
MoMARETO: A CRUISE DEDICATED TO THE SPATIO-TEMPORAL DYNAMICS AND THE ADAPTATIONS OF HYDROTHERMAL VENT FAUNA ON THE MID-ATLANTIC RIDGE

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The Mid Atlantic Ridge (MAR), located close to the Azores Triple Junction (ATJ), has been extensively studied over the past 15 years. Over ten cruises were conducted in the area, leading to the discovery of three major vent fields - Menez Gwen, Lucky Strike and Rainbow -, located on three segments of the south-eastern limb of the ATJ (Ondréas et al. 1997). The vent fields differ by their depths (850 to 2350 m), their tectonic settings, the composition of their host rocks (mantle-derived serpentinized peridotite vs. basalt), the nature of associated volcanism, their fluid composition and the dominance of different key faunal assemblages (Fouquet et al. 1998, Desbruyères et al. 2001).

The drop in hydrostatic pressure due to depth differences between the three fields directly affects the chemistry of high temperature effluents and their mineral particle content. At the shallowest site, Menez Gwen (850 m), phase separation of high temperature fluids produces a metal-depleted fluid and a gas-rich vapour phase (Charlou et

al. 2000, 2002, Douville et al. 2002). The emitted fluids are clear with a low mineral particle content. The geological structures are small, their height restricted to a few meters. Faunal communities are dominated by large patches of the bivalve *Bathymodiolus azoricus*. Important populations of the shrimp *Mirocaris fortunata* colonize active surfaces and live within the mussel assemblages.

Lucky Strike is one of the largest known active vent fields. The vent sites are distributed around a large lava lake at depths varying from 1650 to 1750 m (Fouquet et al., 1995). Well-defined hydrothermal edifices such as Tour Eiffel, Elisabeth and Bairro Alto, as well as diffuse flow areas, are scattered throughout the vent field. The composition of hydrothermal fluids varies significantly from site to site, suggesting the presence of two fluid sources (Charlou et al. 2000). Faunal communities are dominated by extensive mussel beds of *B. azoricus* partially covered by visible microbial mats. The vicinity of active high-temperature

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chimneys, flanges and cracks are colonized by *Chorocaris chacei*/*M. fortunata* shrimp assemblages (Desbruyères et al. 2001).

The Rainbow vent field (2350 m) is located on the deepest active segment of the ATJ area. The nature of the host rocks differs from the two other fields with the presence of ultramafic rocks. Hydrothermal emissions are characterized by acidic fluids enriched in iron and gas (H_2 and CH_4) and relatively impoverished in H_2S (Charlou et al. 2000, 2002). The relief of the field is particularly chaotic. Several sulphide edifice complexes can be found within the 400 m long field. The main hydrothermal structures are often surrounded by numerous thin pinnacles. The walls of large edifices are covered by swarms of *R. exoculata* and *M. fortunata* shrimp assemblages. Small *B. azoricus* patches, encrusted with particles, were sporadically found (Desbruyères et al. 2001).

The MoMARETO cruise was held from August 6 to September 6, 2006 on the new French oceanographic vessel *Pourquoi pas ?*, with the ROV Victor 6000. The cruise was

organised in two legs permitting fifty-six scientists to work on board. At two occasions, shuttles with the Portuguese oceanographic vessel *Arquipélago* allowed visits by scientists and journalists and the transfer of fresh biological samples to the LabHorta land-based laboratory installed in Fayal island (Azores). Although most of the dives were centred on Lucky Strike, two other vent fields, Menez Gwen and Rainbow, were also visited.

The main objective of the MoMARETO cruise was to study the spatial and temporal dynamics of hydrothermal communities colonizing the MoMAR zone, located on the ATJ. The first leg of the cruise was dedicated to the final integration and validation phase of 13 equipment prototypes developed during the European EXOCET/D project. A fine-scale bathymetric study of the Lucky Strike area was also conducted. The proposed approach for the second leg was to study the response of different hydrothermal species to their environment at two temporal scales: a very short-term response of organisms to habitat micro-variations (hours-days) and a longer observatory-type scale where the dynamics of faunal assemblages will be linked to broader-scale habitat variations (months-years).

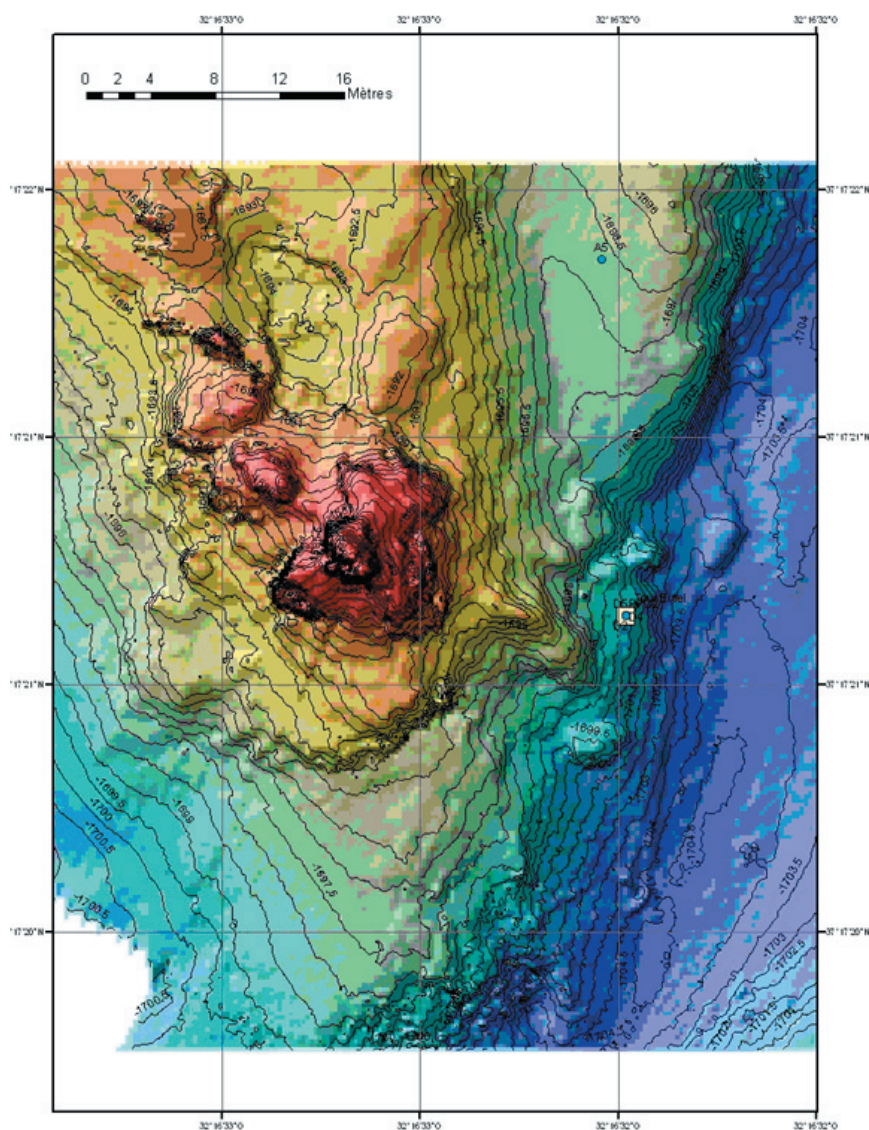


Figure 1: Raw bathymetric map of the 11 m high Tour Eiffel hydrothermal edifice, Lucky Strike vent field. The map was obtained during the MoMARETO cruise using a Reson 7125 multibeam echosounder mounted on Victor 6000 survey module. The transects were done at 8 m above bottom and the resolution attained is approximately 25 cm. The map was plot using the CARAIBES software.

Leg 1 - August 6-17

The first two dives of the cruise were used to gather microbathymetric data of the Lucky Strike vent field with the new survey module of the ROVVictor 6000. This module includes a Reson 7125 multibeam echosounder, an OTUS black-and-white high-resolution photo camera, and a Simrad EK60 fish echosounder. A first survey surrounding the lava lake was done at 30 m altitude (1200 m x 800 m). A raw 50-cm resolution map was obtained with the CARAIBES software. A second passage was done at 8 m to couple the microbathymetric survey with OTUS imagery. A complete coverage of the Tour Eiffel edifice was done (Figure 1). A first image mosaic mapped the distribution of faunal assemblages around the structure.

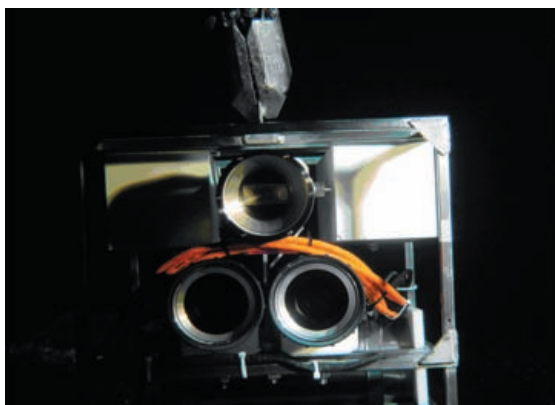
The next four dives were used to test different tools developed during the EXOCET/D project. The general objective of this project was to develop, implement, and test reliable and cost-effective technologies to study deep marine biodiversity in fragmented habitats. Three major themes were identified for instrument improvement and/or development: 1.1) quantitative imaging, 1.2) sampling and *in situ* measurements and, 1.3) faunal sampling and *in vivo* experiments.

1.1 Quantitative imaging

Two stereo-video camera, CAMEREO (Figure 2A) and IRIS, were developed and tested during the cruise. The underwater video imagery obtained by the cameras will be used to make projective 3-D reconstructions of small-scale scenes (Figure 3). This will not only improve the image mosaicking process but will permit the scaling of features present in the image (e.g. organism size and density) and to associate the precise positioning of *in situ* measurements to faunal distribution.

An advanced 3D viewer software (A3DV) was tested during the cruise. For this, real 3D data issued from IRIS stereo camera imagery were to be annotated and visualized by the A3DV. Unfortunately, 3D reconstructions from the stereo camera were too long to process on board and were not available during the trials. A compromise solution was found by using recently acquired bathymetric data as a 3D mesh. The newly-developed A3DV was able to visualize and annotate the 3D bathymetric mesh.

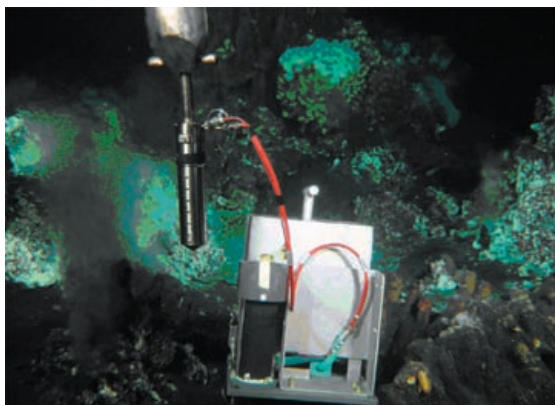
TEMPO, a long-term imaging module was developed to study community dynamics and patterns of succession in remote habitats. This module is composed of a deep-sea autonomous video camera and two LED projectors. The



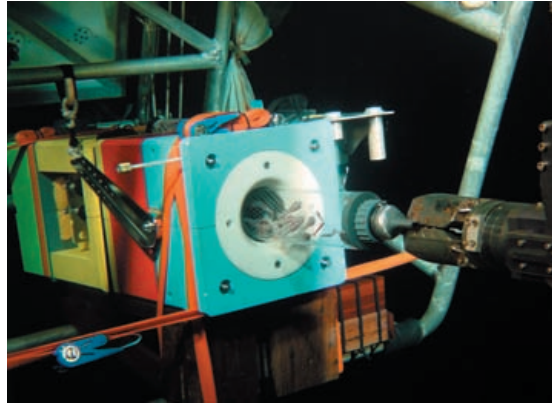
A.



B.



C.



D.

Figure 2: A. CAMEREO autonomous stereo-video camera (AWI, Germany). B. Isosampler (ISObaric SAMPLER) hydrothermal flux sensor (Earth-Ocean Systems Ltd, UK). C. AISICS instrumented microbial colonisation system (Ifremer, France) and D. PERISCOPE hyperbaric sampling device (Université Pierre et Marie Curie).

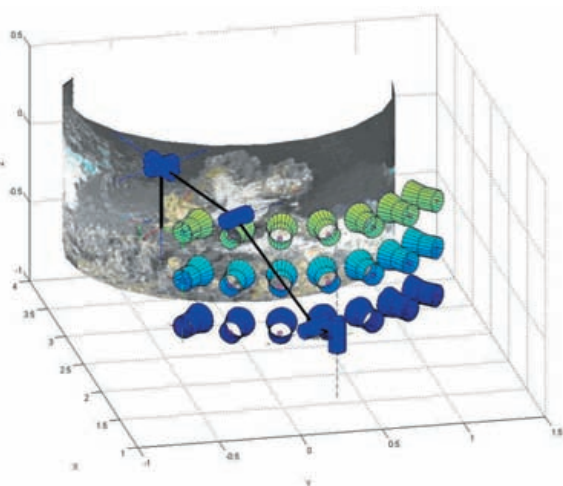


Figure 3: IRIS stereo-video camera (Ifremer, France) was developed to achieve the 3D reconstruction of small underwater structures. This system based on stereovision techniques is operated by Victor 6000 robotic arm. The image acquisition phase was done by visual servoing that allows a precise control of the camera position.

video camera is able to pilot the projectors and to record digital pictures on a hard disk. A biofouling protection, based on localized microchloration, was installed on the camera port hole and on the lights (Delauney et al. in prep.). TEMPO is also equipped with a CHEMINI Fe *in situ* analyzer and with three temperature probes, and is powered by a Sea-Monitoring Node (SEAMON, Blandin et al. 2005). TEMPO was deployed and tested during one dive during the first leg. During the second leg, Victor 6000 was used to precisely position the module near a mussel assemblage and a CLSI link was used to control the complete setting (camera and CHEMINI probe). The operational module was moored for one year and will be recovered during the RECO cruise in the summer 2007.

To evaluate the potential of using acoustic backscatter data to study deep-sea community distribution and dynamics, a Tritech Super SeaKing Dual Frequency Profiler was installed on Victor 6000. The longitudinal axis of the sonar housing was perpendicular to the axis of Victor's main video camera. The sonar was programmed to acquire and log each ping's backscatter at the highest resolution available. A series of tests were performed to acquire acoustic and vision data. Several vertical transects were done on different hydrothermal edifices and habitat types during both legs. Visual data were used to train classification algorithms and validate the results obtained. Future work will include the use of these algorithms to georeference acoustic bottom returns, thus contributing to the development of accurate 3D models of the observed structures.

1.2 Sampling and *in situ* measurements

Deep-sea ecosystems are characterised by limited spatial scales, steep biogeochemical and physical gradients, and

the coexistence of chemically reactive species (hydrogen sulphide, reduced metals) and dissolved gases (oxygen, methane). Appropriate instrumentation is necessary to study the extreme variability of environmental factors (Sarrazin et al. 1999). *In situ* analyses are preferable when possible in order to circumvent sample artefacts and alterations caused by depressurisation and temperature effects. Different analysers and sensors were adapted and optimised from existing instruments and tested during the MoMARETO cruise.

A second-generation *in situ* chemical analyser based on flow analysis and colorimetric detection (Le Bris 2001) was tested. Two CHEMINI modules were used for the analysis of total sulphide and iron II or total iron (II + III, Sarradin et al. 2005). The module is designed around an engraved manifold and a miniaturised photometric system. The first results obtained showed analytical performance comparable to a bench-top system, with detection limits close to $0.2 \mu\text{M}$ for both variables. In parallel, a second *in situ* chemical analyser (Deep Probe Analyser DPA), was developed and tested for the same chemical variables.

Sampling followed by on-board analysis often remains the only analytical way to complement the range of geochemical species covered by *in situ* sensors. It is also used to validate data obtained by *in situ* measurements. PEPITO is a small-volume water sampler enabling the intake of a high number of samples (up to 25). It has been tested and used during the whole cruise for many different purposes: ground-truthing of the CH_4 sensor, *in situ* filtration on $0.45 \mu\text{m}$ filters, water sampling in the vicinity of hydrothermal assemblages (pH, CH_4 , dissolved and particulate Fe, Cu, Cd, Pb and organic matter) and microbial mat sampling.

Capsum (Germany) type methane sensors (METS), that had been modified and optimised within EXOCET/D, were tested during the cruise. Two different sensors were used in a flow system using either a CTD pump or the PEPITO pump. The sensors were installed on the Victor 6000 survey module in line with the PEPITO water sampler. Water samples were taken in parallel with the *in situ* measurements for direct comparison with the sensor signal.

The isosampler (ISObaric SAMPLER) hydrothermal flux sensor (Earth-Ocean Systems Ltd, UK) is designed to determine simultaneously the temperatures and flow rates in the hydrothermal vent habitat (Figure 2B). The isosampler was deployed to quantify the fluxes of fluid and heat within faunal assemblages located on Menez Gwen and Lucky Strike fields. It operated over a wide variety of temperatures and flow rate regimes.

A dedicated sensor package with a CTD and an acoustic Doppler current meter was designed to allow the collection of time series measurement over several days on the seafloor. The instrument consists of a commercially-available CTD (type CTD 48 from SEA & Sun Technology, Germany)

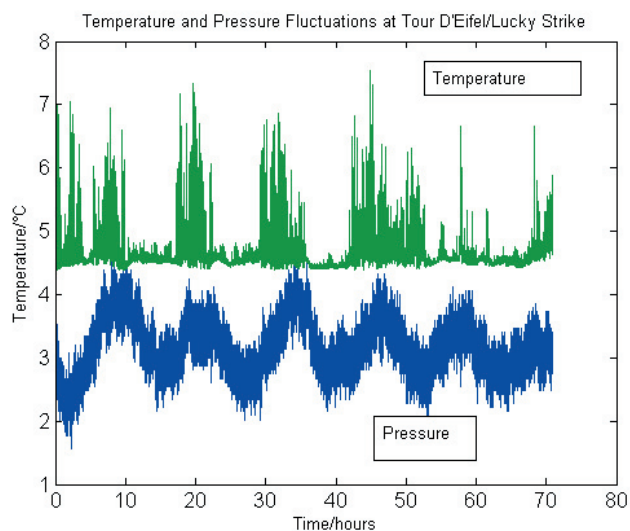


Figure 4: Pressure and temperature data as recorded during three days in the vicinity of Tour Eiffel edifice by the CTD/acoustic Doppler current meter sensor package (University of Bremen, Germany).

upgraded to deployment depths down to 4000 m, a data logger consisting of a PIC controller with an SD-Ram disc as storage medium that was designed at MARUM, and a commercial available Acoustic Doppler Current Meter (Type AQUADOPP working at 2 MHz from NORTEK, Norway). This autonomous package was deployed during three days close to the Tour Eiffel hydrothermal edifice. The data acquired will be used to extract information regarding the influence of tides on the fluid flow and the exchange of heat and salt in the surrounding water mass. Preliminary results clearly show a tidal influence on hydrothermal venting activity in the Lucky Strike region. Figure 4 shows that rising water levels during flood phases are connected with enhanced fluid outflow from hot vents. The current-meter data show an enhanced vertical flow directed towards the bottom from the lower 8 m of the water column, while the horizontal current components were just above the level of detection of the ADCP in use.

1.3 Faunal sampling and *in vivo* experiments

Microorganisms play a crucial role in biogeochemical processes and recent studies, based on molecular biological methods, suggest that less than 5% of marine microbial species have been identified (Sogin et al. 2006). Thus a variety of biochemical pathways and key enzymes remain to be discovered. The development of advanced sampling technologies is required to pursue the discovery of new microorganisms. AISICS is an instrumented microbial colonisation system (Figure 2C). This autonomous device allows the *in situ* colonisation of a specific substratum by indigenous microorganisms. It is coupled with a water sampler and a temperature probe, providing information on temperatures and fluid composition during colonisation events. AISICS was deployed four times (from 50 to 70 h

periods) on an active hydrothermal chimney during the cruise. Temperature was continuously recorded inside the system and fluids were sequentially sampled (4 samples per deployment).

Live organisms studies represent a luxury that biologists studying deep-sea fauna rarely access. Yet, this type of research has proved very valuable towards understanding the biology of such organisms. In the case of hydrothermal vent ecosystems, relevant biological features such as thermotolerance, reproduction, or primary production have been determined using *in vivo* experimentation but at native pressure. Nevertheless, a pre-requisite to successful *in vivo* pressure experiments of collected organisms is to insure their survival throughout recovery. PERISCOP is a hyperbaric sampling device, allowing the collection and recovery of deep-sea organisms at their natural pressure (isobaric collection) at depths reaching 3000 m. It allows an access to live animals or bacteria, by avoiding a traumatic and often lethal decompression. PERISCOP was tested successfully during the first leg. At the end of the cruise, the pressure retention of PERISCOP was almost perfect (95%) and the temperature inside the system remained fairly low (6.5°C for a bottom temperature of 4.5°C). Several types of organisms were collected using the PERISCOP, from two different depths, 1700 and 2300 m (Figure 2D).

In addition to collection devices, high-pressure instruments to study the physiological processes developed by the endemic fauna of vents and other deep-sea habitats are still rare. DESEARES (DEep-SEA RESpirometer) was developed to better understand, by an ecophysiological approach, the mechanisms responsible for the life and evolution of key organisms living in extreme deep-sea habitats. DESEARES is composed of three small pressure chambers, two dedicated to experiments and a third one serving as the control. The temperature inside the chambers is controlled and the circulating fluid is composed of sea water with known concentrations of different gases mimicking the *in situ* conditions of the animals. The system that control fluid composition is called SYRENE. It was developed during the VENTOX European project. DESEARES and SYRENE were installed on-board the ship during the entire cruise.

Leg 2 - August 18-September 6

This leg was entirely dedicated to biological studies. It heavily relied on the instrumentation tested during the first leg. While some studies were dedicated to one particular site, many of them rely on samples collected on the three sites visited.

2.1 Spatial distribution and temporal dynamics of hydrothermal communities

2.1.1 Spatial distribution of *Bathymodiolus azoricus* mussel assemblages in relation to environmental factors on the Tour Eiffel edifice

The principal goal of this ecological study was to examine the spatial distribution of the *B. azoricus* mussel assemblage on the Tour Eiffel edifice in relation to environmental factors. A total of 12 dives were spent to deploy a complex array of twelve instrumented chains (which also served as markers for the sampling units) around the 11 m-high hydrothermal edifice (Figure 5). Each chain was equipped with two autonomous temperature probes. *In situ* measurements with CHEMINI and water sampling with PEPITO were done on each sampling unit. Following that, the fauna was quantitatively collected using both the suction sampler and the submersible grab. Four days of short-term temperature measurements were obtained on each sampling unit. The ecological data will be used to assess and compare the temperature and chemical gradients within the microhabitats colonized by the mussels, to compare the biological descriptors (community composition, density, biomass, condition index, isotopic ratios, etc.) from one assemblage to the other, to relate these descriptors to the measured environmental variables, and finally to assess and compare the short-term variability of temperature within these assemblages. Fluid flow measurements, using both a visual and an hot-film techniques, were simultaneously acquired on several mussel patches around the Tour Eiffel edifice (Sarrazin et al. in prep). Autonomous *in situ* oxygen measurements, coupled with temperature measurements, were also acquired within the mussel assemblages.

Instrumented markers were deployed on the sampled surfaces to study the long-term dynamics of temperature within the faunal assemblages. The temperature data will be analyzed to identify significant scales of variability, common to several sampling units, using PCNM statistical analyses (Borcard and Legendre 2002). The TEMPO module completed the temporal study by monitoring both visually and chemically one of these mussel assemblages during one year. The data obtained will give insights about the natural dynamics of vent mussel communities, on the eventual reaction of the benthos to environmental events (temperature changes, disturbances) as well as on the role of biological interactions on community dynamics. To explore colonization patterns in the vicinity of mussel assemblages, six instrumented substrata (stone and wood) were deployed.

Imagery data (photo and video) was collected to be added to the hydrothermal vent time series investigated through a Marbef Ph.D. study (EU Network of excellence on Marine Biodiversity and Ecosystem Functioning). The major objective of this thesis is to assess the dynamics of hydrothermal ecosystems through time by using data from previous cruises at the same site. The emphasis was put on the Tour Eiffel edifice for which a bulk of historical data is available. This large edifice is one of the major active venting structure of the Lucky Strike vent field. Video transects were surveyed to allow reconstruction of the entire structure, followed by zoomed-in transects near the chimney (2-3 m) to observe the distribution of the different communities. The fine com-



Figure 5: For the ecological study on *Bathymodiolus azoricus* faunal assemblages, twelve instrumented chains were deployed around the Tour Eiffel hydrothermal edifice.

position of the faunal assemblages was visually sub-sampled on the four sides of the structure (N, E, S, W) by taking close-up images at three different depths. The periphery of the edifice was also imaged to see the frontier of life and hydrothermal activity. Variations in the morphology of the structure, venting activity and faunal distribution will be reported, in order to investigate the temporal dynamics of the hydrothermal vent habitat.

TRACS colonization panels, filled with different textures (alfafa, stones and wood), were deployed in the vicinity of two active hydrothermal sites (Lucky Strike and Rainbow) to study larval recruitment and colonization patterns.

2.1.2 Composition and distribution of hydrothermal vent megafauna

The goal of this study was to identify the composition and distribution of the megafauna on different hydrothermal edifices by using two approaches: acoustic sonar imagery and video imagery. Several transects on different edifices from the three vent fields were done, using both imaging systems. The usefulness of acoustic signals to study the composition and the distribution of the fauna in the vent environment will be evaluated.

2.1.3 Temporal variations of *Bathymodiolus azoricus* genetic structure and influence of introgression patterns in the evolution of allelic clines in the ATJ.

The survey of the genetic structure of *B. azoricus* mussel and *M. fortunata* shrimp populations along a bathymetric gradient on the MAR was initiated during the MARVEL (1997) and the ATOS (2001) cruises. To complement this study, biological samples of both species were collected at the three visited vent fields. The regulation of *Bathymodiolus* transcriptome in relation to different vent environmental stressors (pressure, temperature, hydrocarbon, metal and oxygen concentrations) will also be studied. For this, specimens were subjected to *in vivo* experiments in the DESEARES system, using qPCR and micro-array hybridization. Experimented animals will be genotyped at several

enzyme loci and stress proteins will be quantified to analyse the relationships between phenotype and genotype. This will help understanding how natural selection is shaping vent populations and potentially, hybridization patterns. The level of gene expression in mussel individuals using DNA microarrays will be assessed from different mussel assemblages at Lucky Strike to determine how environmental variations can affect mussel physiology (in relation to project 2.1.1). Mantle cavity water of *B. azoricus* was collected from animals of the three vent fields to study protist diversity using sequencing and FISH techniques.

2.1.4 Particulate fluxes and larval dispersal of *Bathymodiolus azoricus*

Larval dispersal of species associated to hydrothermal vents remains obscure, the main difficulty being the collection of larvae throughout the year. The objectives of this project was to study the larval dispersal of *B. azoricus* and associated hydrothermal particles on two hydrothermal sites, and determine the origin of the larvae and young recruits. Larvae of *B. azoricus* were successfully collected during past cruises using a particle trap, equipped with a current meter (Comtet et al. 2000, Khrpounoff et al. 2000). This system was deployed for one year during the Exomar cruise (2005) at Rainbow. The particle trap was picked up during MoMARETO and redeployed on Menez Gwen for one year. The larvae and particles collected will be analysed to assess seasonal larval and recruit distributions on these hydrothermal sites. The origin of larvae and young recruits will be determined with molecular studies to assign individuals to a particular population, in conjunction with the population genetic study (2.1.3). The retention hypothesis will be tested by the determination of the origin population.

2.2 Response and adaptation of hydrothermal organisms to the characteristics and short-term variations of their habitats

2.2.1 Microbial populations of active hydrothermal edifices

The major objective of this project was to study the microbial communities inhabiting active hydrothermal edifices and to identify the thermophilic and hyperthermophilic microorganisms involved in the degradation of organic matter. Most heterotrophic thermophilic species described to-date are coming from the hot part of the hydrothermal ecosystem. Many of these species belong to the archaeal order Thermococcales or to the bacterial order Thermotogales and are strict heterotrophs (Rinker et al. 1999, Godfroy et al. 2000, Adams et al. 2001, Postec et al. 2005a). They use both carbohydrates and proteic carbon sources. While these species are quite easy to grow *in vivo*, it is still not known if they play a key role in the degradation of organic matter at vents.

Even though molecular studies of hydrothermal active structures evidenced a very large diversity in both the

Archaea and Bacteria domains (Takai et al. 2001, Schrenk et al. 2003), the physiological role of these microorganisms is not trivial to infer. To identify the microorganisms involved in the degradation of organic matter in relation with the physico-chemical conditions, the AISICS module was deployed four times on a small active chimney. The colonization module was filled with different organic substrates. Molecular analysis of enriched microbial populations (diversity of both 16SRNA and 16SrRNA genes and FISH experiments) will be performed.

In addition to the colonization experiments, a continuous enrichment culture in a bioreactor was performed on board (Postec et al. 2005b). Hydrothermal fluid (supplemented with organic matter) was used as culture medium and the AISICS-sampled chimney fragment as *inoculum*. This continuous culture was maintained during 19 days at 65°C. The cell concentration was checked daily and samples were regularly conditioned for both molecular and cultural analyses.

During the EXOMAR cruise (2005), specific molecular markers for the anaerobic oxydation of ammonia (ANAMMOX, Jetten et al. 2005) were evidenced in both mineral and animal hydrothermal samples. To complete this work, hydrothermal chimney fragments and faunal samples were used for ANAMMOX activity measurements. The gills of different *Bathymodiolus* individuals from Lucky Strike and Menez Gwen were also dissected and preserved for identification and analysis of their bacterial symbionts.

2.2.2 Bioavailability of trace elements in hydrothermal organisms

The adaptation and tolerance of hydrothermal species to the toxicity and the short-term variations of their habitats influence their spatial distribution (Cosson and Vivier 1997). The objective of this project was to better understand fluid/organisms interactions at the scale of the habitat. For this, certain chemical "stress" biomarkers will be followed and the level of impregnation of trace elements, including radio-elements, will be quantified. The organisms studied came from the twelve sampling units of the ecological study (2.1.1) where the environment was characterised at small spatial scales. Experimental studies will be done to evaluate the penetration pathways of trace elements in the organisms, their distribution in different tissues and their bioaccumulation.

2.2.3 Physiology of hydrothermal organisms in simulated *in situ* conditions

The objective of this project was to study the effect of oxygen partial pressures on antioxidant systems of several hydrothermal vent species. This habitat is naturally rich in "pollutants" and its fauna is submitted to oxidative threat. The high metabolic aerobic activity linked to potentially high *in situ* temperatures lead to the production of high levels of Reactive Oxygen Species (ROS). Four types of experiments were done in the DESEARES respirometer

system (Figure 6) to study i) the physiological response of deep-sea crustaceans (*Segonzacia mesatlantica* and *R. exoculata*) to different concentrations of oxygen in their natural pressure conditions, ii) the adaptation of crabs to temperature and different oxygen partial pressures, iii) the phenotypic plasticity of crustacean hemocyanins to variation of physico-chemical conditions, and iv) the effect of pressure and oxygen concentrations on the mussel *B. azoricus*.



Figure 6: DESEARES (DEep-SEA RESpirometer) was used to perform *in vivo* experiments in simulated conditions on different hydrothermal species.

2.2.4 Stress response of hydrothermal shrimp species in relation to pressure and temperature variations

Previous *in vivo* experiments showed that the thermotolerance of adult hydrothermal shrimps was relatively low (Shillito et al. 2001, Ravaux et al. 2003), which seems paradoxical in this "extreme" ecosystem. Pressure conditions and the presence of toxic compounds in the habitat may influence the response to stress (Feder and Hoffman 1999). Studies on thermal stress response were continued by considering the role of pressure variations. Three species of shrimps (*R. exoculata*, *M. fortunata* and *C. chacei*) were collected with or without PERISCOP isobaric faunal sampler. Their physiological response (heat shock proteins) in relation to pressure and temperature variations will be compared. The activation and/or the transposition of certain elements of the genome in response to temperature and pressure variations will also be studied.

2.2.5 Genome size and transposable elements in hydrothermal species

The major objectives of this project were to study the variability of genome size in hydrothermal species and to characterise transposable elements in vent crustacean species. The size of the genome can be variable between related species or between populations of the same species. This variability could be correlated with the number of transposable elements found in their genome. The genome size will be estimated in several populations of different species (especially in four crustacean species) collected at

Rainbow, Lucky Strike and Menez Gwen. The copy number of some transposable elements, already characterised in the laboratory, will be estimated on the same sample. New transposable elements will also be characterized using DNA and RNA extractions done on board. Correlations between genome size and copy number of different transposable elements will be investigated. Moreover the study of transposable element activity (at the RNA level) and their abundance in the genome will help assessing the role of these elements in species adaptation to hypervariable environments such as hydrothermal vents.

2.2.6 Short-term and long-term experiments on *Bathymodiolus azoricus*

Three cages were moored at Menez Gwen and filled with mussels. These cages will be periodically recovered by the Arquipélago to carry out studies on live *B. azoricus* mussels throughout the year. Cd, Cu and Zn contamination experiments, feeding experiments with microalgae as well as physiological studies at seasonal scales are planned. The dissolution rate of mussel shells will be studied through experimental devices (resin blocks) attached to the cages. These experiments are partially conducted by Ph.D. students through the MoMARNET Marie Curie training network.

2.2.7 Trophic ecology of vent species

The goal of this project was to complement previous trophic studies by evaluating the carbon and energy flux in the vent food web (Colaço et al. 2002). Biological samples (*B. azoricus*, *S. mesatlantica*, *R. exoculata*, *M. fortunata*) were collected and preserved. Total carbon and calorimetric analyses of muscle tissues will be done.

The relative contribution of filter-feeding vs. endosymbiosis-derived food in the diet of *B. azoricus* will be evaluated by a biomarker approach. Fatty acid biomarkers of the two types of endosymbionts (methanotrophic and thiotrophic) will be identified through labelling with ^{13}C stable isotope and GC-IRMS analyses. Once the labelling is optimised, the natural abundance of the biomarkers will be evaluated in wild mussels. The identification of biomarkers will enable the study of *B. azoricus* nutritional plasticity, its response to seasonal events, as well as the differences observed between vent sites. Enrichment experiments ($\text{NaH}^{13}\text{CO}_3$) were done on-board to measure the turnover of incorporated ^{13}C in gills vs. remaining tissues. The results obtained will be used to identify the exact timing for biomarker search. Specific biomarkers for methanotrophic and thio-oxidising symbionts as well as for phytoplankton species chosen for the enrichment will be identified. The particles collected with the particle trap (2.1.4) will be analysed to identify the resources available to the mussels.

2.2.8 Bone structure and gene expression in deep-sea fish

Bone can adapt to reduced pressures (e.g. reduced air pressure during space flight) by modifying its metabolism, pattern of gene expression and structure. What would

happen at high pressures? Deep-sea fish reach depths of several thousand meters where they encounter extremely high pressures. They are therefore a good model to study the adaptation of bone to increased pressure. The objective of the DeepBone project within the MoMARETO cruise was to collect deep-sea fish in order to compare their bony structure and mineralization-related gene expression with those of surface fish. Two different strategies were used to capture fish specimens around hydrothermal vents: baited traps and suction sampler chasing. Despite numerous attempts, only three fish were collected during the cruise, all using the suction device and all from the same species: *Pachycara saldanhai*. One of these specimens, represents the biggest captured so far for this species (total length: 27.8 cm). Various bone tissues have been sampled and conditioned on board for future analysis (e.g. histology, *in situ* hybridization, gene expression, etc).

3. Conclusions and Perspectives

The first leg of MoMARETO cruise was a real success with 13 instrument prototypes tested, developed within the EXOCET/D European project for the study of deep-sea extreme habitats. These instruments for quantitative imaging, *in situ* measurements, faunal sampling and *in vivo* experiments will greatly improve our capacity to explore, observe and monitor these remote ecosystems. The second leg led to a detailed ecological study of Tour Eiffel mussel assemblages. Biological samples were collected on the three vent fields visited to feed genetic, physiological and ecotoxicological studies.

In addition to the acquisition of fundamental insights about the dynamics, functioning and adaptations of hydrothermal communities, the data acquired during this cruise, reinforced by past studies in the same area, constitute a fundamental basis for the establishment of a long-term observatory on the MoMAR zone.

Web sites related to the MoMARETO cruise

<http://www.ifremer.fr/MoMARETO>

<http://www.ifremer.fr/exocetd>

<http://www.ipgp.jussieu.fr/rech/lgm/MOMAR/>

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