HOT 96 NEWS

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A team of French and American biologists has just returned from a joint expedition at 9°50'N and 13°N on the EPR. The expedition, HOT 96, was the latest of a long list of collaborative French/American cruises by hydrothermal vent biologists (Note 1). Five of the participating scientists (J. Childress, H. Felbeck, C. Fisher, F. Gaill and R. Lutz) were at sea together during the first Oasis expedition to 21°N on the EPR in 1982, and D. Desbruyères was the chief scientist of a French cruise with Cyana at 13°N.

HOT 96, was led by F. Gaill (CNRS INSU Roscoff UPMC) within the joint research group URM 7 associating IFREMER, CNRS and the University of Paris 6, on board the *N/O Nadir*, mother-ship to the submersible, Nautile. Also present on site was the US *R/V Wecoma*, with Chief scientist H. Felbeck of Scripps Institute of Oceanography. A total of 26 French and 20 US scientists participated in at least one of the two legs of the expedition and several French and American scientists were exchanged between the two research vessels according to their scientific interests. One dive during this cruise was funded by the European Community and attributed to the young scientist C. Arndt of the Institut für Ostseeforschung in Warnemünde, Germany.

The 31 dives were divided between two venting areas of the EPR. Vent sites of the 13°N area have been intensively studied by French scientists since 1982, including 10 French-American biological expeditions (Note 1). The other dive area was in the vicinity of 9°50'N, at which 100 Alvin dives have been performed since the recent volcanic eruptive event of 1991. Numerous French-American collaborative studies have been initiated during the course of 10 American cruises (Note 2) to the 9°50'N region. French scientists have participated in most of the biological cruises devoted to this area and, during HOT 96, several instruments deployed from the submersible Alvin over the last year were retrieved with the Nautile.

Analysis performed on the *N/O Nadir*

Various *in situ* experiments and measurements of the main chemical and physical vent parameters were conducted by the French team in order to study the organism/environment interactions at different sites.

Water sampling was performed on several selected sites, Totem Parigo and Genesis (13°N), and East Wall and Tube Worm Pillar (9°N), and allowed new 150 ml sampling bottles to be successfully tested. These bottles are based on vacuum depression and were deployed by the Nautile. Analyses were done by P.M. Sarradin and J.C. Caprais to characterize accurately the chemical environment around the vent organisms in order to correlate both the organism and the chemical species distributions. The chemical species studied were pH, sulfide, methane, carbon dioxide, oxygen, nutrients, copper and lead, sulfur compounds and magnesium as tracer. Preliminary results obtained on board show methane traces among the 9°N vent sites and high CO_2 and H_2S concentrations which differ between areas colonised by *Riftia* and *Alvinella*. Animals from selected sites were also collected in order to define a specific biological signature which includes carbon fixation (A.-M. Alayse) and metallic concentrations (R. Cosson)

Hydrothermal vent particles were also sampled at the Totem site (13°N) to study direct sedimentation around chimneys. The settling particles were collected using time series sediment traps on which current meters were fixed. Two traps were deployed by the Nautile at 1 m and 2.5 m from the chimney base. The aim of these studies, conducted by A. Kripounoff, was to measure how the particle flux exported by hydrothermal springs vary at a micro-scale. The third trap was used as a pelagic reference and moored out of the vent region.

Totem was also an excellent site to analyze various ecological aspects of the fauna associated with smokers, especially *Alvinella* worms, and will be used as a population reference in the near future. This work includes population dynamics and genetics studied by P. Chevaldonné and D. Jollivet. Populations of additional smokers (e.g. Elsa and Parigo) were

Notes

1. Oasis in 1982, Guaymas in 1985, Galapagos in 1985, Hydronaut 1987, Galapagos in 1988, Magical Mystery Tour 1990, HERO 91, HERO 92, 9°N in December 1993 and HOT 96.

2. April 1991, November 1991, March 1992, December 1993, March 1994, October 1994, November 1994, February 1995, November 1995 and December 1995 and 2 French expeditions (October 1991 and February 1996).

also sampled for further ecological and genetic analyses in order to study how populations have evolved since 1991 (i.e. the populations that have already been analyzed in 1987 and 1990). This study includes a fine tuned analysis among cohorts already settled. Moreover, mRNA extractions were also performed on board in order to identify genes coding for enzymes sensitive to environmental parameters (e.g. temperature) and to assess adaptive mutations.

A major thrust of the French ecological experiments was the deployment of SAMO, an *in situ* instrument designed to monitor temporal variation of an array of biologically important parameters and equipped with a time-lapse video system. SAMO uses an array of four thermistors which are positioned in the field of view of the video camera which can be aimed and focused by those on board the Nautile. In addition to the temperature measurements, SAMO also yielded data collected by a transismometer, CTD and current-meter. All these data, including digitized video images, were acoustically transmitted to the surface and monitored from the *N/O Nadir* in real time. SAMO was deployed at both the Genesis site (13°N) and the site known as Riftia Field (9°N) and devoted to the ongoing recruitment studies of L. Mullineaux (WHOI), C. Peterson (Univ. N. Carolina) and C. Fisher. Results from SAMO will be a welcome addition to their studies.

Protective surfaces (tubes and body wall) were collected from various vestimentiferan and annelid species by B. Shillito, J.P. Lechaire, C. Durif and J. Ravaux from the F. Gaill team and J. Delachambre (University of Dijon CNRS) for further studies on the properties, molecular structure and phylogeny of collagen and chitin protein complexes. The molecular characteristics and behavior of such biopolymers are specific to the vent species and may be used as environmental markers. Additional *in vitro* experiments were done on the *R/V Wecoma* to analyze various aspects of the tube secretion process including growth rate, polymerization and enzymatic activities. Other tissues were also sampled from a set of various bivalves and crustaceans by A. Pruski from A. Fiala's group (Banyuls, University of Paris 6) and J.Y Toullec (ENS, Univ. Paris 6) for complementary studies related to their ecophysiological adaptations and molecular evolution.

During a recent Alvin expedition in December of 1995, F. Gaill and C. Fisher (Pennsylvania State Univ.) initiated *in situ* tube-worm growth experiments using a staining device, and alvinellid recruitment experiments using titanium and basalt settlement surfaces. Both experiments were collected during HOT 96 and additional short-term experiments on tube-growth and recruitment were conducted by the two scientists during the cruise. Some of these are still underway at 13°N and 9°N vent areas. Similarly, another type of recruitment experiment, "SMAC", was deployed for D. Desbruyères (IFREMER) at 9°50'N during a November 1995 Alvin cruise conducted by R. Lutz (Rutgers Univ.), recovered during HOT '96 and supplemented by 3 additional short-term similar experiments during the cruise.

R. Lutz used a Nautile dive to describe the ecological state of most of the vent fauna aggregations situated along the 1.3-km long stretch of the axial summit caldera known as "Biotransect" (9°49.6'-9°50.3'N), a region which has been intensively studied since 1992 in an attempt to investigate temporal changes in both vent conditions and community structures. Two separate 3-chip video cameras (recording on SVHS and Beta SP PAL formats), combined with a lighting system customized by the Nautile group, were used to provide excellent video coverage of the Biotransect, at altitudes varying from 4 to 6 m, which will be compared to a similar coverage obtained in November 1995. Observations made during the course of the transect run suggest that the amount of iron oxides may have substantially increased since November 1995 in the vicinity of a site recognized as "Bio-9" inside the "Hole-to-Hell" region, associated with a steadily increasing mortality of vestimentiferan tube worms at this site.

Studies on the *R/V Wecoma*

J. Childress and his group (Univ. of California, Santa Barbara) were on board the *R/V Wecoma*. They had an array of pressure aquarium systems capable of simulating *in situ* vent conditions for the maintenance of, and experiments on, living vent animals. Their studies focused on estimating processes and rates of important metabolite uptake by *Riftia*. Measurements, under a high pressure respirometer system, demonstrated rapid rates of net inorganic carbon uptake by *Riftia* and *Tevnia* and the effects of temperature, oxygen concentration, sulfide concentration, pH, and PCO_2 on these rates. Experiments were also conducted using pressure aquaria to determine the relationships between external conditions and internal pools of metabolites using inhibitors to elucidate the uptake processes involved. Collaborative studies were carried out with French scientists on: tube accretion in *Riftia* by F. Gaill and B. Shillito; crab population biology by D. Desbruyères; and *Riftia* physiology by A. Toulmond and F. Lallier.

H. Felbeck's group concentrated on molecular and metabolic studies of vent fauna using their pressure aquarium systems, *in situ* experiments and on board laboratory experiments. Excretion of succinate by endosymbionts was investigated and new studies on the immunological reactions between symbionts and hosts were initiated. The recently discovered potential for nitrate respiration and nitrate concentration mechanisms by *Riftia pachyptila* was further investigated. *In vitro* egg fertilizations were also performed to study larval development in *Riftia* both on board (pressure chambers) and on the seabed (*in situ* experiments). Collaborative efforts overseen by the German researcher C. Arndt were focused on the elucidation of a switch in metabolic pathways towards anaerobic metabolism in vent organisms. Initial results indicate that *Riftia pachyptila* can survive extended periods of anoxia and can overcome these conditions by producing extremely high concentrations of succinate in all its body tissues. In addition, a collaborative

work has started on carboxylating enzymes with the French researcher A.-M. Alayse (IFREMER).

A. Toulmond and F. Lallier (Univ. of Paris VI and CNRS at Roscoff, France) were on board the *R/V Wecoma*, collecting blood from various vestimentiferan and annelid species for further studies on the physiological properties, molecular structure and phylogeny of the giant extracellular hemoglobins. *Riftia* tissues were also sampled and fixed in various conditions for immunological studies on the so-called "band 3 proteins", constituents of the chloride-bicarbonate transport system in the vertebrate red blood cell. Previous observations have shown that these proteins were present in various parts of *Riftia*'s body. Experiments were done on board, using a Ussing chamber, to tentatively measure bicarbonate fluxes through the body wall. These studies are part of a collaboration between J. Childress's and A. Toulmond-F. Lallier's teams. Another member of the group, S. Hourdez, was on the *N/O Nadir* to sample blood and tissue from Alvinellids and Branchipolynoids worms in order to continue ongoing work on their elaborate coelomic-vascular oxygen transfer systems.

G. Hervé (CNRS and Univ. of Paris 6) also invited on board the *R/V Wecoma*, pursued his group's studies on the localisation and properties of enzymes of the pyrimidine nucleotides pathway in *Riftia* and its related symbionts. Previous studies on samples collected by F. Gaill had shown that aspartate transcarbamylase (ATCase) was only found in the trophosome. Preparations of symbionts and vestimentiferan gonads were made during the cruise in order to precisely locate the cellular origin of such a restricted activity. If this enzyme is indeed located inside the symbiont, its high specific activity would suggest that the symbiont is growing actively. Carbamylphosphate synthetase (CPSase), the enzyme which provides carbamylphosphate to ATCase, was not found in the trophosome but in the vestimentum and the plume and raises the problem of a carbamylphosphate source in the trophosome. This problem has been reinvestigated by tests of CPSase activity performed immediately after sample collection. Numerous samples were prepared for further enzyme assays of both the "*de novo* pathway" and the "salvage pathway". The lack of ATCase in *Riftia* itself suggests that CPSase is only operating in adult, non-growing individuals. To test this hypothesis samples of young *Riftia* (15 cm) were also prepared.

The future of French-American cooperation

The cruise was very successful for all members of the scientific party involved and was a model for international collaboration. A variety of very sensitive experiments were conducted and are still underway at these sites and utmost care needs to be taken by all the scientists involved to respect the ongoing work of others. In these days of dwindling resources, truly collaborative expeditions like this are an excellent way for the participating countries to get the most out of the limited funds available for submersible expeditions.

Since the April 1991 eruption, the region of the EPR crest between 9°49'N and 9°51'N has been extensively studied by French and American scientists from a wide variety of disciplines and considerable equipment and experiments are currently deployed on the bottom in this region. On-going geological and chemical studies are being conducted by D. Fornari and T. Gregg of Woods Hole Oceanographic Institution, R. Haymon of the University of California at Santa Barbara, K. Von Damm of the University of New Hampshire and M. Lilley of the University of Washington. On-going time-series imaging studies of temporal changes in biological community structure within the area of the Biotransect are being conducted by R. Lutz and T. Shank of Rutgers University. Numerous ecological experiments have been deployed on the bottom (or are being conducted periodically at certain sites) in the region as part of the ongoing studies of the following scientists: L. Mullineaux, C. Fisher, C. Peterson, F. Gaill, D. Desbruyères, C Cary of the University of Delaware, as well as others.

To date, all the above scientists have respected these long-term experiments and/or observations and it is requested that all individuals having an interest in working in this intensively studied region respect them. Questions concerning the nature or the location of these ongoing studies should therefore be addressed to some or all of the listed persons in order not to disturb or destroy these studies.