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Biogeochemical interactions in extreme environmental conditions: an integrated study (PHARE 2002/ EPR 13°N)

N. Le Bris[1], F. Gaill[2], Alain K.[3], Cambon M.-A.[3], Desbruyères D.[1], Fabri M.-C.[1], Jeanthon Ch.[4], Jollivet D.[5], Lallier F.[5], Lopez-Garcia P.[2], Mevel C.[6], Phillipot P.[6], Piccino P.[5], Pradillon F.[2], Prieur D.[4], Ravaux J.[2], Rees J.-F.[7], Sarradin P.-M.[1], Shillito B.[2], Thiebault E., Zal F.[5], Zbinden M.[2].

[1] Ifremer DRO/EP, Plouzané, France
[2] CNRS-UMR 7622, UPMC, Paris , France
[3] Ifremer DRV/VP/LMBE, Plouzané, France
[4] CNRS-UMR 6539, IUEM, UBO, Brest, France
[5] CNRS-UPR 9042, UPMC, Roscoff, France
[6] UMR-CNRS 7097- IPGP, Paris, France
[7] Univ. libre de Louvain, Belgique

Introduction

Over the last 20 years, a set of biological cruises have been dedicated to the study of hydrothermal vent communities on the 13°N East Pacific Rise segment. These cruises have emphasized some of the most striking features of these communities. As reviewed in (Desbruyères *et al.*, 1998), the two alvinellid species, *Alvinella pompejana* (Fig. 1) and *Alvinella caudata* are especially remarked for their ability to dwell in the hottest and, presumably, most hypoxic and toxic conditions found in this biotope. Although *Alvinella* spp. are also known as the very first metazoans colonizing newly grown hydrothermal substrates, in a second stage, other invertebrates such as the polychaete worms *Paralvinella grasslei, Hesiolyra bergi* or the crabs *Bythogrea thermydron* and *Cyanagrea predator* are found, in various abundance, in this extreme habitat. The abundance and the close association of microbial communities to *Alvinella* spp., as epibiotic bacteria or in their immediate surrounding, was also particularly underlined (Taylor *et al.*, 1999; Jeanthon, 2000).

In these harsh environmental conditions, biological development and adaptation as well as community structuring are expected to be mostly driven by biogeochemical processes. Still, the genetic, biological, ecological, and geochemical features of these interactions are poorly known. The PHARE 2002 (Peuplements Hydrothermaux, leurs Associations et Relations avec l'Environnement) cruise was devoted to an integrated study of this "hot pole". From 2002 April 30 to June 3, a multidisciplinary project was held including biologists, ecologists, chemists, microbiologists, and geologists from different French research institutions. This cruise was planned under the frame of the national Dorsales program.

Figure 1. *Alvinella pompejana,* the Pompeii worm

Instrumentation used at depth and on-board

The work at sea was based on innovative instrumentation, developed by the PHARE participants for *in situ* and *in vivo* approaches: *in situ* chemical analysis (ALCHIMIST) coupled to imaging and scaling devices, bacteria and larvae collection and alvinellid colonization devices (TRAC), pressurized aquaria equipped with a chemical and thermal monitoring system (IPOCAMP and SYRENE) and pressurized incubation chambers (PICCEL).

ROV, Victor 6000 - The new French deep-sea ROV, VICTOR 6000 was operated from the *R/V Atalante* (Fig. 2) for its first implementation on the EPR. It was equipped with watertight boxes to collect geological and biological samples and their associated microflora, minimizing contamination from shallow waters. Chemical samples were collected with titanium syringes (for black smoker hot fluids) or a sequential sampler equipped with 200 ml bottles or large volume bags with 0.45 µm filtering units.

VICTOR 6000 was also equipped with various imaging systems (digital camera, 3-CCD video camera) and 4 lasers for scaling. Dives were planned overnight for 12 to 14 hour duration. A total of 20 dives provided 250 hours working time on the seafloor.

In situ chemical analyser and temperature probes - The chemical analyser, ALCHIMIST (Le Bris *et al.*, 2000) was deployed on the VICTOR 6000 and was operated from onboard. It enabled the determination of profiles of dissolved ferrous iron and sulphide content, together with pH and temperature, over the studied alvinellid communities. The analyser inlet tube was coupled to the VICTOR's temperature probe, and handled by the submersible arm. The inlet was also equipped with a small video camera. In addition to short term measurements, autonomous temperature probes (Micrel S.A.) were deployed to monitor the medium-term variability of temperature at the experiment sites.

High pressure vessels - Together with ambient pressure aquaria, two high pressure IPOCAMP devices, with 20 litres thermoregulated chambers, were operated on board (Shillito *et al.*, 2001). The systems were coupled to a chemical regulation system (SYRENE) to control dissolved gases content (O_2, CO_2) and pH. Pressure chambers for egg and embryo incubations (PICCEL) were also implemented on board (Pradillon *et al.*, 2001).

Figure 2. The deep-sea ROV, VICTOR 6000

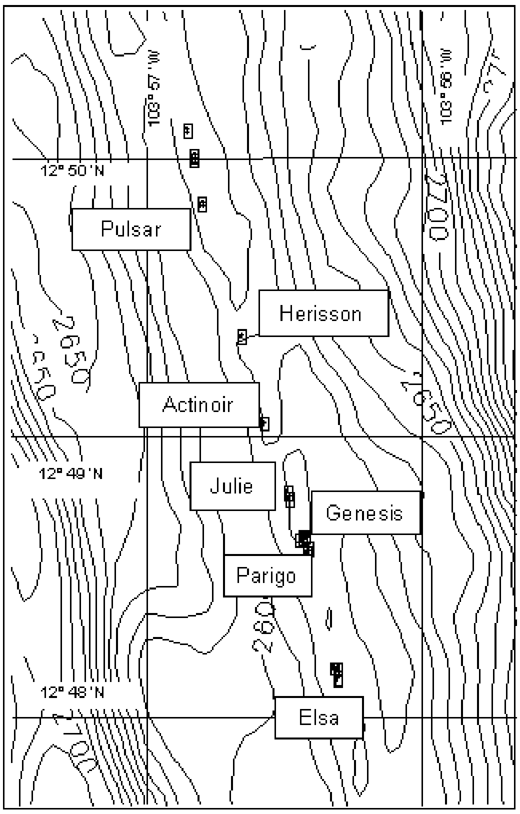

Figure 3. Sites of the PHARE 2002 integrated study

In situ experimentation

Segment-scale variability - Identification and description of previously known actives sites from 12°48'N 103°56'W to 12°50'N 103°57'W was assessed. Seven different sites were then repeatedly visited (Fig. 3). These sites, consisting in one or a group of active chimneys, exhibited marked differences in their dimension (from less than 1m to 20m high) and morphologies. Contrasting faunal assemblages were described at these sites. They displayed important changes in their morphologies and/or fauna distribution with respect to the last 1999 cruises. These data will complete the long-term evolution pattern of this segment (Jollivet, 1993).

Focused, *in situ* chemical analysis and temperature measurement over the alvinellid dominated communities suggested marked difference in the thermal and chemical conditions sustained at the different sites. A combination of chemical and thermal characterisation with photographic and videoscopic

International Research: **Biological Studies:** Le Bris *et al.,* cont...

records, and animal sampling will enable to relate the composition of faunal assemblages as well as genetic and demographic population structures, of *Alvinella* spp. or some associated species, to environmental features.

Colonisation dynamic and biogeochemical interaction - To describe the temporal succession of microbial and biological communities colonising a new substrate, TRAC devices (Titanium Ring for Alvinella Colonisation) were implemented. Seven deployments were performed over selected alvinellid colonies, over different chimneys. Each TRAC was equipped with autonomous temperature probes. Chemical and thermal profiles associated to the fluid-seawater mixing gradients in the immediate vicinity of a TRAC were investigated by *in situ* techniques and fluid sampling. Pompeii worm tubes, associated bacterial mats and mineral deposits, formed after 5 to 21 days, were recovered from the TRAC. The mineral composition as well as the mineral content of particles associated with the tubes will be examined in detail to investigate biogeochemical interactions at the nanoscale level between the worm and its epibiotic bacteria. Mineral structures associated to the Alvinella tubes on chimney walls will also be considered through a combined biological, geological and chemical approach.

"Microscale" diversity - The microscale variations of faunal distribution and of alvinellids population genetic differentiation were studied over single edifices. Thermal and chemical measurements were carried out in conjunction with biological sampling. High resolution photography and video were used to complete this approach.

Additionally, the deployment of microorganism collection devices at different positions within the temperature gradient will enable the investigation of microbial diversity in relation to environmental factors. The diversity of microorganisms associated to the Pompeii worms and the nearby minerals and their metabolic activities will be considered by cultural and DNA-based approaches. For the first time on this 13°N EPR site, the microeucaryote diversity will also be considered.

In vivo experimentation

Dives were also partly dedicated to the collection of live animals for *in vivo* experiments. Special attention was paid to try and recover these animals in the best possible physiological condition, particularly by limiting the delay between collection and recovery on board. When not associated to an extended study of chemical, thermal and faunal characteristics, the biological samples dedicated to *in vivo* experimentation were related to *in situ* temperature measurement, immediately before collection, with the VICTOR temperature sensor.

Temperature tolerance - In this highly turbulent environment, large spatial and temporal temperature variations were confirmed. The tolerance of metazoans to high temperature, however, remains enigmatic and the mechanisms of adaptation to such a constraint still poorly understood. The behavioural and biochemical response of different species of the *Alvinella* spp. habitat to thermal stresses will be analysed, from the video-recordings and samples of on-board heat shock experiments under simulated pressure conditions

Chemical stress - The resistance of animals to chemical toxicity was another topic of *in vivo* experimentation. The effect of hypercapnia (excess CO_2) on the respiratory function of the crab *B. thermydron* was investigated at ambient atmospheric pressure and at 260 bar. Potential adaptation to anoxic and oxic stresses was also investigated in the pressurised aquaria.

Embryo development - To study the environmental conditions that may determine the early stages of *Alvinella pompejana* development, *in vitro* fertilization experiments were performed on board. Embryos were then incubated under simulated or natural conditions.

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Biological studies using *Mir* submersibles at six North Atlantic hydrothermal sites in 2002

A.L. Vereshchaka[1], S.V. Galkin[1], A.V. Gebruk[1], E.M. Krylova[1], G.M. Vinogradov[1], C. Borowski[2] and the "Mir" submersibles team

[1] *P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, Russia*
[2] *Max Planck Institute for Marine Microbiology, Bremen, Germany*

The 47th cruise of the R/V *Akademik Mstislav Keldysh* with 2 manned submersibles *Mir* started on 17th May 2002 from Kaliningrad, Russia, and ended on 14th August in Hamburg, Germany. The cruise was aimed at the studies of geological and biological processes at 6 hydrothermal vent fields on the Mid-Atlantic Ridge (MAR) and at the wreck site of the battleship "Bismarck" that lies at 4500 m depth in the Porcupine abyssal plain. In the present paper we focus on biological studies at the hydrothermal fields Snake Pit, TAG, Broken Spur, Lost City, Lucky Strike, and Rainbow.

Overall attention was focused on the entire hydrothermal ecosystems: benthic, benthopelagic, and planktonic components were examined with equal priority. The biological team consisted of Vladimir Dyakonov (computation biologist), Vladimir Gagarin (primary production, deck work), Sergey Galkin (benthos distribution, mapping and landscape approach to the vent communities), Andrey Gebruk (benthos distribution and ecology), Elena Krylova (benthos distribution, mytilid biology), Alexander Vereshchaka (coordinator of biological studies, water column and near-bottom ecology, shrimp biology), Georgy Vinogradov (water column and near-bottom ecology, amphipod biology). Three guest researchers participated in biological works: Christian Borowski (Max Planck Institute, Bremen, bacteria-animal symbiosis), Joshua Osterberg (Duke University Marine Laboratory, behaviour of scavengers), and Diane Poehls (Woods Hole Oceanographic Institution, shrimp genetics).

Water column studies

The Mid-Atlantic vent fields between 23° N and 30° N are located in the areas where the primary photosynthetic production is estimated as ca. 200 mgC_{org}/m^2/day. Seven dives were devoted to a plankton research program at the MAR vents. During these dives, large planktonic animals (0.5 cm and larger) were visually studied (see details of the method in Vereshchaka and Vinogradov, 1999). In addition to the visual observations, a vertically hauled BR $^{113}/_{140}$ plankton net (opening area 1 m^2, mesh size 500 ìm) was used. Above all vent fields the near-bottom layer was sampled, the net position was controlled with a pinger ("Benthos"). The sampled layers corresponded to different water masses recorded by preceding vertical probing with a CTD Rozette.

The MAR vent sites can be divided into 2 principal groups: the southern abyssal vents below the central part of the North Atlantic halistatic area (Snake Pit, TAG, Broken Spur) and the northern bathyal vents below the periphery of the halistatic area (Lost City, Lucky Strike, Rainbow).

Southern abyssal vent fields

The plankton distribution above these sites showed three maxima (Fig. 1). The main maximum was positioned in the lower part of the main pycnocline at 700-1000 m. This is a common phenomenon in the deep North Atlantic and usually related to a high abundance of various mesopelagic animals. The second, much less prominent, maximum was located at ~2000 m and might be associated with the presence of Mediterranean water masses, which were well marked by the salinity curve. Appendicularians and chaetognaths dominated in the plankton community in the second maximum.

The third maximum was observed adjacent to the hydrothermal plumes and was mainly composed of gelatinous animals (including voluminous appendicularian "houses"). Similar increase of the appendicularian abundance near the bottom has been previously reported from the Rainbow vent field, and from a non-vent site off Newfoundland near the wreck of the *Titanic* (Vinogradov and Vinogradov, 2002 a, b). Later in the cruise, we found a similar pattern at