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MANAGING IMPACTS OF DEEP SEA RESOURCE EXPLOITATION

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Assessment report on the use of avoidance behaviour monitoring for real time impact assessment of mining activity

Deliverable 3.3

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Assessment report on the use of avoidance behaviour monitoring for real time impact assessment of mining activity

Foreword

This report presents several studies and associated instruments. The description of these studies were deliberately kept short, to preserve the clarity of the report. However, the entire set of cited references are available upon request from the author of this report: Bruce Shillito, e-mail: Bruce.Shillito@upmc.fr

Abstract

Using *in situ* remote monitoring (video, still photographic imagery) of avoidance behaviours of macro- and megafauna may provide ‘early warning’ evidence of the impacts of mining on hydrothermal ecosystems. Pioneering deep-sea observatories at vents have installed video-recording modules that provide essential information on natural behaviour of native species; this report shows is that *in situ* observations should be completed by experiments and analyses on live samples of these fauna. Laboratory investigations on live vent fauna are described, some of which are still underway and supported by the MIDAS project. The different instruments and facilities supporting such studies are also presented. The report concludes on the necessity of *in situ* visual monitoring, combined with a more generalised review of equipment allowing studies of live fauna at the laboratory. A final point calls for new generations of deeper- and longer-operating pressure instruments, to improve sampling and studies of live vent fauna.

Contents

1. Introduction

An essential outcome of MIDAS will be to identify low cost methods to monitor the ecological impact of mining in real time. These recommendations will be essential for the production of explicit recommendations in WP10. For real-time monitoring of mining activity, remote monitoring (video, still photograph imagery) of avoidance behaviours exhibited by macro- and megafauna may well provide 'early warning' evidence of toxic effects or other disturbance impacts of mining. Such *in situ* monitoring approaches have recently been transposed to the deep ocean, thanks to pioneering multidisciplinary observatories at mid-ocean tridges (reviewed in Juniper et al., 2007). However, a key point in this report underlines the necessity of complementing field monitoring with laboratory-based studies of live deep-sea fauna, to fill fundamental gaps in our knowledge of these remote ecosystems.

This report focuses on hydrothermal vent macrofauna of the Mid-Atlantic Ridge (one of the target zones for mining activity), and their possible responses to disturbance (behavioural, physiological, molecular levels). Macrofaunal assemblages at Mid-Atlantic vent fields are dominated in abundance by vent mussels (*Bathymodiolus azoricus* and *B. puteoserpentis*) and alvinocaridid shrimp (*Rimicaris exoculata* and *Mirocaris fortunata*) (**Figure 1**, Desbruyères et al. 2000, Desbruyères et al. 2006, Sarrazin et al. 2015). These assemblages are distributed along a gradient of fluid flow intensity, with shrimp-dominated communities colonising warmer habitats, while mussel communities colonise cooler microhabitats (Cuvelier et al. 2009, De Busserolles et al. 2009, Sarrazin et al. 2015). Due to their relatively large size, these species (and also others such as crabs, *Segonzacia* sp.) are readily observable *in situ*, and are therefore interesting indicators of the state of these faunal communities in response to environmental change (Matabos et al. 2015). Pioneering deep-sea observatories (Juniper et al. 2007, Sarrazin et al. 1997) have used direct *in situ* observations (i.e. cameras deployed on the seabed) to help understand the factors influencing community dynamics and species biology at vents as well as their underlying mechanisms (Bates et al. 2005; Chevaldonné and Jollivet, 1993; Grelon et al. 2006; Robert et al. 2012). However, these studies indicate the need to combine such *in situ* observations with laboratory investigations on species' behavioural, physiological and molecular responses under controlled conditions (van Dover and Lutz 2004).

Biological responses that can be observed or measured *in situ* (behavioural traits, levels of stress molecules, and/or toxicants measured on freshly-collected samples), need to be "calibrated" in the laboratory by exposing a set of live specimens to a range of controlled environmental conditions (Shillito et al. 2014). Several laboratory approaches may be envisaged that rely on the study of live samples, meaning the use of pressurised equipment (aquaria, sampling cells). Indeed, it is a well-known fact that freshly-collected deep-sea fauna (i.e. from depths exceeding 2000 m) are severely impacted by decompression and exposure to atmospheric pressure. The use of instruments to restore the pressure prevailing at depth is therefore a prerequisite for a variety of *in vivo* experiments (Pradillon et al. 2004 and Shillito et al. 2014 illustrate this variety). In the frame of the MIDAS project, several partners have made use of such facilities to study toxicology (partners 4, 23 & 25; Auguste et al. 2016), and symbiosis plasticity in vent model organisms, in response to environmental perturbation (partner 25; Szafranski et al. 2015; Duperron et al. 2016) (see Section 2). An overview of these pressure instruments and their necessary developments are provided in this report (Section 3).

In some cases, when considering vent fields of intermediate depth (less than 2000 m), sampled fauna may survive at atmospheric pressure for long times (weeks or months), thereby facilitating laboratory experiments, allowing larger numbers of samples and cheaper maintenance conditions. This is the case for some fauna from the Menez Gwen and Lucky Strike MAR vent fields (850 and 1750 m depths respectively), which allowed long-term maintenance and studies to be initiated (presented in Section 3; Kadar et al. 2005, Colaço et al. 2006, Smith et al. 2013, Matabos et al. 2015, Shillito et al. 2015). While these initiatives are valuable approaches and have been used in the MIDAS (see Section 2), it should be kept in mind that they are restricted to fauna of intermediate depth, and that many possible mining zones are likely to be well beyond 2000 m depth.

***Figure 1:** Close-up views of typical MAR vent macrofauna (copyright Ifremer): Left: shrimp gathering on a vent chimney, close to an active smoker. Right: Shrimp (*Rimicaris exoculata, Mirocaris fortunata*), mussels (*Bathymodiolus azoricus*), and crabs (*Segonzacia mesatlantica*). Sizes of the animals range from 1 to 5 cm.*

2. Experimental perspectives for laboratory studies of live animals

2.1 Combining behavioural studies *in situ* and at the laboratory

This section presents original work by Matabos et al. 2015. This work was undertaken a few months before the start of the MIDAS project, but the deep-sea sites and fauna, and the methods employed are directly linked to this report's main subject.

Identifying the factors driving community dynamics in hydrothermal vent communities, in particular biological interactions, are challenged by scientists' ability to make direct observations and the difficulty to conduct experiments in those remote ecosystems. As a result, little is known on species' behaviour and interactions, and how they in turn influence community dynamics. Interactions such as competition or predation significantly affect community structure in vent communities, and *in situ* imaging has successfully been used to gain insights in biological interactions and species behaviour, including responses to short-term changes in temperature or feeding strategies. In the work of Matabos et al. (2015), *in situ* and *ex situ* approaches were combined to characterise the behaviour and interactions among and between two key species encountered along the Mid-Atlantic Ridge (MAR): the shrimp *Mirocaris fortunata* and the crab *Segonzacia mesatlantica*. *In situ,* species' small scale distribution, interactions and behaviour were studied using the TEMPO observatory module (Sarrazin et al. 2007) deployed on the seafloor at the base of the active Eiffel Tower edifice in the Lucky Strike vent field as part of the EMSO-Açores MoMAR observatory (Cannat et al. 2011). TEMPO sampled 2 minutes of video several times a day from July 2011 to April 2012. One week of observations per month was used for 'long-term' variations, and the full video data set was analysed for January 2012. In addition, observations of crab and shrimp individuals maintained for the first time under controlled conditions in atmospheric pressure and pressurised aquaria (AbyssBox, at the Oceanopolis public aquarium in Brest) allowed better characterisation and description of the different types of behaviour and interactions observed in nature. While *in situ* spatial distribution pattern was stable over several months, both crab and shrimp species displayed a significant preference for mussel bed and anhydrite substrata, and preferentially occupied areas located in the fluid flow axis. The aggregation behaviour of *M. fortunata* resulted in the occurrence of numerous intraspecific interactions mainly involving the use of sensory organs (antenna/antennule) and fleeing behaviours when in contact or close to individuals of *S. mesatlantica*. The higher level of passiveness observed in the AbyssBox artificial environment compared to the *in situ* environment may be due to the lack of stimulation related to low densities of congeners and/or of sympatric species compared to the natural environment and the absence of continuous food supply, as both species displayed a significant higher level of activity during feeding time. This result emphasises the role of food supply as a driver of species distribution and behaviour. This original combination of *in situ* observations using cameras deployed on deep-sea observatories, with experimental set-up in pressurised aquaria, will help understand factors influencing community dynamics and species biology at vents, as well as their underlying mechanisms.

2.2 Laboratory exposures to toxicants

2.2.1 At in situ pressure, at sea *(ship laboratory, MIDAS work; Auguste et al, submitted)*

The aim of this MIDAS study was to assess the natural background levels of biomarkers in the hydrothermal vent shrimp *Rimicaris exoculata* and its response to copper exposure at *in situ* pressure (3 days at 30 MPa) as well as the effects of depressurisation and pressurisation of the high-pressure aquarium IPOCAMP, inside which the exposures took place. *R. exoculata* specimens were collected from the chimney walls of the hydrothermal vent site TAG (Mid Atlantic Ridge) at 3630 m depth during the BICOSE cruise in 2014. Given the important depth of sampling, isobaric sampling ("pressure recovery") using the PERISCOP cell was essential. Tissue metal accumulation was quantified in different tissues and a battery of biomarkers was measured. Data show a higher concentration of Cu in some tissues after incubation (details in Auguste et al., submitted, and in MIDAS Deliverable 3.2). Significant induction of metal-exposure biomarkers was observed in the gills of shrimps exposed to copper compared to the control group. Moreover, enzyme activity was detected for the *in situ* group showing a background protection against metal toxicity. Results suggest that the proposed method, including a physiologically critical step of pressurising and depressurising the test chamber to enable the seawater exchange during exposure to contaminants, does not affect metal accumulation or biomarker response, and may prove a useful method to assess toxicity to contaminants in deep-sea species.

2.2.2 At atmospheric pressure, at the Oceanopolis aquarium *(MIDAS work in progress, partners 23 and 25)*

The toxicant (copper) exposures described above were repeated (Partner 25), but this time with another vent shrimp species, *Mirocaris fortunata,* maintained at atmospheric pressure and a longer exposure time (10 days). In parallel, another shrimp, this time from coastal origin (*Palaemon serratus*) was submitted to a similar treatment for comparison. The advantage of this approach (as opposed to that described above in 2.2.1) is that longer exposures were achievable, mainly because there were no cruise-schedule constraints. Larger numbers of specimens were also available (10 individuals per treatment, as opposed to 3 in the shipboard study), but it should be noted that this may not always be the case given the need to maintain a sufficient stock of live specimens for public exhibition. As for the *Rimicaris* shipboard pressure experiment (Section 2.2.1), the samples were later processed for various analyses (Partner 23).

2.2.3 At atmospheric pressure, at the LabHorta facilities *(Compani et al, 2004, 2006)*

Unlike the two methods mentioned above, the work from Company et al. (2004, 2006) was carried out before the MIDAS project started. However, it is important to mention this pioneering work because this was the first attempt to achieve toxic incubations in live vent fauna, using IPOCAMP to simulate *in situ* pressure, back on shore at the LabHorta facilities (see Section 3.2.1 in this report) in the Azores (partner 5, 6, 23 and 25 contributed to this work). Carried out in the frame of EC project VENTOX (FP5, Contract No. EVK3-CT1999-00003), the study focused on vent mussels (*B. azoricus*) from the Menez Gwen, which were incubated under different copper, cadmium, and mercury concentrations in seawater. Results showed that vent mussels possess antioxidant enzymatic protection in the gills. Cd and Cu had an inhibitory effect in the enzymatic defence system, contrarily to Hg. These enzymatic systems are not completely understood in *B. azoricus* since reactive oxygen species might be produced through other processes than natural redox cycling, due to hydrogen sulphide and oxygen content present.

2.3 Electrophysiology

One physiological skill that might be adversely affected by sulphide excavation at vents is chemical sensing, which is crucial in mediating important patterns of behaviour for Crustaceans, such as social interactions, location and evaluation of food, navigation and perception of the habitat (Rittschof et al., 1992). The sensory abilities of hydrothermal species may play a major role in their life cycle, dispersion, maintenance and long-term evolution. They face recognition of active hydrothermal emissions both as adults to locate fluids that contain reduced chemicals compounds for their symbiotic bacteria, or as larvae when they disperse in the water column to find new sites to settle in. A commonly accepted potential attractant is the chemical composition of the fluid. In decapods, the chemoreceptor neurons located in the antennules are responsible for the primarily mediate responses to odorant chemicals (Ache and Derby, 1985). Only one paper was published on chemoreception on hydrothermal vent species, on the vent shrimp *Rimicaris exoculata* (Renninger et al., 1995), and suggested with electrophysiological recordings from the antennal nerve that it could detect sulphides and bacterias homogenates via olfaction. We developed a modified electroantennography (EAG) technique for shrimp, originally used for Insects (Schneider, 1957), to record the electrical field emitted by the chemoreceptor neurons (**Figure 2**). This method allows us to measure the global olfactory response of the antennules to chemical stimuli, and so to test which fluid compounds hydrothermal shrimp are able to detect via olfaction. Preliminary results showed that the vent shrimp *Mirocaris fortunata* can detect iron, manganese and sulphide solutions via the antennules. The technical validation of EAG on the *Mirocaris* model (Machon et al. in prep., partner 25) opens a field of possibilities to further explore hydrothermal shrimp chemosensory detection modalities, as well as thermoreception (Puri and Faulkes, 2015), and thus to better understand which parameters play a key role in the perception of the environment in deep sea shrimp.

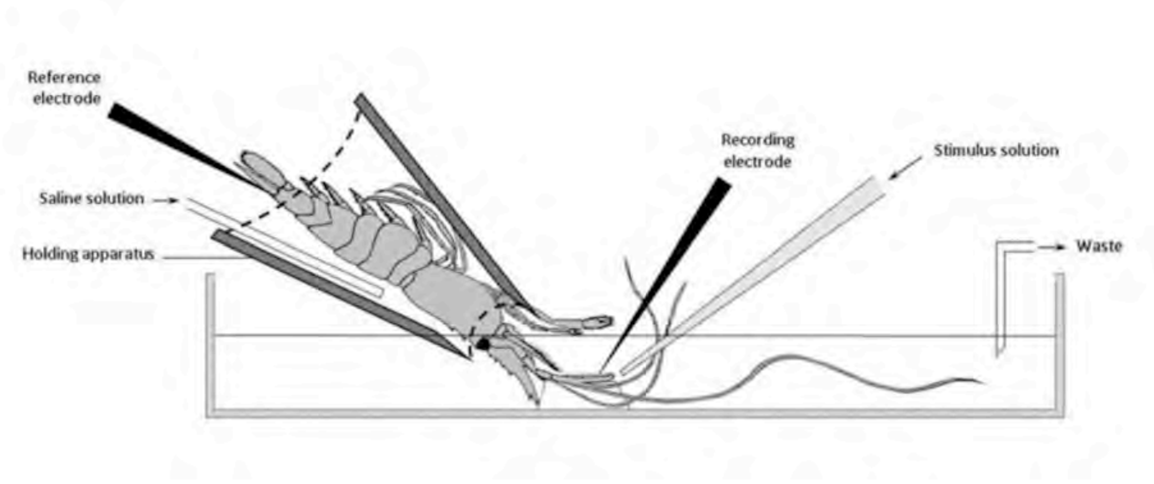

***Figure 2**: Experimental design for Electro-Antennography of live shrimp (Machon et al, in prep.)*

2.4 The study of symbiotic plasticity

The deep-sea mussels *Bathymodiolus azoricus* and *B. puteoserpentis* dominate hydrothermal vent fauna in the Azores region. Results presented here are a first step towards integrating the dynamics of mussel symbioses into the broader picture of vent ecosystem functioning, and provide a basis for the future evaluation of potential impacts of deep-sea exploitation on endemic symbiotic metazoans (Moskvitch 2014). The gills of this species house methane- and sulphur-oxidising bacteria that fulfill most of the mussel's nutritional requirements. Previous studies suggested that the ratio between methane- and sulphur-oxidisers could vary in response to the availability of electron donors (methane and sulphide) in their environment, and this flexibility is considered a key factor in explaining the ecological success of vent mussels. Attempts were made to quantify and characterise this flexibility, but previous studies were based on non-isobaric recovery of specimens, with experiments at atmospheric pressure, which may have induced artefacts. This study investigates the effect of pressure-related stress during recovery and experimentation on the relative abundances of bacterial symbionts. Mussel specimens were recovered for the first time using the pressure-maintaining device PERISCOP. Specimens were subsequently transferred into pressurised vessels and exposed to various chemical conditions. Using optimised fluorescence in situ hybridisation-based (FISH) approaches, relative abundance of symbionts were measured. The results show that the recovery method (isobaric versus non-isobaric) does not influence the abundance of bacterial symbionts. Significant differences occur among specimens sampled from three contrasting sites, with those from methane-rich sites displaying higher abundances of methanotrophic symbionts versus sulphide oxidisers. Exposure of mussels from the deeper site (Rainbow, 2300 m) to sulphide and bicarbonate, and to bicarbonate alone, both resulted in a rapid and significant increase in the relative abundance of sulphur-oxidisers. Results reported herein are in line with those from previous reports investigating mussels originating from shallow sites and kept at ambient pressure. Isobaric recovery and maintenance allowed *in vivo* experiments to be performed on specimens from a deeper site that could not be maintained alive at ambient pressure, and will greatly improve the chances of identifying the molecular mechanisms underlying the dialogue between mussel hosts and their symbionts.

The second Mid-Atlantic Ridge vent mussel species *Bathymodiolus puteoserpentis* also hosts sulphur- and methane-oxidising bacteria in its gills; however this species occurs at depths exceeding 3000 m, significantly deeper than its congener *B. azoricus*. In this study undertaken within the MIDAS project, pressurised recovery and incubations in pressure vessels were therefore mandatory and were used to test whether *B. puteoserpentis* displayed similar behaviour in the presence of symbiont substrates.

Symbiont relative abundances were analysed using fluorescence *in situ* hybridization and qPCR, and results indicate a slower variation of symbiont densities than in *B. azoricus*. Total gill surface areas and total bacterial numbers in specimens were estimated for the first time. Symbiont-bearing mussels display exchange surfaces about 20-fold higher than those found in similar-sized coastal mussels, and mean bacterial numbers of $2.5*10^{12}$ per specimen were estimated. This emphasises that symbiotic mussels are a major reservoir of bacteria in vent ecosystems, and should not be neglected in impact studies. The flexibility of symbiont abundances in response to environmental variations may provide a promising biomarker in order to evaluate the resilience and capacity of Bathymodiolus mussels to cope with potential impacts of deep-sea exploitation.

2.5 Studying the thermal biology of MAR vent fauna (thermal limits, preferendum)

These studies were undertaken before the MIDAS project (by partners 4, 5, 25, overview in Shillito et al. 2014). However, they illustrate how *in vivo* lab experiments can highly complement *in situ* observations. Behavioural studies are very important here, and the advantage over *in situ* observations is that environmental parameters inside an experimental mesocosm are accurately known and controlled. Furthermore, the importance of temperature in biology and ecology, added to the likely issue of thermal perturbation due to mining activities, demand improvement in our knowledge of thermal biology of vent fauna.

The general picture emerging here is that deep-sea vent animals are definitely adapted to temperatures much higher than those encountered throughout most of the deep-sea environment (1 to 3 °C range). However, as far as thermal limits are concerned, vent animals are not quite as exceptional as once presumed in the early days of hydrothermal vent discovery, in fact many shallow-water species stand the comparison very well. The Mid-Atlantic vent shrimp *Rimicaris exoculata* appears to live at the “hot end” of the hydrothermal habitat, almost in contact with the pure (and hot) hydrothermal fluid emitted by black smokers. Still, *in vivo* experiments in pressurised aquaria show 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 (Ravaux et al. 2003). Efficient behavioural responses may be sufficient to allow avoiding intense heat bursts, and if not, biochemical repair responses may allow the species to withstand the consequences of short-term exposures to such bursts.

Beyond the measurement of lethal thermal limits, and in order to understand the complex relationship between these fauna and their thermal environment, the molecular response of *Rimicaris exoculata* upon hyperthermia was investigated. The heat-shock protein response of this shrimp was quantified, after a sub-lethal temperature exposure. The results are compared to measurements achieved on shrimps freshly collected at their natural pressure, using isobaric collection (PERISCOP, Ravaux et al. 2009). Again, while *in situ* observations suggest that these shrimp encounter temperatures very near their lethal limits, the 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, revealing these creatures as thermal “acrobats”, constantly oscillating between hot and cold.

Data on the temperature resistance (CTmax), lethal temperature (LT50: exposure of a given duration, at a given temperature, leading to 50% mortality), and thermal preference of another MAR vent shrimp, *Mirocaris fortunata,* were also provided. Its preference was determined by monitoring the behaviour of shrimp positioned in an experimental thermal-gradient, at atmospheric pressure (at the National Oceanography Centre in Southampton, see section 3.2.2), and was found to be about 19 ºC (Smith et al. 2013). *Mirocaris fortunata's* critical thermal maximum is about 36°C, and was measured by observing the onset of specific behavioural responses (loss of equilibrium and/or onset of spasms) at *in situ* pressure (use of IPOCAMP pressure aquarium see section 3.3.1, and Shillito et al. 2006). Studies on this species molecular response to hyperthermia have yet to be undertaken.

To conclude on thermal biology studies of vent fauna and to highlight the importance of laboratory *in vivo* investigations to complete *in situ* work, the reader is reminded that the early (and long-lasting) vision of "outstanding" thermophilic fauna was built from *in situ* observations and measurements, until laboratory experiments on live animals came along to moderate this belief. In 2012, after more than 20 years of debate (Chevaldonné et al. 2000), Ravaux and collaborators (2013) used isobaric sampling and transfer for studying live specimens of the vent tubeworm *Alvinella pompejana*. While early *in situ* observations (Chevaldonné et al. 1992) further backed up by *in situ* measurements (Cary et al, 1998), had proposed that this East-Pacific Rise annelid thrived at sustained habitat temperatures of 60°C or more, Ravaux et al. (2013) showed that it did not survive short thermal exposures above 50°C.

3. Instruments for *in situ* and laboratory studies

3.1 The Momar Mid-Atlantic Ridge deep-sea observatory

The Lucky Strike vent field (371180 N, 321160 W) is a well-known field with a central lava lake surrounded several active hydrothermal edifices (Sarrazin et al. 2014 and references therein). In the south-eastern section of the field, the Eiffel Tower hydrothermal edifice is one of the most visited sites in the vent field, and has been the subject of several ecological studies (De Busserolles et al. 2009; Cuvelier et al. 2009). The base of the Eiffel Tower edifice was extensively monitored during the MoMARETO 2006 cruise and deemed suitable for deployment of the MoMAR interdisciplinary deep-sea observatory. Primarily operated by French research teams, MoMAR is an international programme aiming at monitoring hydrothermal vent processes at the MAR. This observatory combines long-term observations (see Matabos et al. 2015 in section 2.1 of this report), detailed site studies and experimental work and includes a variety of seafloor and water column sensors. Collected data are periodically transmitted to a relay buoy moored nearby and further onshore via satellite. MoMAR has been active since 2006, with yearly maintenance cruises and regular upgrades (Cannat et al. 2011). These yearly cruises are of great importance to insure regular sampling opportunities for physiological studies of vent fauna. This was the case several times in relation to the AbyssBox project in Oceanopolis (see section 3.2.3 in this report), and in relation to the MIDAS project (see section 2.2.2 in this report). MoMAR recently joined the EMSO European programme as the EMSO-Açores observatory (www.emso-eu.org, EMSO stands for "European Multidisciplinary Seafloor and water-column Observatory").

3.2 Land-based aquaria for long term studies at atmospheric pressure :

Here we describe three initiatives that involve long-term maintenance of MAR vent fauna in shore-based laboratories. They were all initiated before the MIDAS project arose, but nevertheless directly involve scientists who are MIDAS partners. They demonstrate that it is possible to sample and bring back to land live hydrothermal vent fauna, thereby opening the way to experiments such as described in Section 2 of this report.

*3.2.1 **LabHorta** (detailed in Colaço et al 2006, Kadar et al, 2005, partner 6)*

Based in the Azores and funded by the European VENTOX (EVK3-CT1999-00003) programme and the Azorean Regional Directorate for Science and Technology, LabHorta infrastructures provided a pool of vent mussels (*Bathymodiolus azoricus*) originating from 850 m depth (the MAR Menez Gwen site, **Figure 3**), maintained alive for several months at atmospheric pressure. During the Momareto cruise (2001), mussels were first placed *in situ* in retrievable cages, which were to be later recovered, and brought to LabHorta facilities. Cages recovered in January 2003 contained mussels with ripe gonads while those recovered in July, August and November 2001 and in April 2003 did not. Mussels collected post-spawning in April 2003 spawned in the aquaria in January 2004. Young mussels recruited to the cages in April 2003. The data suggested a main single period of spawning and of juvenile recruitment for *B. azoricus* (from Colaço et al. 2006).

Figure 3*: The LabHorta facilities for live maintenance of vent mussels* B. azoricus *(from http://www.horta.uac.pt/projectos/fisiovent/labhorta.htm)*

To study cessation and re-establishment of symbiosis in vent mussels, another laboratory experiment was conducted over a period of 45 days in LabHorta. Animals were exposed to conditions lacking inorganic sulphur supply for 30 days (sulphur is vital for their symbionts), and then re-acclimatised in sulphide-supplied seawater for an additional 15 days. Gradual disappearance of bacteria from the symbiont-bearing gill cells was observed for animals deprived of sulphide. Following re-acclimatisation in sulphur-supplied seawater, proliferation of sulphur-bacteria in the mussels' gills confirmed the functionality of the sulphide-feeding system in supporting chemoautotrophic symbionts. This work reported the first laboratory set-up successfully used to maintain the hydrothermal vent bivalves *B. azoricus* for prolonged periods of time by supplying inorganic sulphur as an energy source for its endosymbionts. The methods reported in (Colaço et al. 2006 and Kadar et al. 2005) therefore represent great potential for future studies of the dynamics of symbioses and for post-capture experimental investigations at the laboratory (from Kadar et al. 2005).

3.2.2 National Oceanography Centre, Southampton *(Smith et al. 2013, partner 5)*

Specimens of *Mirocaris fortunata* were collected in September 2009, from the Lucky Strike vent field (371180 N, 321160 W) along the Mid-Atlantic ridge from a depth of 1617 m using the ROV Victor 6000 during the Bathyluck'09 cruise (Escartin and Cannat, 2009). While onboard, shrimp were maintained in seawater aquaria at atmospheric pressure and a constant temperature of 10ºC. The shrimp were transferred to the laboratory in Southampton and were kept in a temperature controlled laboratory at a temperature of 8 +/- 0.5ºC at the National Oceanography Centre, UK. Shrimp were acclimated to ambient pressure and maintained in complete darkness from September 2009 until experimental work began in November 2010 (Smith et al., see section 2.5 in this report), more than one year later.

3.2.3 The Oceanopolis public aquarium (Shillito et al. 2015; partners 4 and 25)

The AbyssBox project aims to provide the first permanent public exhibition of live deep-sea hydrothermal fauna maintained at *in situ* pressure (**Figure 4**). AbyssBox is a set of two pressurised aquaria (exposed to the public) designed to function permanently. Along with these pressure aquaria, a dedicated dark room (unaccessible to the public) provides a set of aquaria for maintenance at atmospheric pressure. Started in 2012, the exhibition has functioned for more than three years at the Océanopolis public aquarium in Brest, France. Vent shrimp (*Mirocaris fortunata*) and crabs (*Segonzacia mesatlantica*) were sampled from 1700 m depth at the Lucky Strike vent field (Mid-Atlantic Ridge) during different MOMAR cruises. Because of their moderate depth of origin, shrimp tolerate prolonged exposure at atmospheric pressure (see also Smith et al. 2013), but nevertheless experience significant mortalities in the first days following sampling. While mortalities exceeded 50% during the first days at atmospheric pressure, the remaining animals appeared to acclimate fairly well. For crabs, pressure maintenance proved mandatory. Some crabs have now been kept for more than 2 years, and some shrimp have spent more than 3 years in captivity. Primarily designed for a public exhibition, the AbyssBox may be used for scientific purposes (Oceanopolis collaborates with partners 4 and 25), since it provides one of the most effective tools for long-term rearing of deep-sea fauna. AbyssBox is a first step towards maintaining a variety of deep-sea fauna year-round at *in situ* pressure, which will serve both scientific and public interests. By providing access to a live stock of *Mirocaris fortunata*, Oceanopolis infrastructures significantly contributed to experiments of the MIDAS WP3 (partners 4, 23, and 25; see sections 2.1 to 2.3 in this report).

Figure 4 *(next page): The Oceanopolis facilities*

*Top: Front view of the AbyssBox (i.e. what is seen by the public), displaying vent crabs (*Segonzacia mesatlantica*) from the Lucky Strike vent field, 1700 m depth, maintained at in situ pressure. Size of the animals: about 4 cm carapace width.*

Middle: Dedicated darkroom for the maintenance of live stocks of the vent shrimp Mirocaris fortunata *at atmospheric pressure. In each tank, a heater wrapped in PVC foam plays the role of a "hotspot".*

Bottom: Close-up view of Mirocaris *specimens (from the Lucky Strike vent field) gathering on one of the heaters. Size of the animals: 1 to 4 cm length (photographic credits : D. Barthelemy, Oceanopolis)*

3.3 Pressurised instruments (sampling cells and aquaria) used in MIDAS

3.3.1 IPOCAMP pressure aquarium

Generally speaking, animal biology studies have always benefited from the achievement of experiments on live animals, which allow investigation of aspects such as behaviour 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 (MacDonald 1997; Shillito et al. 2004), and following exposure at atmospheric pressure. While some fauna originating from moderate depths may survive at atmospheric pressure (see the Oceanopolis and LabHorta experiments in this report), *in vivo* investigations on organisms from depths greater than 2000 m clearly require pressure simulation in experimental mesocosms. Provided such equipment is available, freshly collected deep fauna may be maintained alive and studied in good condition, thereby offering unique opportunities to understand how deep-sea species respond to environmental perturbation. he demand for such ecophysiological studies are supported by growing evidence that human activities may seriously impact the deep sea, mining prospection being only one of many threats (Ramirez-Llodra et al. 2011).

The pressure aquarium named "Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds", or IPOCAMP (**Figure 5**), is inspired from pressure devices designed by Childress (Quetin and Childress, 1980). It provides a constant renewal of seawater at working pressure (so-called "flow-through" system), capable of simulating 3000 m depths, 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. IPOCAMP was used for the MIDAS project (see sections 2.2 and 2.4). Shillito et al. (2014) provides an overview of IPOCAMP's features, and presents the numerous studies that were achieved using this pressure equipment. Seven IPOCAMP vessels are today available in four research institutes (Portugal, United Kingdom, Newfoundland, France), including three MIDAS partner instituions (partners 5, 6, 25). Although the IPOCAMP offers a unique combination of large volume and high pressure, it is likely that higher working pressures would be required to encompass all depths at which hydrothermal venting is encountered.

Figure 5*: The IPOCAMP pressure aquarium. Left: IPOCAMP #3, in service at the National Oceanography Centre (Partner 5). Right: IPOCAMP #1 (Partner 25), mounted onboard R/V "Pourquoi Pas?" (Ifremer) during a MoMAR cruise in 2012.*

3.3.2 PERISCOP pressure-recovery cell

While some organisms may be studied in pressurised aquaria, others remain inaccessible to *in vivo* investigation. 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 preclude *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 into experimental facilities without pressure loss. Partner 25 has designed and tested a pressurised recovery device named PERISCOP ("Projet d'Enceinte de Remontée Isobare pour la Collecte d'Organismes Profonds" (**Figure 6),** described in Shillito et al. 2008): It offers a 7-litre volume, 10 cm diameter aperture, and may be operated at depths of 3000m. This prototype aims at making pressurised recovery become a more efficient and practical process, while also enabling the transfer of freshly caught animals without decompression, into a larger ship-based pressure aquarium named BALIST, which was recently used to study the thermal biology of the vent worm *Alvinella pompejana* (described in Ravaux et al. 2013). PERISCOP has been used in about 60 dives across 6 cruises since 2006, and has contributed to MIDAS work (sections 2.2 and 2.4 in this report; Bicose cruise 2104; Auguste et al., submitted; Duperron et al, submitted; partners 4, 23, 25).

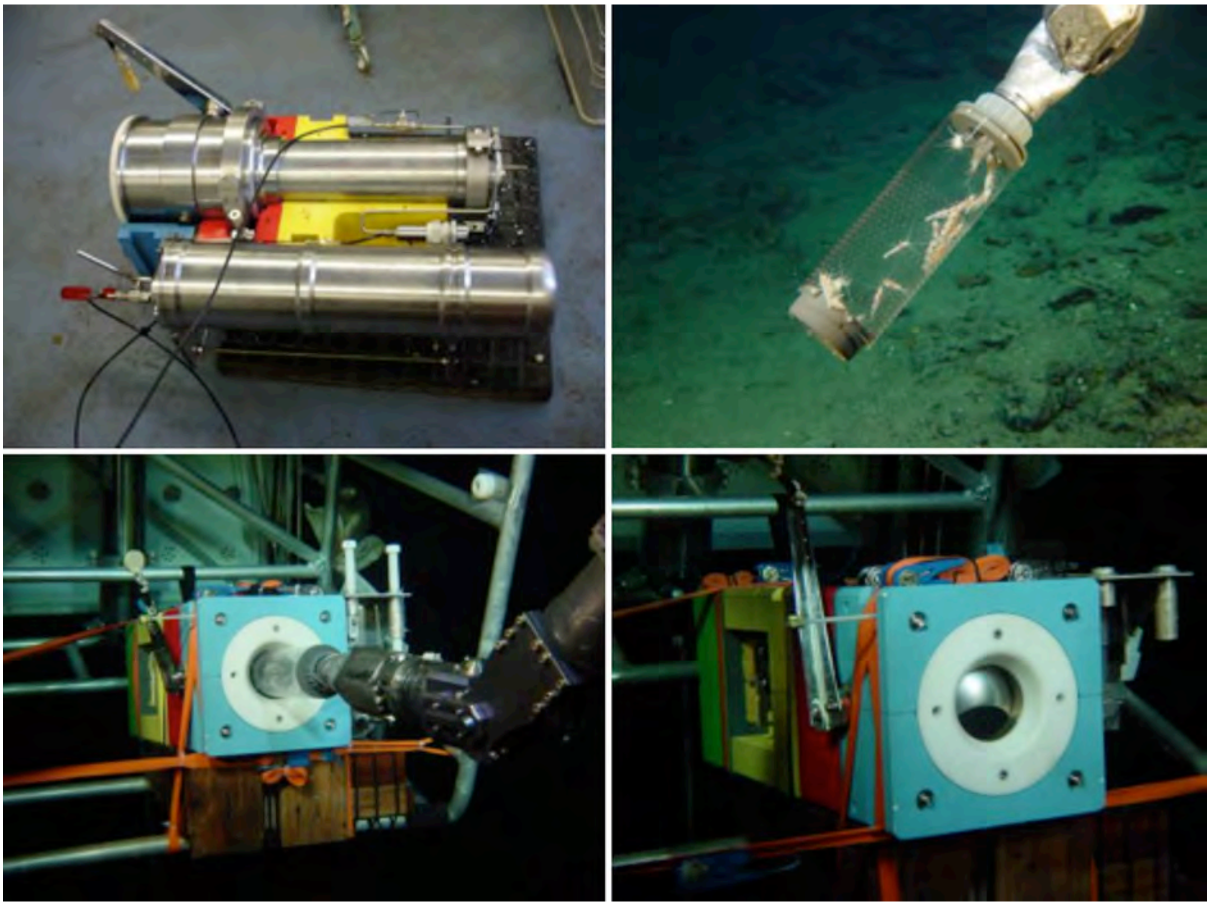

***Figure 6**: The PERISCOP isobaric sampling cell. Upper left: partially unmounted at the laboratory. Upper right: Vent shrimp are sampled in a transparent cell, using the submersible's suction device. Lower left: this transparent cell is further stored inside the PERISCOP, which has been moored on a shuttle device, at proximity of the sampling site. Lower right: Once the samples are stored inside the PERISCOP, its quarter-turn ball-valve may be closed prior to the ascent through the water column.*

4. Conclusions and recommendations

Deep sea observatories such as EMSO-Açores MoMAR are a vital step in our understanding of hydrothermal vent field dynamics, particularly in regard to future mining activities. They demonstrate scientists' ability to install and maintain video-monitoring facilities (such as the TEMPO module) over the longer term, and clearly improve our knowledge on these remote ecosystems. However, in terms of using these tools to give early warnings on ecosystem perturbation caused by mining activity, questions remain on the way such autonomous modules should be deployed: how many and where, with respect to a given extraction site? Implicit to answering these questions is the **need to better know the natural unperturbed state of vent ecosystems**.

***In situ* behavioural studies must be completed by sampling of fauna**. This would at first allow correlation of environmental perturbations to biological responses by working on live samples at the laboratory. In return, this could allow detection of perturbations on animals analysed directly after their sampling. Finally, *in situ* sampling also opens the possibility of investigating meiofauna, which are by definition not visible using *in situ* imagery, but are nevertheless very important in obtaining the global picture of a vent ecosystem.

Regarding the tools needed, **efforts should be made towards making *in vivo* experiments possible over a longer term** (weeks or months). When dealing with fauna of moderate depths (<2000 m), which may be studied for several months at atmospheric pressure, the initiatives described in Section 3.2 are very valuable, because they allow long-term studies to be carried out with relatively large numbers of live samples. A special mention should be made to acknowledge public sites such as the Oceanopolis aquarium, which play an important mediatic role in addition to adding scientific value, and thereby promote such initiatives.

Finally, pressurised tools have long demonstrated their potential to improve our knowledge on deep-sea biota. **The use of pressurised mesocosms** such as the IPOCAMP **should be expanded**, **and a new generation of similar instruments**, upgraded to higher pressures (to encompass deeper study areas, i.e. deeper than 3000 m) and adapted to long-term experiments, **should be promoted**. This would have to develop alongside a **more generalised use of pressure recovery** (isobaric sampling), with instruments such as PERISCOP (or "deeper" versions of such equipment), to ensure that sampled fauna reach the surface in a good physiological state.

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