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THE IPOCAMP PRESSURE INCUBATOR FOR DEEP-SEA FAUNA

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ABSTRACT

Animal Biology studies have always benefited from the achievement of experiments on live animals, which obviously provide data on dynamic aspects of physiology. When it comes to deep-sea fauna, *in vivo* experiments are impaired, and in some cases impossible, due to the severe and often lethal stress experienced by animals throughout the sampling process. However, freshly collected deep fauna may be maintained alive and in good condition, by using specific aquaria which restore environmental conditions prevailing at depth *in situ*. Here we describe the pressure device named "Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds", or IPOCAMP, and provide an overview of the studies which were achieved using this pressure aquarium, with a particular focus on thermal biology of deep-sea vent fauna.

I. INTRODUCTION

The Deep Sea is the largest environment on the planet, with 75% of the Ocean's volume lying below 1000 m depths [18]. Because access to the deep-sea for scientific investigation is rare, difficult, and expensive, our knowledge of the biology and ecology of deep-sea fauna remains relatively poor [27]. Generally speaking, Animal Biology studies have always benefited from the achievement of experiments on live animals, which allow research to investigate aspects such as behavior or physiology. In the case of deep-sea fauna, biologists have to deal with the severe and often lethal stress experienced by animals throughout the sampling process [21, 33]. Investigations on organisms in good physiological condition are therefore impossible, thus adding another obstacle to the study of deep-sea life. However, freshly collected deep fauna may be maintained alive and in good condition, by using specific aquaria which restore pressure and temperature conditions prevailing at depth *in situ*. Studies of live fauna under controlled experimental conditions may then be undertaken, thereby offering unique opportunities to understand how deep-sea species respond to environmental perturbation. Such ecophysiological studies are urged by growing evidence that human activities exert serious impact on the Deep Sea [28].

Here we describe the pressure aquarium named "Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds", or IPOCAMP. The general principle of this equipment is inspired from pressure devices designed by Childress [26], which provided a constant renewal of seawater at working pressure (so-called "flow-through" system). Based on this principle, IPOCAMP aimed at higher working pressures, capable of simulating situation at 3000 m depth, while offering a large working volume (19 litres) and aperture (20 cm diameter). Additionally, IPOCAMP offers the possibility to record visual observations of the pressurized samples. We here provide an overview of IPOCAMP's features, and present the studies which were achieved using this pressure equipment, with a particular focus on thermal biology of deep-sea vent fauna. This field of study illustrates well the important contribution of *in vivo* experiments to our understanding of vent ecology (a complete overview of hydrothermal vent fauna and ecology may be found elsewhere [14]). Indeed, early *in situ* temperature measurements had suggested that some vent organisms were exceptionally thermophilic [3, 6]. Surprisingly, direct *in vivo* tests revealed that outstanding thermal limits were not a pre-requisite for life at the hot end of the hydrothermal habitat, other traits such as appropriate behavioural and molecular responses were more likely involved [29-32].

II. HISTORY OF THE IPOCAMP PROJECT

Born in 1997, the I.P.O.C.A.M.P. project aimed at providing the French community of deep-sea vent biologists, with means of simulating depths of up to 3000 m, in order to investigate *in vivo*, the biological responses of fauna originally from 1000 m depths or deeper, with respect to environmental stimuli. The IPOCAMP 1 prototype was constructed in 1999 and first tested during the HOPE cruise (1999, Biology of East Pacific Rise vent fauna). Its success during the cruise led to the construction of IPOCAMP 2 which was first used during

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the ATOS cruise (2001, Biology of Mid-Atlantic Ridge fauna). Both devices were later used throughout 6 cruises, all focused on the Biology of Hydrothermal fauna: PHARE (2002), BIOSPEEDO (2003), MESCAL (2010, 2012) for the East-Pacific Rise, EXOMAR (2005), MOMARDREAM (2007) for the Mid-Atlantic Ridge. Today, 3 more IPOCAMP aquaria are being operated in other marine research institutes: The National Oceanographic Centre of Southampton (NOCS) (IPOCAMP n°3, 4, since 2002), and the University of the Azores (IMAR-DOP) (IPOCAMP n°5, since 2007).

III. DESCRIPTION OF THE IPOCAMP PRESSURE AQUARIUM

IPOCAMP is a large pressurized container (about 19 litres), which may function at pressures up to 30 MPa (equivalent to approx. 3000 m depth), and at temperatures ranging from 2 to 100°C. IPOCAMP is a flow-through system which circulates seawater, with flow rates that may reach 20 l/hour. It is designed to maintain live deep-sea fauna, therefore allowing *in vivo* experimentation. IPOCAMP permits to visually control the content of the pressurized vessel while in function. Schematically, the system divides in 5 parts (see Fig. 1):

1. A seawater feeding line, which provides filtered water to the system, at atmospheric pressure. A high-pressure pump pressurizes the water, which is further circulated through a heat-exchange device prior to its entry in the vessel.
2. A stainless Steel 19-litre vessel, providing a experimental chamber of 20 cm diameter, and 60 cm height. It is equipped with an O-ring closing lid, and with temperature-regulating outer envelopes. Four viewports in the lid each provide a 1cm optical diameter, for visual access to the experimental chamber. Its Maximum Working Pressure (MWP) is 30 MPa at 100°C, and its test pressure is 48 MPa at room temperature.
   The vessel is made of 316 Ti Stainless steel, and is composed of 2 main parts (lower and upper) which are assembled by 12 screws, and sealed together by an O-ring. The lower part bears a 1/4 inch connection allowing seawater inlet. The upper part bears 7 connections: two of 1 inch diameter, situated on the lower half, and five 1/4 inch connections. One of the latter is designed to allow water outlet, whereas the four others, and the two larger connections, are blocked with plugs, and available for further developments.
3. A downstream line, equipped with a manually-operated back-pressure valve, designed to set and control the working pressure. The circulating water returns to atmospheric pressure once past the back-pressure valve.
4. A temperature-regulation unit, designed to circulate cooling/heating fluid around the experimental vessel (2) and the feeding line (1). 2 K-type probes measure the temperature of the seawater when it enters the vessel (upstream), and when it leaves it (downstream). These probes are connected to LCD readings on the general electrical supply box.
5. A visualization system: an endoscope, coupled to a camera, a TV monitor and a video-recorder. Visualization of the experimental chamber is achieved vertically, through viewports that are disposed in the lid of the pressure vessel (2). Illumination is also achieved through these windows, by means of optical-fiber light-guides (Fig. 2).

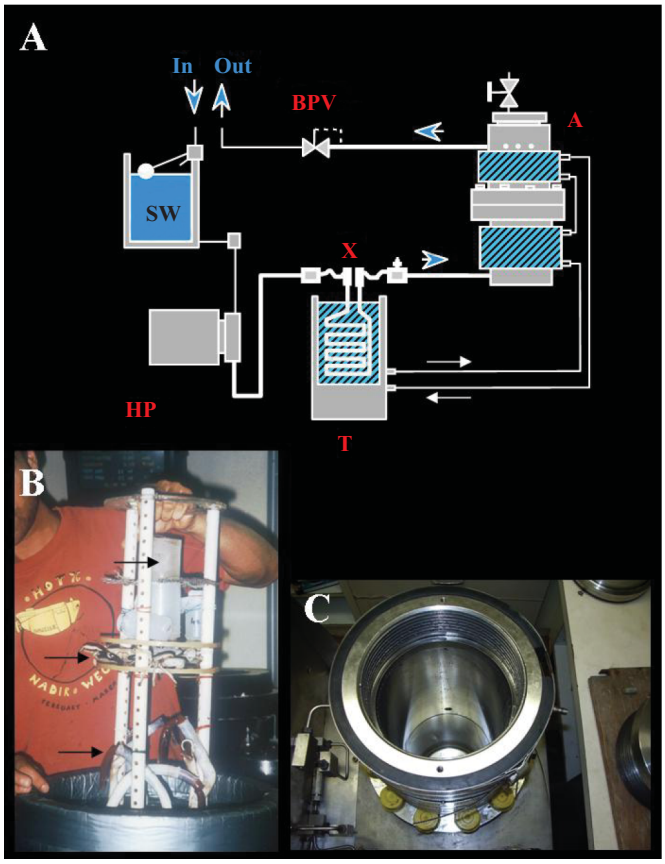

Fig. 1. A: Schematic representation of IPOCAMP. A seawater tank (SW) provides filtered water to the system, at atmospheric pressure. A high-pressure pump (HP) pressurizes the water, which is further circulated (blue arrows) through a heat-exchange device (X) prior to its entry in the 19 litre-vessel (A), which is equipped with temperature-regulating outer envelopes. The circulating water returns to atmospheric pressure once past the back-pressure valve (BPV) which is designed to set and control the working pressure. A temperature-regulation unit (T) circulates cooling/heating fluid (white arrows) around the aquarium and the heat-exchange device (hatched areas). B: An internal sample-holder allows maintenance and separation of different biota, from bottom to top (arrows): Riftia tubeworms, Bythograeaid crabs, and a PVC cage for video survey of smaller animals (see Fig. 2). C: IPOCAMP's internal volume is 20 cm diameter, for 60 cm height.

In addition, the IPOCAMP system is designed to allow mounting and un-mounting in order to facilitate transport between land-based and ship-based laboratories.

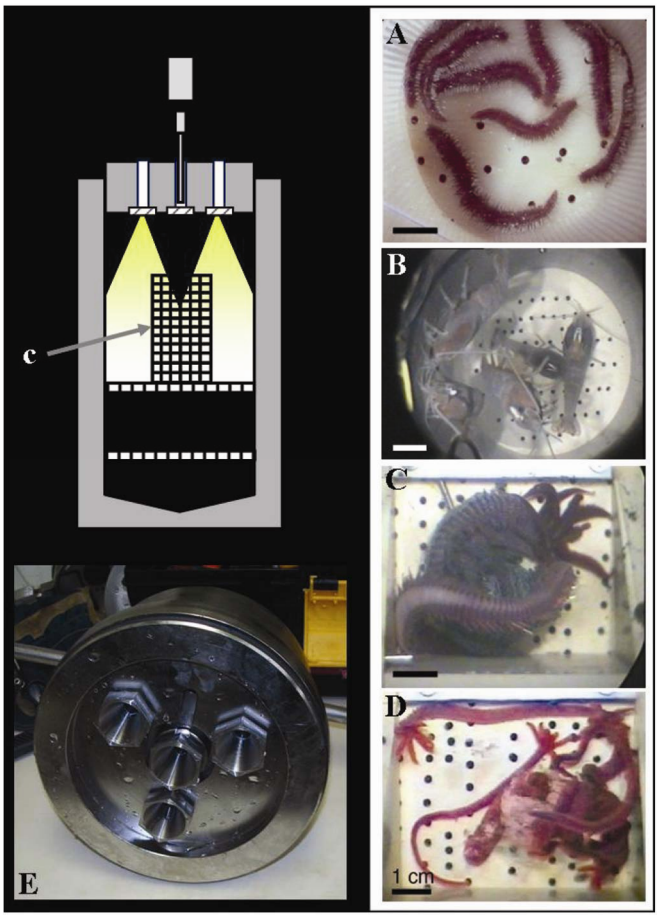

Fig. 2. IPOCAMP allows to observe and record the behaviour of re-pressurised animals, by combining an endoscope, a high-sensitivity camera, and a TV monitor. Illumination is provided by flexible optical-fiber lightguides. Endoscope or lightguides are positioned in front of the viewports which are situated in the IPOCAMP lid. Animals may be kept in the field of view by using experimental cages (c). A: *Hesiolyra bergi*, 26 MPa pressure (adapted from [32]). B: *Rimicaris exoculata*, 23 MPa pressure. C: *Alvinella pompejana*, 26 MPa pressure. D: *Paralvinella grasslei*, 26 MPa pressure (adapted from [9]). Scale bars are 1 cm. E: The IPOCAMP lid (lower side, facing the water), showing 4 viewports.

IV. BIOLOGICAL STUDIES USING IPOCAMP

1. Thermal Biology of Deep-Sea Vent Metazoans

IPOCAMP has allowed to test the biological responses of some deep-sea hydrothermal vent animals, with respect to experimentally imposed hyperthermia. IPOCAMP has also been used for various other purposes, which are presented further in this manuscript. The general picture emerging here is that deep-sea vent animals are definitely adapted to temperatures much higher than those encountered in the bulk of the deep sea (i.e. in the 1 to 3°C range). However, as far as thermal limits are concerned, the animals we have studied so far are not quite as exceptional as once presumed in the early days of hydrothermal vents' discovery, in fact many shallow-water species stand the comparison very well. The East-Pacific Rise worm *Hesiolyra bergi* and the Mid-Atlantic vent shrimp *Rimicaris exoculata* are two animals which appear to live at the "hot end" of the hydrothermal habitat. The first cited lives within the colonies of alvinellid worms [32], and may be observed wandering in and out of *Alvinella* sp. tubes, a micro-habitat where sustained temperatures exceeding 60°C have been reported [3, 6]. The second cited forms swarms of thousands of individuals [29, 30] which seem almost in contact with the pure (and hot) hydrothermal fluid emitted by black smokers. Still, our group has shown that these animals do not survive sustained temperatures above 40°C, meaning that biochemical functioning at exceptionally high temperature is not a pre-requisite for fauna inhabiting the "hot end" of the vent biotope. Efficient behavioural responses may be sufficient to allow avoiding intense heat bursts, and if not, biochemical repair responses may allow to withstand the consequences of short-term exposures to such bursts.

Additional data on the temperature resistance (CTmax) and lethal temperature (LT50: exposure of a given duration, at a given temperature, leading to 50% mortality) of another vent shrimp, *Mirocaris fortunata* were provided [34]. As for *Rimicaris* and *Hesiolyra*, the critical thermal maximum is lower than 40°C, which is less surprising for this particular species. Indeed, *Mirocaris* is rarely observed close to hot emissions, and more likely seen scavenging upon mussel beds, at cooler temperatures. This particular work pleads for the use of pressurised equipments, by the determination of LT50s, which illustrates very simply the fact that *in vivo* investigations should be carried out at *in situ* pressure, even when studying species which appear to tolerate quite well the collection trauma. The shrimps studied here were sampled from various depths along the Mid-Atlantic Ridge, 850 m (Menez Gwen site), 1750 m (Lucky Strike), and 2300 m (Rainbow site). When maintained in the cold room at atmospheric pressure on board, even the "deepest" of these individuals may survive several days. However, when exposing two pools of shrimps, differing by depth of habitat (850 m *vs*. 2300 m), to the same set of experimental temperatures (10-16-21 °C), the mortalities are much more important for the deeper-originating specimens [34].

Beyond the measurement of lethal thermal limits, and in order to understand the complex relationship between these fauna and their thermal environment, the broad molecular response of vent fauna upon hyperthermia was investigated [2, 11]. Investigations focused on the heat-shock protein response were also undertaken [10-12, 30, 31]: identification of Heat Shock Proteins (HSPs) and quantification of their expression after heat stress, using molecular biology methods. In Ravaux *et al*. [30], The aim was to quantify the HSP70 response (HSP70 is universally one of the major heat shock proteins involved in the heat shock response) of the shrimp *Rimicaris exoculata*, after a temperature exposure (30°C for one hour, using the pressure vessel IPOCAMP) only a few degrees below the critical thermal maximum (35 to 38°C).

The results are compared to measurements achieved on shrimps freshly collected at their natural pressure, using the isobaric collection device PERISCOP [35]. A comparison with coastal shrimp species was also undertaken [11]. The vent shrimp's response is important after the experimental heat exposure, and although this may be efficient with respect to heat damage, it might not be viable regarding energy budgets for "housekeeping" functions, should this type of exposure be too frequent. *In situ* observations suggest that these shrimps encounter temperatures very near (or above) their lethal limits, but results from isobaric collection suggest that *Rimicaris* is not heat-stressed *in situ*. Overall, this work points at exposure duration being crucial in terms of minutes or perhaps seconds, picturing these creatures as thermal "acrobats" constantly oscillating between hot and cold.

2. Other Studies

IPOCAMP has been used for various studies of deep-sea vent fauna. One study investigated the salinity tolerance and the pattern of osmotic and ionic regulation of *Bythograea thermydron*, a brachyuran crab endemic to the deep-sea hydrothermal vent habitat [20]. Further work investigated this crab's ability to regulate glycemia [38]. Other vent crabs (*Segonzacia mesatlantica*) were studied for their respiratory adaptations [4, 5].

Studies on the larval development of the vent annelid *Alvinella pompejana* were also carried out, using both IPOCAMP and smaller pressure incubators [24, 25], while the survival of this animal after collection was also evaluated for the first time [33]. Ecotoxicology studies of vent fauna were also achieved: effect of exposure to heavy metals on antioxidant activity and expression of metal-binding protein of vent mussels (*B. azoricus*), [1, 7, 8], or evaluation of DNA damage upon exposure to hydrogen peroxide in the case of vent polychaetes [16]. In the case of the vent tubeworm *Riftia pachyptila*, IPOCAMP incubations at *in situ* pressure improved the subsequent isolation of bacteriocyte cell suspensions, in comparison to isolations achieved just after *in situ* collection [13].

In addition to investigations of thermal biology, the vent shrimp *Rimicaris exoculata* was also studied regarding the epibiotic microbial community it hosts in both its gill chamber and guts, following incubations in IPOCAMP in different conditions [17, 23, 39]. These studies revealed an original nutritional mode from epibionts to host, with organic compounds being transferred through the tegument of the shrimp's gill chamber, as opposed to a classic gut transit.

Finally, recent studies conducted at the National Oceanography Centre of Southampton (IPOCAMP n°3 and 4) have focused on the pressure tolerance and biological responses of non-vent species, such as the amphipod *Stephonyx biscayensis*, with some experiments also including shallow-water coastal fauna: the shrimp *Palaemonetes varians* [22], the crab *Maja brachydactyla* [37], or the hermit crab *Pagurus cuanensis* [36]. Various biological responses to pressure were here examined, but a general trend observed in these works is that these coastal species tolerate pressures significantly higher than those encountered in the wild.

V. CONCLUSIONS

Laboratory- or ship-based pressure aquaria such as IPOCAMP offer many experimental perspectives, by allowing "pressure resuscitation" of freshly collected deep sea fauna, provided that exposure to atmospheric pressure does not last too long. Unfortunately, many deep-living creatures avoid *in vivo* investigation, due to lethal decompression effects upon sampling. Addressing these issues requires that target species are recovered at their natural pressure, by using isobaric collection chambers, before transferring these fauna towards experimental facilities, without pressure loss. As mentioned previously, our group has designed and tested a pressurised recovery device named PERISCOP [35]. This prototype aims at making pressurised recovery become a more efficient and practical process, while also envisaging the transfer of freshly caught animals, without decompression, towards a larger ship-based pressure aquarium named BALIST, which was recently used to study the thermal biology of the vent worm *Alvinella pompejana* [31]. Such developments are urged by the growing impact of human activities on the deep sea [19, 28], and by the risk of extinguishing species [15], before science has a chance to discover their existence, let alone study their biology.

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