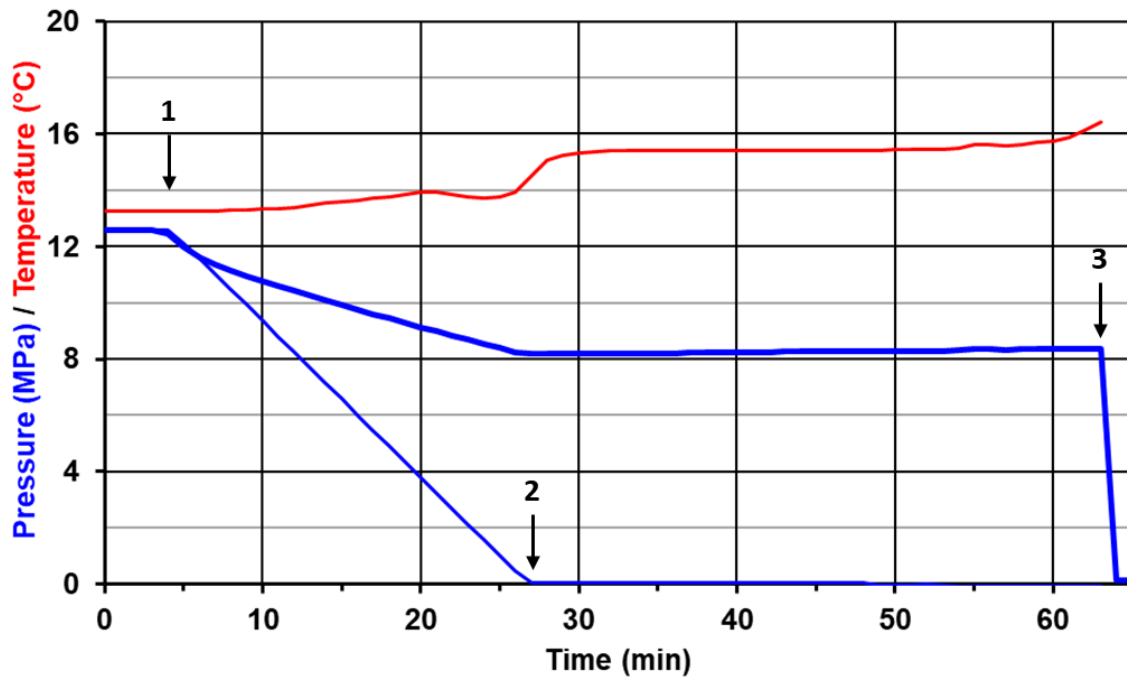
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Figure 6 : Various animal samples collected with the PERISCOP PRD, on the MAR Rainbow vent field, at a depth of 2300 m (photographic credits Ifremer).
 A and B : vent shrimps, *Rimicaris exoculata* and *Mirocaris fortunata*.
 C and D : zoarcid fishes *Pachycara saldanhai*.
 E and F : mussels, *Bathymodiolus azoricus*, collected with the Croco sampling cell.



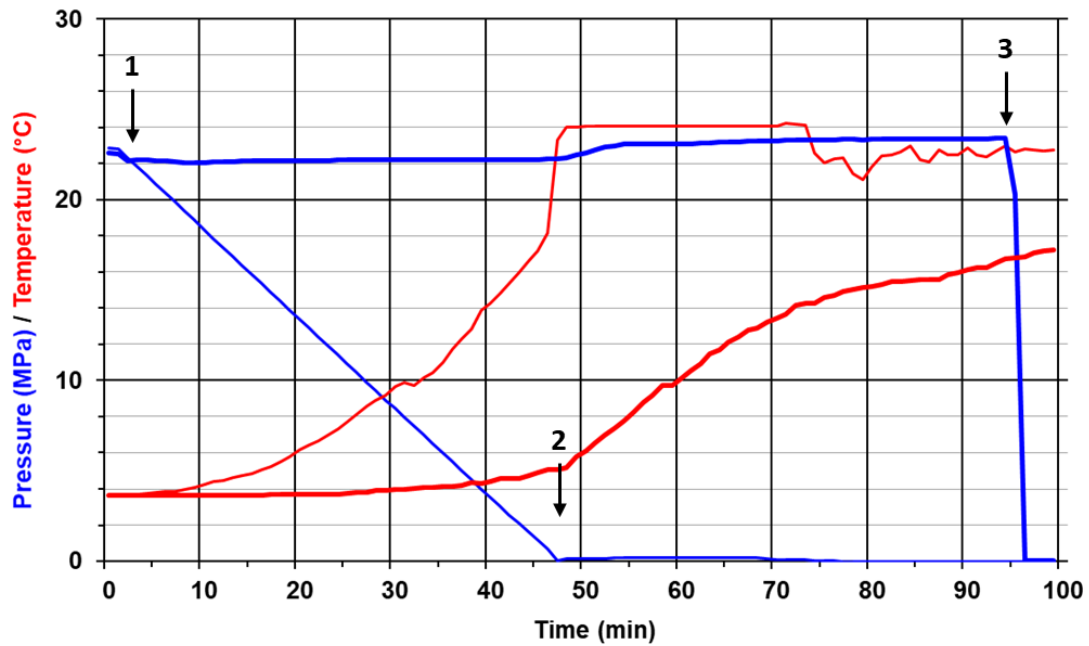
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Figure 7 : Pressure (MPa) and Temperature (°C) monitoring during the recovery of the PERISCOP from about 2350 m depth in the Mediterranean Sea, using the active compensation system, as a function of time. Blue lines account for Pressure, either inside the PRD (bold line), or in the surrounding water column (thin line). The red line accounts for Temperature in the surrounding water column. Arrows indicate from 1 to 3 respectively : beginning of shuttle ascent, shuttle reaching the surface, releasing pressure of the PRD on ship deck.



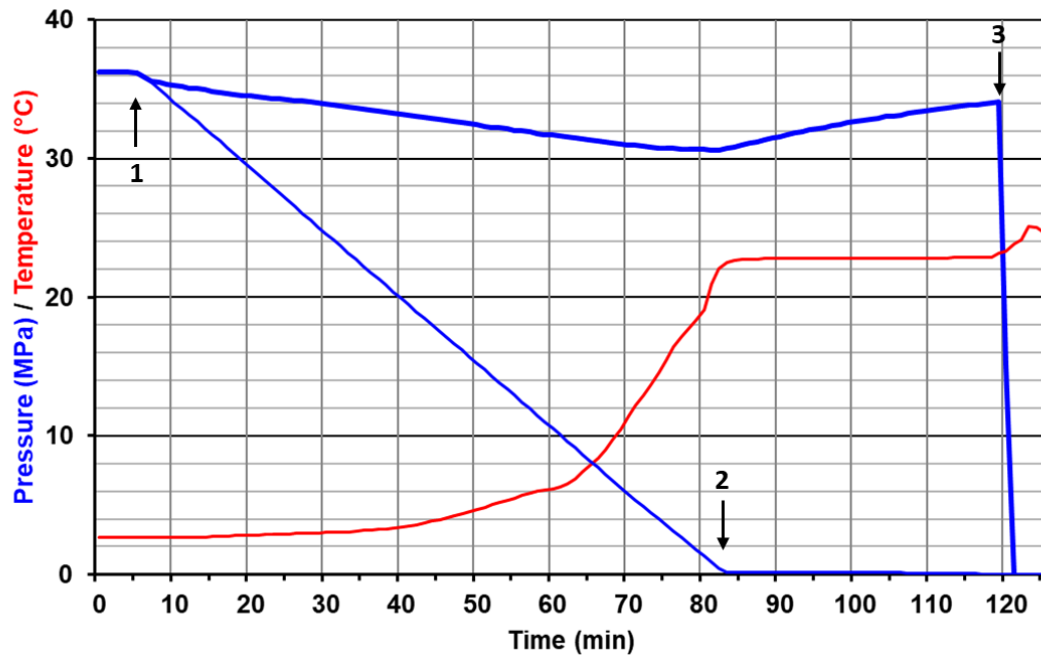
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Figure 8 : Pressure (MPa) and Temperature (°C) monitoring during the recovery of the PERISCOP from about 1250 m depth in the Mediterranean Sea, with no compensation, as a function of time. Blue lines account for Pressure, either inside the PRD (bold line), or in the surrounding water column (thin line). The red line accounts for Temperature in the surrounding water column. Arrows indicate from 1 to 3 respectively : beginning of shuttle ascent, shuttle reaching the surface, releasing pressure of the PRD on ship deck.



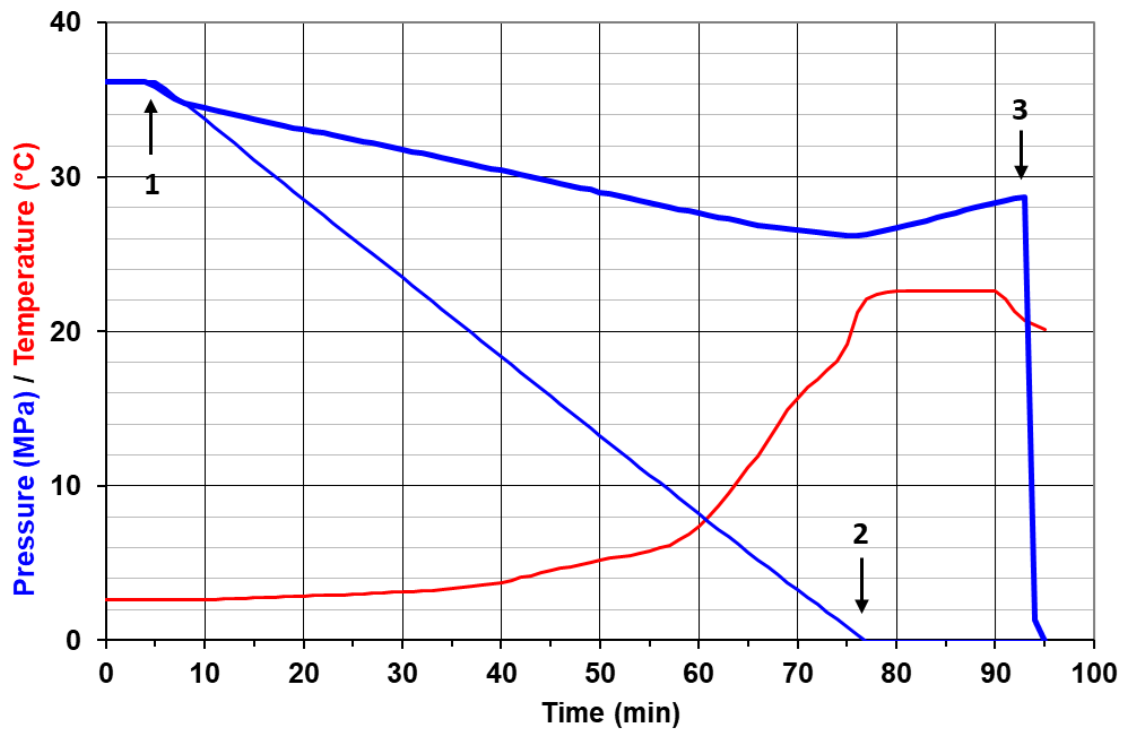
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Figure 9 : Pressure (MPa) and Temperature (°C) monitoring during the recovery of the PERISCOP from about 2300 m depth at the MAR Rainbow site, using the active compensation system, as a function of time. Blue lines account for Pressure, either inside the PRD (bold line), or in the surrounding water column (thin line). Red lines account for Temperature, either inside the PRD (bold line), or in the surrounding water column (thin line). Accordingly, thick lines are recordings of pressure and temperature conditions inside the PRD, while thin lines correspond to recordings of data loggers mounted on the shuttle device which carries the PRD. Arrows indicate from 1 to 3 respectively: beginning of shuttle ascent, shuttle reaching the surface, releasing pressure of the PRD on ship deck.



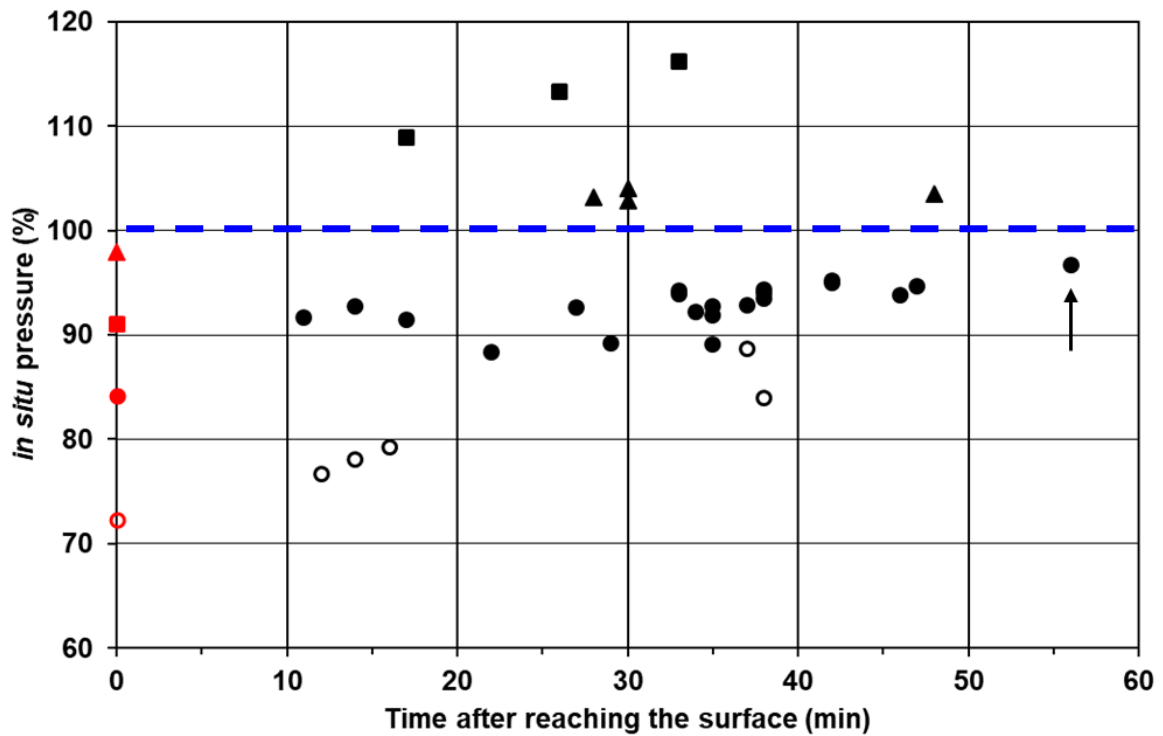
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Figure 10 : Pressure (MPa) and Temperature (°C) monitoring during the recovery of the PERISCOP from about 3600 m depth at the MAR TAG site, using the passive compensation system, as a function of time. Blue lines account for Pressure, either inside the PRD (bold line), or in the surrounding water column. The Red line accounts for Temperature in the surrounding water column. Arrows indicate from 1 to 3 respectively : beginning of shuttle ascent, shuttle reaching the surface, releasing pressure of the PRD on ship deck.



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Figure 11 : Pressure (MPa) and Temperature (°C) monitoring during the recovery of the PERISCOP from about 3600 m depth at the MAR TAG site, with no compensation, as a function of time. Blue lines account for Pressure (Mpa), either inside the PRD (bold line), or in the surrounding water column (thin line). The Red line accounts for Temperature in the surrounding water column. Arrows indicate from 1 to 3 respectively : beginning of shuttle ascent, shuttle reaching the surface, shuttle being hauled out of the water, releasing pressure of the PRD on ship deck.



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Figure 12 : Percentage of pressure retention (% of *in situ* pressure) just before releasing the pressure in the PRD, as a function of time spent in warm surface waters, for several pressurised recoveries. For example, the sampling with an arrow had a pressure of 97% of *in situ* pressure, just before pressure release and opening of the PRD on ship deck, 56 minutes after reaching the surface. All the data correspond to MAR vent fields, depths ranging from 3600 to 2300 m, with *in situ* water temperature ranging from 2.5 to 4.5 °C, and surface waters ranging from 23 to 26 °C. Empty circles : no compensation. Plain circles : water-filled passive compensation. Plain squares : oil-filled passive compensation. Plain triangles : active compensation. Red points on the vertical axis represent the mean of minimum pressure retention, which occurs upon reaching the surface (Time = 0 min).

cruise	trial #	sampling depth (m)	pressure compensation	minimum pressure (%)	final pressure (%)
EssNaut 2021	P3	1200	none	63,5	60,1
	P5	1250		65,2	66,60
	P1	2700	passive (oil)	84,1	86,7
	P2	2700		84,4	86,80
	P4	1250		83,3	89,08
EssNaut 2016	P1	2430	active	94,5	94,90
	P2	2420		95,3	96,24
	P3	1170		94,3	95,90
	P4	1180		95,3	96,26
	P5	1180		94,1	94,47
	P6	2350		97,6	98,30
	P7	2410		93,8	93,94

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Table S1 : Data for several technical deployments of the PERISCOP PRD, in an isothermal column (Mediterranean Sea in winter, less than 2°C gradient between sampling site and surface water, EssNaut cruises). Pressures are given as percentage of *in situ* pressure. The deployments were undertaken either with no compensation, or oil-filled passive compensation, or active compensation.

cruise	trial #	site	sampling depth (m)	pressure compensation	minimum pressure (%)	final pressure (%)	time spent at surface (min)	comment
MomarDream	D1	Rainbow	2300	active	98,2	102,9	30	
	D2	Rainbow	2290		98,1	104,1	30	
	D4	Rainbow	2210		97,8	103,2	28	
	D5	Rainbow	2220		97,6	103,5	48	
	D3	Rainbow	2250		73,0	89,1	37	
Bicose 1	P13	TAG	3610	none	72,3	83,9	38	
	P14	TAG	3640		72,0	78,0	14	
	P15	TAG	3640		71,7	76,6	12	
	P16	TAG	3620		72,4	79,3	16	
Bicose 2	P1	TAG	3600	passive (water)	84,2	93,5	38	
	P2	TAG	3570		84,5	94,2	33	
	P3	TAG	3620		83,3	91,9	35	
	P4	TAG	3590		83,7	93,8	46	
	P5	TAG	3580		84,9	96,7	56	
	P6	TAG	3630		84,5	94,0	38	
	P7	TAG	3600		84,6	92,6	27	
	P8	TAG	3580		83,9	94,4	38	
	P9	TAG	3570		85,0	93,9	33	
	P10	TAG	3560		85,3	94,9	42	
	P11	Snake Pit	3390		84,6	95,2	42	
	P12	Snake Pit	3430		no data	no data	no data	probe failure
	P13	Snake Pit	3450		83,1	94,6	47	
	P14	Snake Pit	3440		83,1	92,2	34	
	P15	Snake Pit	3380		84,2	92,7	35	
	P16	Snake Pit	3390		84,1	92,9	37	
	P17	Snake Pit	3530		80,9	89,1	35	
	P18	Snake Pit	3480		81,4	89,2	29	
Transect	P6	Broken Spur	3100	passive (water)	83,5	88,3	22	
	P7	Broken Spur	3120		85,6	91,4	17	
	P8V	Lost City	760		71	89,2	25	leak during ascent
	P9	Broken Spur	3130		86,6	92,7	14	
	P10	Broken Spur	3090		86,4	91,7	11	
	P11	Broken Spur	3120		84,4	91,40	17	
	P1	Rainbow	2250	passive (oil)	91,1	108,9	17	
	P2V	Rainbow	2360		91,3	113,3	26	
	P3	Rainbow	2330		90,7	116,2	33	
	P4	Rainbow	2350		88,3	100	17	leak at surface
	P5V	Rainbow	2350		85,4	90,5	39	leak during ascent

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Table S2 : Data for several deployments of the PERISCOP PRD, in a non-isothermal water column (MAR sites with similar temperature gradients of about 20°C between sampling site and surface water). Pressures are given as percentage of *in situ* pressure. The deployments were undertaken either with no compensation, or water-filled passive compensation, or oil-filled passive compensation.

Mean values (+/- SD) for minimum pressure (upon reaching the surface) are 72.3 +/- 0.5 for no compensation (n = 5), 84.2 +/-1.3 for water-filled compensation (n = 22), 91.0 +/- 0.3 for oil-filled compensation (n = 3), and 97.9 +/-0.3 for active compensation (n = 4). P4, P5V and P8V deployments were discarded because of leaks during ascent, or at the surface.

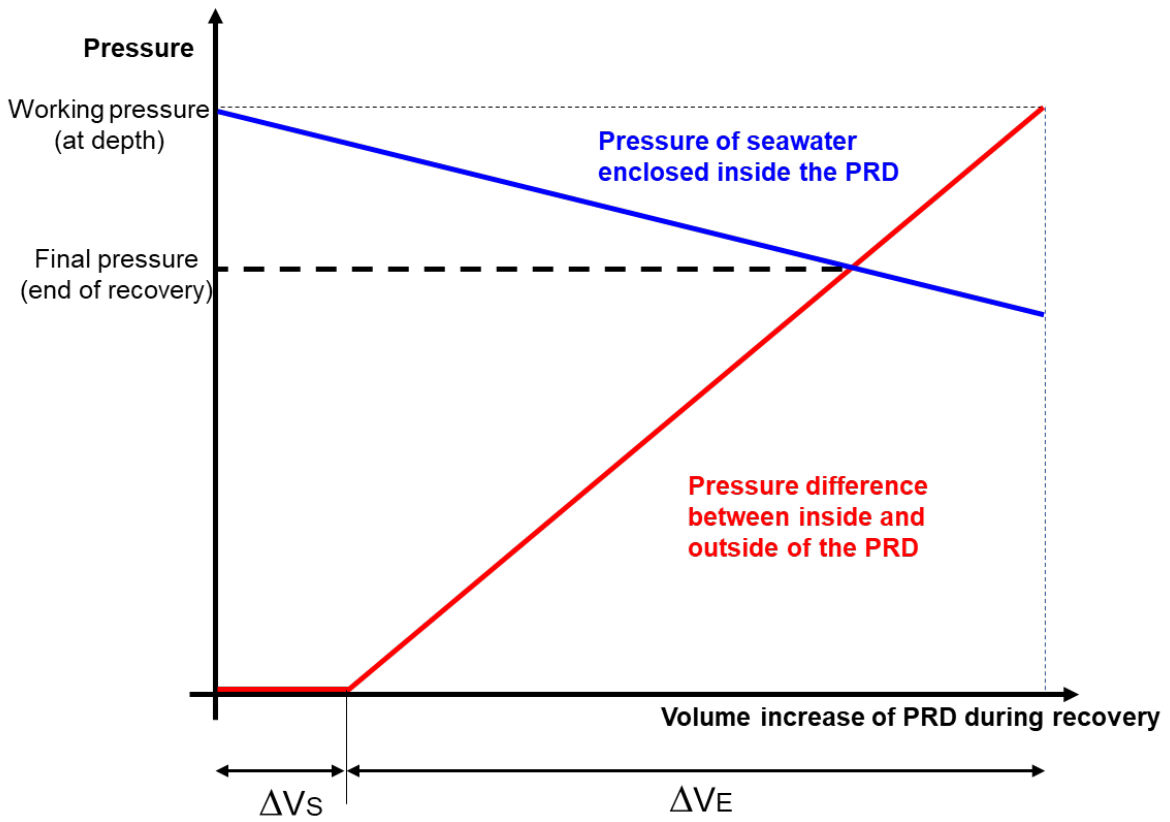


Figure S1 : Estimating the pressure-retaining performance of a PRD

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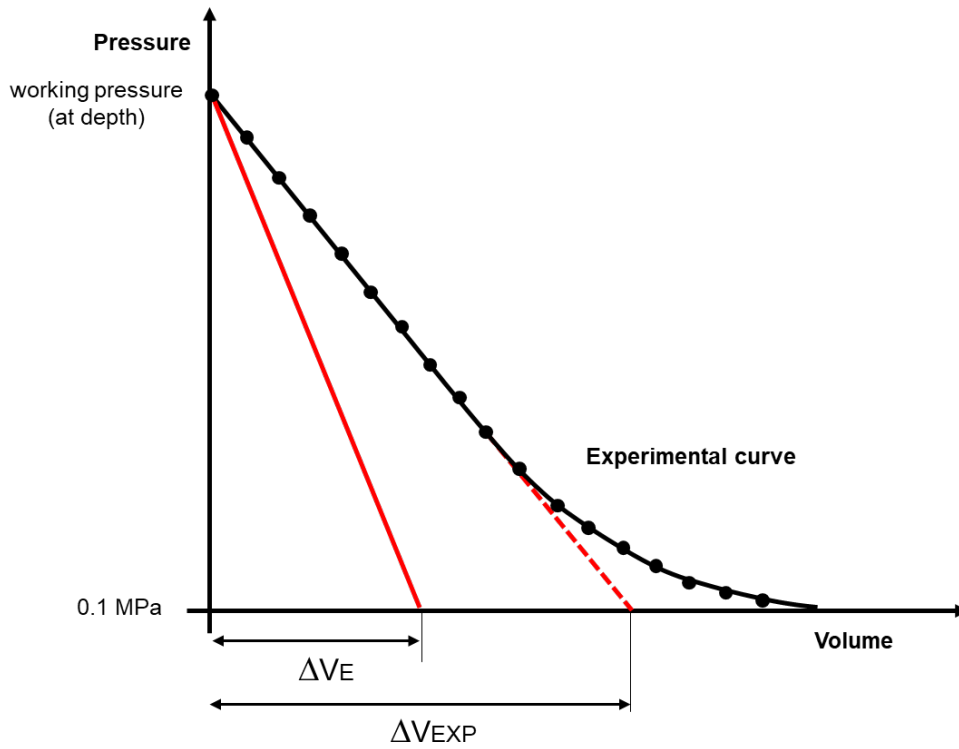


Figure S2 : Experimental determination of the expansion of PRD walls

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1002 **ANNEX 1 : Estimation of pressure-retaining capacity of the PERISCOP PRD**
1003 **(figures S1 and S2)**

1004 Before choosing/designing the type of compensator needed for a PRD, it is necessary to first
1005 estimate its pressure-retaining performance, without any compensation. During PRD recovery
1006 from deep water, the surrounding hydrostatic pressure decreases due to the ascent through the
1007 water column. Consequently, a difference in pressure builds up between the water inside the
1008 PRD and the external environment (Figure S1, red curve). This difference pushes seals within
1009 their grooves, and further expands the walls of the PRD. In turn, the volume of the seawater
1010 trapped inside the PRD increases, thereby causing a drop in hydrostatic pressure (Figure S1,
1011 blue curve). The internal pressure will further decrease as long as it is higher than needed to
1012 expand the PRD walls, before reaching the final pressure, at the intercept between the two
1013 considered curves.

1014 The expansion curve of the trapped seawater (blue curve) may be established by knowing the
1015 initial volume of the PRD, the mass of seawater trapped inside the PRD, according to pressure
1016 and temperature at depth, and the variations of seawater density as a function of pressure
1017 (McDougall and Barker, 2011). Temperature may first be considered constant throughout the
1018 process, simulating deep-sea conditions (e.g. 4°C), although it should be kept in mind that
1019 gradual heating (when approaching warm surface waters) would increase the internal
1020 pressure.

1021 The expansion curve of the volume inside the PRD (red curve) has two contributions : seal
1022 movement and expansion of PRD walls.

1023 Seal movement: the volume variation due to seals depends mainly on the volume
1024 displacement of seals (ΔV_S), and at lesser extent on volume change of seals while being
1025 compressed. The assumption made here is that the seawater enclosed inside the PRD expands
1026 as the external pressure is decreasing, and pushes the seals until they reach their locking
1027 position. During this process, the pressure difference between inside and outside the PRD
1028 remains small, but sufficient to push the seals.

1029 In our study, based on the size of the seals and their maximum displacements within their
1030 respective grooves, we estimated the volume displacement to be around 3 to 5 mL. Compared
1031 to volume variations due to expansion of PRD walls (see below), this contribution of seal
1032 displacement may be considered constant, i.e. independent from pressure at which the PRD
1033 was closed.

1034 Expansion of PRD walls: once the seals are in sealing position, the difference in pressure
1035 (inside vs. outside the PRD) builds up, therefore expanding the PRD walls. Such an expansion
1036 may be experimentally estimated (Figure S2), by repeating pressurisation/depressurisation
1037 experiments of the PRD at the laboratory. The assumption made here is that the variation in
1038 volume of the PRD when being pressurised from atmospheric pressure to working pressure at
1039 the laboratory, is similar to that experienced when the pressure surrounding the PRD
1040 decreases from working pressure to atmospheric pressure, while working pressure is
1041 maintained inside the PRD (a situation closer to operational sampling conditions). Although
1042 both final situations are identical (PRD pressurised, and surrounded by atmospheric pressure),
1043 the initial situations differ by the fact that the entire PRD (inside and outside) is either at
1044 atmospheric pressure (laboratory experiment), or at working pressure (*in situ* deployment),
1045 meaning the compressibility of steel is neglected in the laboratory experiment. Measurements
1046 of successive water volumes released from the PRD are achieved (typically in the 5-10 mL
1047 range at each sampling, for the PERISCOP PRD), upon gradual depressurisation from
1048 working pressure (pressure at which the PRD would be deployed) to atmospheric pressure.
1049 Repeated cycles allow to determine the behaviour of pressure as a function of recovered water
1050 volumes, which is almost linear, except when approaching lower pressures (below 10 MPa),
1051 due to expansion of air bubbles possibly trapped inside the PRD. Nevertheless, the linear

1052 behaviour obtained at higher pressures may be virtually extrapolated (dotted line in figure S2)
1053 in order to intercept the volume co-ordinate ΔV_{EXP} at the 0.1 MPa origin (atmospheric
1054 pressure). This volume (measured at atmospheric pressure) is converted to a volume ΔV_E ,
1055 taking in account the densities of water (D_{ATM} and D_{WP}) at both atmospheric and working
1056 pressures, the temperature, and the volume of the PRD at rest (V_0 , or initial volume):

$$1057 \Delta V_E = ((V_0 + \Delta V_{EXP}) \times D_{ATM} / D_{WP}) - V_0$$

1058 Back to Figure S1, this will lead to a linear relation of Pressure as a function of volume, $P = k$
1059 $\times \Delta V + P_{ATM}$ with a slope $k = (WP - P_{ATM}) / \Delta V_E$, corresponding to the true volume variation
1060 of the PRD submitted to internal pressure, independent from the liquid experimentally
1061 employed (provided the equation of state is known, liquids other than seawater may be used).

1062 Finally, the red curve in Figure S1 is a combination of the contribution of seals (horizontal
1063 part corresponding to ΔV_S), and the contribution of PRD wall expansion (pressure increase
1064 following the slope k , corresponding to ΔV_E). It should be noted that because ΔV_S remains
1065 constant (as opposed to ΔV_E which decreases at shallower deployment depths), this term will
1066 increasingly alter pressure retention performance as shallower deployment depths are
1067 considered.

1068 Such an approach allowed us to estimate the expansion of the PERISCOP (ΔV_E) to be about
1069 31 mL upon pressurisation at 30 MPa. Adding about 3-5 mL of seal movement (ΔV_S), this
1070 lead to predictions ranging from about 70% pressure retention in a simulated deployment at
1071 3600 m depth and 4°C temperature, to about 60% for a simulated deployment at 1000 m
1072 depth.

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1074 McDougall T. J. and P. M. Barker, 2011: Getting started with TEOS-10 and the Gibbs
1075 Seawater (GSW) Oceanographic Toolbox, 28pp., SCOR/IAPSO WG127, ISBN 978-0-646-
1076 -55621-5.