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Instruments and Methods

Live capture of megafauna from 2300 m depth, using a newly designed Pressurized Recovery Device

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ABSTRACT

The deep sea is an extremely diverse habitat, which is now threatened by human activity. Means for evaluating the response of deep-sea creatures to environmental perturbation are limited because of lethal decompression effects during sampling. The addressing of this issue requires that target species be (i) captured at depth, (ii) recovered at natural pressure, (iii) submitted to *in vivo* investigations. Although a single container may meet these requirements, we believe that using several dedicated cells greatly expands experimental possibilities. Accordingly, we have designed a new sampling system which has been named PERISCOP and which has accounted for the selective capture and recovery of live animals from depths exceeding 2000 m. Three hydrothermal vent shrimp species were sampled on the Mid-Atlantic Ridge, from depths of 1700 and 2300 m. In addition, a fish caught at 2300 m depth reached the surface in very good condition. This is by far the deepest record for the pressurized recovery of a live deep-sea fish. Our prototype aims at making pressurized recovery a more efficient and practical process. Finally, future evolutions of sampling methods are discussed based on the present design of the PERISCOP.

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1. Introduction

The last 30 years have seen the discovery of several deep-sea ecosystems (hydrothermal vents, cold seeps, whale-falls, sunken woods, and seamounts (Tyler, 1995)) which have modified our vision of the deep sea. Once considered as a homogeneous biological desert (Menzies, 1965), it is now seen as a patchwork of habitats where life may show diverse forms, with locally high biomasses (Van Dover et al., 2002). At the same time, the interest for fishing resources in the deep sea has grown to an alarming degree (Devine et al., 2006; Glover and Smith, 2003), pointing to the risk of severe ecosystem perturbation. Several other forms of human activity are threatening the largest habitat of the biosphere, such as CO_2 sequestration projects, waste dumping, mineral prospecting, and their impact on deep-sea fauna has barely been evaluated (Glover and Smith, 2005). It is therefore a matter of urgency to learn more about the ecology and the biology of deep-sea species, especially concerning their response to environmental changes. Although *in situ* experiments have yielded some interesting results (Bailey et al., 2002; Vardaro et al., 2007), laboratory studies of live animals are a precious tool here, since they allow physiological investigation at various levels, from genetic expression to organismal, physiological, and behavioural responses.

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Unfortunately, while some organisms may be studied in pressurized aquaria (Childress et al., 1993; Shillito et al., 2001; Girguis and Lee, 2006), many deep-living creatures preclude *in vivo* investigation because of lethal decompression effects upon sampling (MacDonald, 1997). The addressing of these issues requires that target species be (i) captured at depth, (ii) recovered at their natural pressure, and (iii) studied *in vivo* at the laboratory. In most previous attempts involving pressurized recovery (MacDonald and Gilchrist, 1972; Yayanos, 1978; Drazen et al., 2005; and references herein), a single container fulfilled these three tasks. This may lead to contradicting technical requirements and we believe that experimental possibilities would be greatly expanded by using dedicated cells for each of these tasks. Here we give account of the recovery of live animals from depths exceeding 2000 m, using a new Pressurized Recovery Device (PRD) named PERISCOP (Projet d'Enceinte de Remontée Isobare Servant la Capture d'Organismes Profonds). This prototype aims at making pressurized recovery a more efficient and practical process. Additionally, its present design is adapted to future evolutions: the transfer of freshly caught animals, without decompression, towards larger ship-based pressure aquaria.

2. Material and methods

2.1. General principle

The PERISCOP system is composed of: (1) an *in situ* sampling cell (Fig. 1) and (2) a PRD, designed to maintain *in situ* pressure during recovery (Fig. 2). The PRD is installed on a "shuttle" device, which is moored and recovered away from the submersible. Once fauna have been confined inside the sampling cell, the latter is stored inside the PRD, which is then sealed and later recovered after ascent through the water column. In addition, the main aperture of the PRD is designed to permit future transfer of the sampling cell, without decompression, towards other surface-based pressure devices (isobaric transfer, Fig. 3).
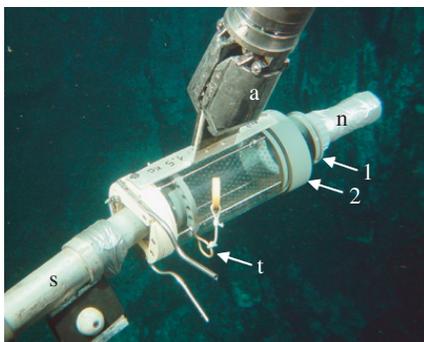

Fig. 1. Operation of the sampling cell. The cell is held by the submersible's arm (a), and is connected to a suction device (s). It is composed of two concentric cylinders (1, 2). The inner cylinder is 35 cm long, and 10 cm in diameter, and bears many 2 mm diameter holes, therefore acting as a filter, when suction is transmitted from the suction device to the nozzle (n), through which fauna are sampled. Once organisms are captured, the suction device is disconnected, and cylinders 1 and 2 are dis-assembled by pulling a trigger (t).
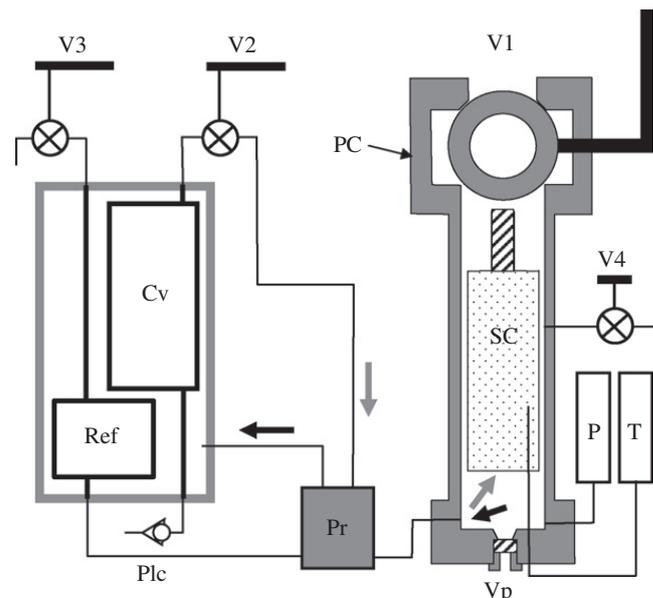

Fig. 2. Principle of the PRD and of pressure compensation. The sampling cell (SC, see also Figs. 1 and 4) is stored in the Pressure Cell (PC), before closing the ball valve V1. The submersible then opens V2 and closes V3. During the PRD's ascent back to the surface, a pressure regulator (Pr) compares the pressure in PC with that prevailing in a reference container (Ref), corresponding to the depth at which V3 was closed. If the pressure in PC decreases (see text), Pr opens its link with a reservoir container (Cv), initially loaded with seawater, at a pressure of 45 MPa: the pressure in PC therefore increases until it is again equal to that of Ref (grey arrows). Conversely, if the pressure in PC exceeds that in Ref, excess water is evacuated (black arrows), towards a stainless-steel container which remains at atmospheric pressure throughout the whole process, and inside which both Cv and Ref are stored. Vp: viewport. P, T: autonomous pressure and temperature probes. Plc: loading connector.

2.2. The sampling cell (Fig. 1)

The sampling cell is made from PVC and transparent Polycarbonate. Its apparent weight in water is 1.5 kg, and it mainly consists of a cylinder for confinement of fauna, with an internal diameter of 95 mm and length of 350 mm (Fig. 1). Fauna are sampled using the suction power of the submersible, through a 50 mm diameter flexible nozzle. The suction device of the submersible is connected to the sampling cell *in situ* immediately prior to sampling, and disconnected afterwards. Once this has been done, the submersible dis-assembles the two cylinders composing the sampling cell. The inner cylinder contains the samples, and will be stored further inside the PRD. The inferior part of the inner cylinder displays a circular slit, allowing the temperature probe of the PRD to penetrate inside the cylinder (i.e. where the samples are held) when the latter is stored inside the PRD. Furthermore, the bottom of the cylinder is transparent, allowing visual observation of the samples through the PRD view-ports, once they are back on-board the ship's deck (Fig. 4D).

2.3. Principle of the Pressurized Recovery Device (PRD) and of pressure compensation (Fig. 2)

Constructed with 316 or APX4 stainless-steel type components, the internal volume of the PRD is 6.6 l. It has
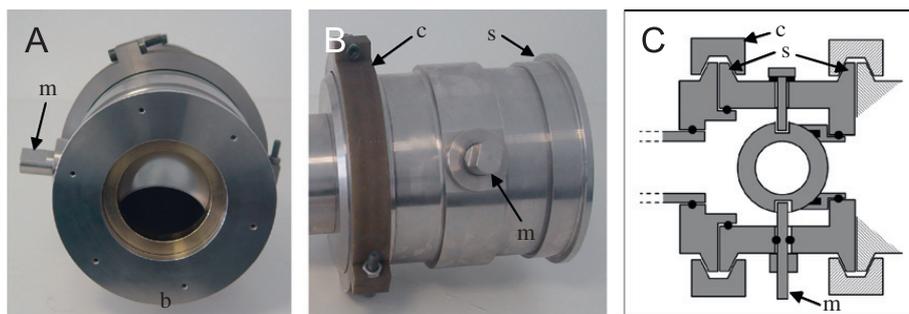
Fig. 3. The ball-valve. Face (A) and side (B) views of the PRD's ball-valve (V1 in Fig. 2). The ball (b) allows a 10 cm diameter passage. The valve is opened or closed using the manoeuvring axis (m), away from the front face of the valve body. The valve body is connected to the rest of the PRD by means of three collar-shaped clamps (c), which fasten around shoulderings (s) of the assembled parts. The valve body displays these shoulderings at both extremities (B and C). Therefore, the valve may be connected to other pressure equipments (hatched domain) using the same method (C).
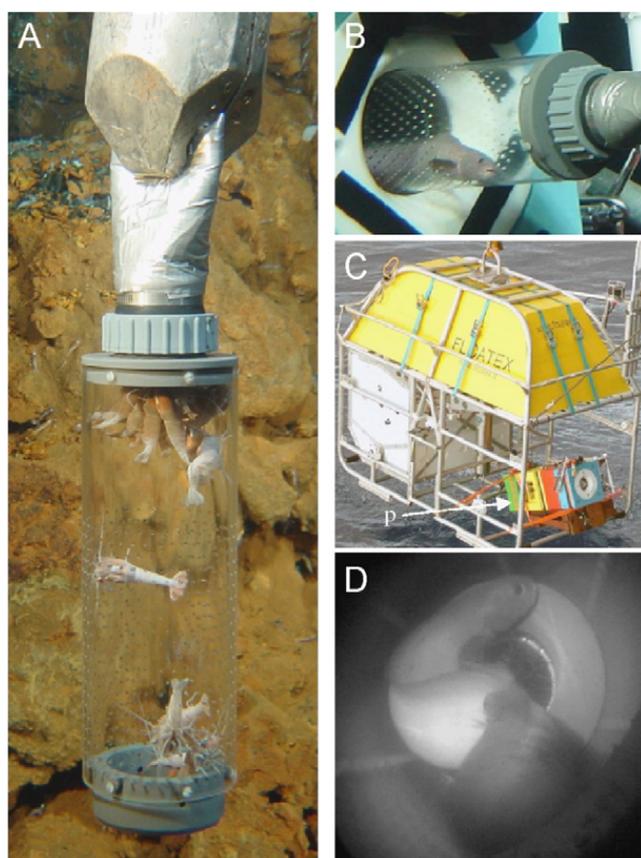

Fig. 4. The sampling process. (A and B) Once the sampling cell has been dis-assembled, cylinder 2 (see Fig. 1) is inserted into the PRD. The lower part of cylinder 2 is equipped with a circular slit, allowing internal temperature of the PRD to be measured inside the cylinder 2 (see also Fig. 2). Further operations, i.e. sealing the PRD and activating the compensation system, took less than 10 min. The captive organisms are hydrothermal vent shrimps (*Rimicaris exoculata*) (A), and a hydrothermal zoarcid fish, *Pachycara saldanhai* (B). (C) Recovery of the shuttle device, on which the PRD (p) is fixed. The syntactic foam casing (multicoloured) contributes both to thermal insulation and floatability. (D) Back on the ship's deck, a *Pachycara saldanhai* specimen (28 cm length) is observed through the PRD's view-ports.

a test pressure of 45 MPa, allowing for a maximum operating depth of 3000 m. Pressure and temperature inside the PRD are recorded by two autonomous probes (NKE instruments, Hennebont, France). Its main aperture is a quarter-turn ball-valve allowing a 10 cm diameter free

passage (valve V1, see Figs. 2 and 3), whereas the bulk of the PRD is a cylinder of 10.6 cm internal diameter and 50 cm length. The PRD is enclosed in a rectangular-shaped syntactic foam casing (BMTI, Toulon, France), reaching 80 cm in length, and 32×32 cm sideways (Fig. 4C). Therefore, the thickness of the syntactic foam is not constant around the cylindrical-shaped PRD, it ranges from 25 to 90 mm around the main cylinder. As a result, the weight of the entire device is 130 kg, appearing at 60 kg under water.

During ascent, changes in environmental pressure and temperature may seriously modify the pressure prevailing inside the cell, because of metal expansion and seal movements, thereby justifying the use of a pressure compensation unit. Unlike most other PRDs, our pressure compensation unit does not rely on pressurized gas. It is described in Fig. 2, and functions as follows: after samples have been stored inside the Pressure Cell (PC), the latter is sealed (valve V1). At the same time, and immediately prior to ascent to the surface, a reference container, composed of braided synthetic fiber (Saint-Gobain, Courbevoie, France) is also sealed. Because it is stored inside a larger stainless-steel container (Hydroem, La Rochelle, France), the inside of which remains at atmospheric pressure (in air) throughout the whole process, the reference container is not submitted to changes in external pressure upon ascent through the water column. Consequently, seal movements and metal expansion (of the reference container) do not occur. The reference container is therefore expected to maintain constant pressure during the recovery process. Thereafter, a double-valve mechanical pressure regulator (Tescom, Elk River, Minnesota, USA) compares the pressure in PC with that prevailing in the reference container, corresponding to the depth at which it was closed. This regulator is set to function when pressure differences exceed around 1.0 Mpa at the most. When the pressure in PC decreases below this limit, due to its expansion or to seal movements, the pressure regulator allows seawater to be injected from a reservoir container (same composition as the reference container, Saint-Gobain, Courbevoie, France) which has previously been pressurized at 45 MPa (the rupture pressure of both reservoir and reference containers is 83 MPa): the pressure in PC therefore increases until it is again 1.0 MPa or less below that of the reference

volume. Conversely, if the pressure in PC increases by more than 1.0 MPa above that in the reference container, excess water is evacuated towards the stainless-steel container inside which both reservoir and reference containers are stored.

2.4. Video monitoring

Once the PRD has been recovered on the ship's deck, visual observations of the samples may be recorded through 26 mm-diameter view-ports (Vp, see Fig. 2), and through the transparent extremity of the sampling cell, using both illumination fibre optics (Sibylux, Volvic, France), and an endoscope (Kinoptik, Dourdan, France) coupled to a black-and-white camera (Kappa, Gleichen, Germany). Resulting images are recorded on a DVO-1000MD Sony DVD recorder.

2.5. The aperture of the PRD (Fig. 3, V1 in Fig. 2)

The main aperture of the PRD, through which samples are introduced, is a quarter-turn ball-valve, constructed with APX4 stainless steel. The ball itself weighs approximately 12 kg, and provides a 10 cm passage diameter. In closed position, pressure sealing of the PRD is provided by a rubber/Teflon composite seal, between the ball and the brass seat on which it rests. As described in Fig. 3, the valve body is connected to the rest of the PRD by means of three collars, which fasten around shoulderings. Such shoulderings are provided on both sides of the valve body, allowing for pressure connections between the PRD and another pressurized device.

3. Results

3.1. The sampling process (Fig. 4)

During the MOMARETO cruise in August 2006 (Mid-Atlantic Ridge), we made six attempts at recovering hydrothermal vent organisms at their native pressure (Table 1), by using the Remotely Operated Vehicle (R.O.V.) Victor 6000 (Ifremer). Two additional "control" attempts were made using the PRD, but with no pressure retention (i.e. V4 had been left open, see Fig. 2). Three shrimp and one fish species were sampled, at depths of 1700 (*Mirocaris fortunata* and *Chorocaris chacei*) and 2300 m (*Rimicaris exoculata* and *Pachycara saldanhai*), at two vent fields, Lucky Strike and Rainbow, respectively (Desbruyères et al., 2006). The sampling sequence was as follows: (1) Connecting the sampling cell to the submersible's suction device, sampling of fauna, disconnecting the sampling cell. (2) Dis-assembling the sampling cell, and moving towards the shuttle device, usually moored at a distance of less than 150 m from the sampling site. (3) Inserting the inner part of the sampling cell inside the PRD fixed to the shuttle, sealing the PRD (V1 in Fig. 2), triggering the compensation unit (V2 and V3 in Fig. 2). (4) Shuttle ascent towards the surface, and subsequent recovery on the ship's deck. The whole process (steps 1–4) lasted less than 3 h in all cases, with steps 2–3 lasting less than 1 h, and with an ascent duration of ca. 20–30 min depending on the depth of each trial. Finally, 10–20 min passed between the time the PRD reached the surface, and the time it was landed safely on the ship's deck.

3.2. Pressure and temperature data throughout recoveries (Fig. 5 and Table 1)

During all trials, both pressure and temperature recorders were set at a recording rate of 1 measurement/10 s. Final recovery pressures on the ship's deck ranged from 82% to 111% of the natural pressure. Two types of pressure history profiles were obtained throughout the six pressure-recovery attempts. The first profile (trials nos. 1–3 and 6, see Table 1 and Fig. 5) displayed a pressure loss during ascent through the water column, down to 74–77% of *in situ* pressure, followed by a pressure increase up to 82–90% (of *in situ* pressure). The second profile (trials nos. 4 and 7), obtained after some adjustments to the compensation unit, displayed a small pressure loss down to 95–97%, followed by a pressure increase up to 104–111% of *in situ* pressure. In all cases, the final pressure increase seemed almost to be synchronized to the PRD's arrival at the surface (see Fig. 5). Pressure, however, soon stabilised when the PRD was retrieved from the water.

Immediately prior to the ascent of the PRD, the temperature measured inside the sampling cell, corresponding

Table 1
Attempts to collect hydrothermal vent fauna (last column, with number of individuals caught: except for *Pachycara saldanhai*, all are shrimp species) using PERISCOP, presented in their temporal order (first to last trial from top to bottom)

No./site	Depth (m)/pressure (MPa)	Recovery pressure (lowest/final, MPa)	% of *in situ* pressure	Species
1/Lucky Strike	1655/17.0	12.8/15.3	75/90	*Mirocaris fortunata* (33)
2/Lucky Strike	1670/17.1	13.0/15.1	76/88	*Mirocaris fortunata* (5)
3/Lucky Strike	1680/17.2	13.2/15.3	77/89	*Mirocaris fortunata* (29)
4/Rainbow	2290/23.5	22.8/26.1	97/111	*Rimicaris exoculata* (30)
5/Rainbow	2280/23.4	Atmospheric Pressure	Control experiment	*Rimicaris exoculata* (19)
6/Rainbow	2290/23.5	17.4/18.8	74/82	*Pachycara saldanhai* (1)
7/Lucky Strike	1675/17.2	16.3/17.9	95/104	*Chorocaris chacei* (28)
8/Lucky Strike	1660/17.0	Atmospheric Pressure	Control experiment	*Chorocaris chacei* (27)

In situ depths and pressures (rounded to the nearest 5 m and 0.1 Mpa, respectively) are provided, followed by the lowest pressure experienced during recovery, and the final pressure (in MPa, or % of *in situ* pressure, third and fourth columns, respectively). Pressure was always minimal upon reaching the surface, and gradually increased.
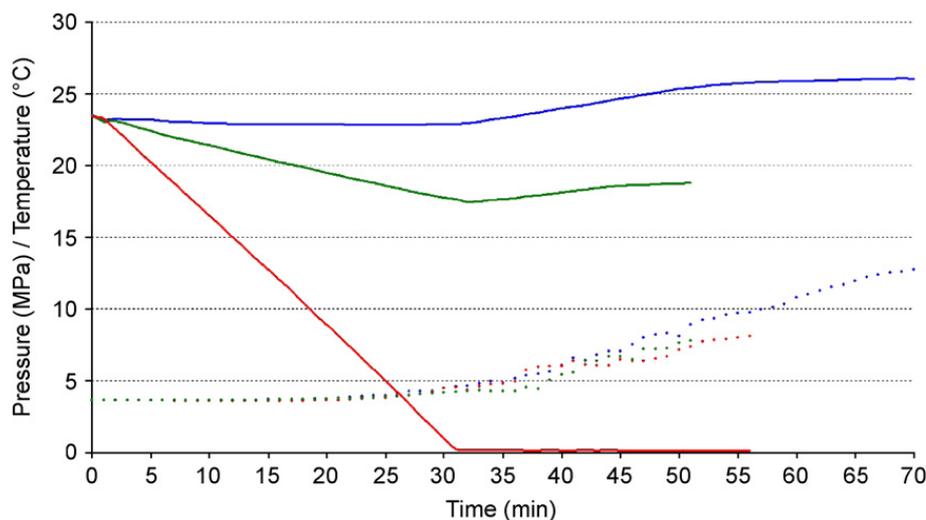
Fig. 5. Pressure and temperature recordings inside the PRD after sampling at a depth of 2300 m, as a function of time after shuttle release from the bottom. Dotted and plain lines correspond to temperature and pressure profiles, respectively. For the red curves, sampling procedure was identical to others, except that the relief valve V4 was left open (see Fig. 2), therefore preventing pressure retention (trial no. 5, Table 1). Temperature upon reaching the surface (after about 30 min of ascent) was always below 5 °C, and later increased due to prolonged contact with warm (~24 °C) surface water. The green and blue pressure curves correspond to the sampling of a fish (trial no. 6), and of shrimps (trial no. 4), respectively (Table 1).

to background water temperature at depth, was 4.4 °C for samplings at Lucky Strike (1700 m depth). When the PRD reached the surface, internal temperature was below 6 °C. Twenty minutes later (longest recovery time of the PRD on the ship's deck), temperature was still below 10.5 °C. For sampling at Rainbow (2300 m depth), initial temperature was 3.6 °C, it remained below 5 °C on reaching the surface, and below 9 °C 20 min after. Fig. 5 shows that temperature still increased after recovery on the ship's deck, reaching almost 13 °C 40 min after the PRD had reached the surface. Surface water temperatures were in the 23–25 °C range throughout the cruise.

3.3. Sampling of deep-sea shrimps

Details regarding species and numbers are given in Table 1. All are endemic vent species, and were captured near to hydrothermal vent emissions. The shrimps *R. exoculata* form dense swarms which can easily be identified. *M. fortunata* and *C. chacei* shrimps are less gregarious, but may nevertheless be found in groups large enough to allow suction sampling. These two species are difficult to distinguish *in situ*. Even on-board ship, identification of the two species required examination under binocular magnifying lenses, the selection criterion being the presence (*M. fortunata*) or absence (*C. chacei*) of a sharp post-orbital prominence on the cephalothorax (Desbruyères et al., 2006). However, each of our samples contained only one species, and provided the sampling area was the same from one attempt to the other, it was possible to repeat sampling of the same species (as for attempts 1–3, or attempts 7 and 8).

3.3.1. Attempts 1–3
M. fortunata shrimps were targeted from a depth of 1700 m. We recorded observations of these samples inside

the PRD, from when it was back on the ship's deck until pressure was released, 10–20 min later. Most shrimps rested in an upward position, on their legs, in the lower part of the sampling cell (effect of gravity). Frequently, shrimps would swim around the cell once or twice, and return to a resting position. Upon pressure release, however, the level of swimming activity suddenly increased, and was accompanied by frequent violent single convulsions of the abdomen. This phenomenon gradually decreased in intensity and frequency within a few minutes after decompression.

3.3.2. Attempts 4 and 5
R. exoculata from a depth of 2300 m (Fig. 4A). We recorded the behaviour of the shrimps inside the PRD, once back on the ship's deck, after a pressurized recovery at 26.1 MPa, and also a "control" recovery, using exactly the same procedure, except for pressure retention (Table 1). For the control experiment, shrimps had lost their balance (i.e. resting sideways or upside down, at the bottom of the cell), displaying few movements, at the exception of occasional single convulsions of their abdomen. For the pressurized recovery, the shrimps were swimming actively and continuously, in what appeared to be normal "swarming" behaviour, as observed *in situ*.

3.3.3. Attempts 7 and 8
C. chacei from a depth of 1700 m. We effected a pressurized recovery at 17.9 MPa, and a control recovery, using exactly the same procedure, except for pressure retention (Table 1). Samples were not observed while inside the PRD, back on the ship's deck, priority here being given to immediate conditioning of biota. Pressure was therefore released as soon as possible, and freshly caught shrimps conditioned for further studies.

All shrimps were still alive when they were recovered from the PRD, whether pressure had been retained or not.

They were conditioned for further studies, either by direct freezing at −80 °C, or dissected and treated with an RNA-later solution (24 h at 4 °C), prior to freezing at −80 °C.

3.4. Sampling of a deep-sea fish

We also succeeded in recovering a fish alive from a depth of 2300 m (Attempt 6, Table 1). This female zoarcid was 28 cm long and belonged to the species *P. saldanhai* (Desbruyères et al., 2006; Biscoito and Almeida, 2005) (Fig. 4B and D). Back on the ship's deck, we observed the animal swimming actively back and forth inside the PRD. Compared to *in situ* observations, this fish appeared somewhat hyperactive. It always remained upright, showing very good movement coordination, although it had been submitted to partial decompression (18–26% pressure loss, see Table 1). We ended our test by finally releasing pressure inside the PRD about 50 min after the PRD had reached the surface; the internal temperature was then 10.4 °C. The fish almost immediately lost its movement coordination, and became totally motionless within a few minutes.

4. Discussion

4.1. Sampling efficiency and specificity, normal and safe operation

There have been previous successful attempts to recover deep-sea organisms at their native pressure, despite the difficulties involved in achieving this task (Yayanos, 1978; MacDonald and Gilchrist, 1982; Jannasch et al., 1982; Wilson and Smith, 1985; Bianchi et al., 1999; Koyama et al., 2002; Drazen et al., 2005). With regard to megafauna, the first challenge is to manage efficient capture. Most existing devices act as baited traps. One main drawback here is that the target species must be mobile and attracted to bait. This problem was recently faced (Koyama et al., 2002), by active sampling, using a submersible. However, given the cost and the tight schedule of a typical submersible dive, it is desirable that such an operation should not monopolize the majority of the vehicle's carrying capacity, if it has to transport the hyperbaric cell during the dive. We opted for the submersible-assisted capture, but decided it would be a more practical approach to first capture the target species using a small PVC cell of negligible weight and size, coupled to the suction device of the submersible (Fig. 1). In a second step, this cell would be inserted inside the PRD (Fig. 4B), installed on a "shuttle" (Fig. 4C), a general purpose autonomous system which may be moored and recovered at a distance from the submersible.

Since it does not possess pressure-retaining capacities, the sampling cell is light and manoeuvrable; therefore the manipulating submersible is able to sample specific fauna efficiently. Although the present work was achieved using an ROV as the submersible, an earlier version of our sampling cell had previously been tested with success, using a manned submersible, the Nautile (Ifremer, Biospeedo cruise, 2004). Moreover, our sampling system can be adapted to all submersibles equipped with a suction device. A PVC adaptor must be fixed to the extremity of the suction gun, in order to allow reversible connection to the sampling cell. However, provided the initial diameter of the suction device is 5 cm or less, the adaptor will not restrict this diameter when the sampling cell is not connected, thereby allowing use of the suction device for other purposes during the same dive.

Finally, unlike with all previously existing systems, we designed a compensation unit, which avoids the use of pressurized gas as compensation potential. Instead, our system uses pressurized seawater (see Fig. 2). This choice reflects our objective, which is to allow hyperbaric recovery for normal use by marine biologists, thus gaining in simplicity of use and in safety. Firstly, the loading pressure is constant for all PERISCOP trials, from the surface to a 3000 m depth, unlike gas-loaded pressure compensators, which need specific pre-settings relating to the depth of operation of a given sampling attempt (usually 70–90% of pressure at depth). Secondly, with regard to normal and repeated use, the absence of pressurized gas is clearly a safety option, should there be mis-manipulation, or fatigue of the system due to corrosion.

4.2. Pressure and temperature maintenance during recovery

With regard to pressure control during recovery of the PRD, two main features are to be discussed: pressure losses during ascent through the water column, and pressure increases once the PRD had reached the surface. Although the first three trials were very successful regarding the *in situ* sampling process, they all displayed a ~25% pressure loss during ascent, which showed that the compensation unit was not playing its role (Table 1, Fig. 5). The problem was identified, i.e. an effect of surrounding pressure on the pressure regulator valve (Pr, Fig. 2). The casing of this regulator was therefore modified accordingly on-board ship. The result was that trial nos. 4 and 7 were a success regarding pressure compensation, with no more than 5% pressure loss. The pressure loss observed during trial no. 6, similar to what occurred in the first three trials, allowed us to determine the origin of the problem with precision, thus allowing for appropriate modifications for future cruises.

The pressure increase observed once the PRD had reached the surface was the result of a thermal effect. Before being landed on deck, the PRD remained 10–20 min at the surface, surrounded by water at a temperature of about 24 °C. This resulted in a pressure increase inside the PRD, which soon reached a plateau after the PRD had been landed on deck (Fig. 5). This increase was expected, but the compensation unit should have restricted it to less than 1.0 MPa (with respect to the pressure prevailing once the PRD was at the surface) according to the pressure regulator specification. However, pressure increases varied from 1.4 to 3.3 MPa. The reason for this is not clear, however, a possible explanation is that the pressure of the reference container itself also increased due to heating in surface waters, via a thermal bridge with the regulator. This higher pressure in the reference container would

have led the compensator to inject more water in the PRD, therefore resulting in a pressure increase following that of the reference container. Another possibility would be that the regulator's over-pressure valve did not function properly, while the pressure inside the PRD was increasing due to prolonged contact with warm surface waters. Indeed, early calculations had predicted that without compensation, a 15–20% pressure increase would occur, upon changing the internal temperature from 5 to 15 °C. Future modifications will therefore focus on improving both mechanical and thermal protection of the regulator.

The two types of pressure profiles obtained with our PRD may be compared to the performances of other recent equipments. During trial nos. 1–3 and 6, the ca. 25% pressure loss is similar to that obtained by Koyama et al. (2002), when they retrieved a deep-sea fish from a depth of 1171 m. Although these authors had fitted a gas-filled balloon in their system, in order to compensate for pressure losses due to metal expansion during recovery, it seems that this feature was not efficient enough. Although comparisons should be made cautiously when considering different designs (the PRD of Koyama et al. (2002) is a sphere) and different depths of sampling, their data are consistent with our pressure-retaining performances during trial nos. 1–3 and 6, when our compensation system had failed to function. During trial nos. 5 and 7, however, the compensator played its role, and we obtained less than 5% pressure losses during ascent, which compare quite well with the 5–7% losses obtained during the deployments of a PRD by Drazen et al. (2005), at depths of about 1400 m. This comparison shows that our pressure compensation system competes well with gas-loaded pressure compensators (Drazen et al., 2005), while being safer to use.

Throughout the recovery process, the temperature profiles inside the PRD show that biological samples reach the surface at a temperature ~1.5 °C above *in situ* temperature (referring here to background water, away from hydrothermal influence). Subsequently, as shown in Fig. 5, and as discussed in previous work (Childress et al., 1978), the temperature of surface waters plays a critical role. In the worst cases (trial no. 4, blue curve in Fig. 5), when the PRD remained immersed in surface waters as much as 20 min, the temperature was then 5–6 °C above *in situ* temperature. Although temperature kept increasing later (when we took time to record observations inside the PRD before decompression for example), it is the previous temperature increase that seems relevant for discussion, at the moment when samples are first accessible to investigators, immediately after reaching the ship's deck. In comparison, Childress et al. (1978) measured a 5–6 °C increase when retrieving deep-sea fauna from a 5 °C environment, through a 28 °C tropical surface layer, using a thermally protected sampler. Recently, in the case of other pressurized recoveries, Koyama et al. (2002) obtained a ~2 °C increase upon recovery from 2.8 °C, through 18 °C surface waters. Drazen et al. (2005) also obtained similar results (~3 °C increase, bottom water temperature of 2.8 °C, surface water temperature not provided). These performances appear slightly better than those obtained in this study. They may be explained by the larger volumes of the PRDs involved in these two

latter studies (30 and 89 l respectively), yielding higher thermal inertia. Our PRD's thermal performance nevertheless would appear sufficient for maintaining high survival rates, even in the case of stenothermal deep-sea species (Childress et al., 1978).

Finally, our data underline the importance of witnessing pressure and temperature histories throughout the entire recovery process. The data provided by these probes helped both to optimize the pressure compensation during ascent, and to evidence the importance of the time spent in surface waters with regard to heating phenomena (Fig. 5). We suggest that providing final pressure alone, as done elsewhere (to our knowledge, there is only one exception, Bianchi et al., 1999), may provide an incomplete view of the recovery process.

4.3. Behaviour of deep-sea Vent Fauna inside the PRD

Two trials (nos. 4 and 5) involving the same species, the vent shrimps *R. exoculata*, with both samples originating from the same site, allowed to evidence decompression effects on behaviour. The difference in behaviour arose most clearly from different pressure conditions, since both recoveries had similar temperature profiles (Fig. 5). In addition, the highest temperature values (around 13 °C, 40 min after reaching the surface) were still far from being critical for these vent species (Ravaux et al., 2003), although temperature increase during recovery has long been identified as a major cause of trauma during deep-sea sampling (Childress et al., 1978). Further biochemical analyses of the tissues of these animals will allow investigation of possible differential expression of stress proteins.

For the first time, a fish caught at 2300 m depth reached the surface in very good condition. This is by far the deepest record for the pressurized recovery of a live deep-sea fish, a type of organism adversely affected by decompression (Sebert, 2002). Indeed, it is a common observation that deep-sea fishes do not tolerate conventional sampling, being either dead, or moribund when reaching the surface (Bailey et al., 2002; Drazen et al., 2005). Although species bearing gasbladders are most affected, other fishes, such as zoarcids, also show very low survival rates throughout sampling (Sebert, 2002). The immediate loss of movement coordination of the fish we sampled, followed within a few minutes by what seemed to be either death or total paralysis at the less, seems to confirm this sensitivity to full decompression. Recent attempts by other workers have reported live recoveries, at *in situ* pressure, from a depth of 1450 m at the most (Wilson and Smith, 1985; Koyama et al., 2002; Drazen et al., 2005). The present achievement may be regarded as a progress towards our urgent need to understand deep-sea ecosystems, given that fishes are both important members of deep-sea communities (Bailey et al., 2002) and immediate targets of anthropogenic threats (Devine et al., 2006; Glover and Smith, 2003). The apparent active behaviour of the recovered specimen is likely to be due to either thermal or capture stress. However, such an activity may also suggest a good physiological state, at least good enough to allow a hyperactive behavioural response.

4.4. Perspectives towards isobaric transfer

The design of the PERISCOP aims at achieving efficient and practical sampling and recovery of deep-sea megafauna. Furthermore, some technical choices were made bearing in mind the possibility of transferring megafauna, without pressure loss, from the hyperbaric cell to a fully equipped lab-based pressure aquarium, in the future. The crucial choice lies in the type of aperture for the hyperbaric cell: ball valves have already been proposed for isobaric transfer, but only in the case of small organisms (plankton, MacDonald and Gilchrist, 1972). Unlike all other systems, their operation is located away from the front face of the aperture itself, which is then free of physical obstacles (lid, or lever, or wedge), thereby allowing coupling to other pressurized devices facing the valve. We propose the same principle, but adapted to a larger size scale: PERISCOP's aperture provides a 10 cm diameter entrance, big enough to sample a great variety of deep-sea fauna. Another technical choice was to confine fauna inside a PVC sampling cell, prior to storage inside the actual PRD. As discussed above, this improves the manoeuvrability of the submersible during the capture process. Advantageously, in view of isobaric transfer, moving fauna from one cell to another can be achieved by transferring the sampling cell itself, rather than attempting to force (or lure) captive fauna to move from one compartment to another.

The present field results illustrate how isobaric sampling may be efficient when the two functions of sampling and pressure recovery are each managed by one dedicated device. Back on the ship's deck, a PRD may be connected to a peripheral pressurized circuit (Koyama et al., 2002), allowing investigators to control flow, pressure, and temperature. Original data have thus been provided recently with regard to oxygen consumption or behavioural responses of un-decompressed deep-sea fauna (Drazen et al., 2005). However, we believe that a PRD may reach its limits regarding some experimental approaches, because its design is somewhat constrained by the pressure recovery function, implying practical limits in size and weight (for manoeuvrability). Additional equipment required for achieving long-term maintenance and elaborate *in vivo* studies at *in situ* pressure (Childress et al., 1993; Pradillon et al., 2001; Shillito et al., 2006; Girguis and Lee, 2006; Holden, 1998) may surpass these limits. In addition, such equipment should tolerate mooring at depth, implying more engineering constraints. Conversely, the development of isobaric transfer methods should allow the optimizing of both steps separately (isobaric recovery and ship-board studies). Isobaric recovery could then be standardized, allowing for ship-board studies to occur in a variety of specific pressure aquaria, provided the latter are equipped with a connecting point matching that of the PRD.

5. Conclusion

The success of the PERISCOP provides a new step towards allowing biologists normal and practical access to live deep-sea megafauna. Three major improvements are proposed, with respect to previous devices. Their aim is to: (i) allow choice of sampling by a submersible, without requiring exclusive dedication of the dive towards this task; (ii) improve safety and simplicity of pressure compensation, as well as recording pressure and temperature history during recovery; and (iii) propose a system designed to evolve towards isobaric transfer.

Fishes are not the only deep-sea organisms for which hyperbaric recovery is a pre-requisite to *in vivo* physiological investigation. Other intriguing creatures, such as the hydrothermal vent worm *Alvinella pompejana*, will most probably require such developments in order to reveal their peculiar biological features, with regard to their presumed record resistance to heat (Cary et al., 1998; Chevaldonné et al., 2000; Shillito et al., 2004) or with regard to the extraordinary bacterial symbiosis which they host. The transfer without decompression of megafauna from pressurized collection devices to larger experimental facilities, will be one of the major issues in future deep-sea biology engineering. Once this has been achieved, we are convinced that new avenues of research will open up, based on the opportunity to study live deep-sea biota at the laboratory. This will undoubtedly improve our knowledge on the molecular, cellular, and organismal responses to environmental perturbation in the deep sea.

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