

# A Biology Laboratory on the Seafloor

BY NADINE LE BRIS

Although occupied submersibles have played an essential role in the discovery and study of hydrothermal ecosystems at depths ranging from 1500–3500 m, operational constraints at great depths have meant that the wide chemical and thermal diversity of the hydrothermal environments have long remained poorly defined. In the last decade, use of remotely operated vehicles (ROVs) to substantially extend dive time and development of a new set of dedicated instruments have greatly expanded our capacity to characterize seafloor hydrothermal habitats at the interface between hydrothermal fluid and seawater. In particular, major breakthroughs in the field of in situ chemical sensing and high-pressure experimentation have led to a much better understanding of the adaptation of invertebrate species to their extreme environment.

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## IN SITU CHEMICAL ANALYZERS AND SENSORS

Physico-chemical parameters of vent habitats are characterized by steep, dynamic gradients. Because these gradients cannot be sampled and preserved for analysis at the surface and are in any case constantly and rapidly changing, the only way to understand these habitats is to perform chemical measurements directly at depth. For this reason, even the very first, prototype, in situ chemical analyzer, the “Scanner,” developed by Johnson et al. (1986) and installed on the submersible *Alvin*, constituted a great technological improvement for the study of tubeworm and bivalve habitats of the first site discovered, the Rose Garden along the Galápagos Spreading Center.

Since the late 1980s, colorimetric flow analyzers have been intensively used (e.g., Sarrazin and Juniper [1999] on the Juan de Fuca Ridge and Le Bris et al. [2003, 2006] on the East Pacific Rise). These complex instruments (Figure 1) continuously sample the medium to be studied, after which online reagent

injection and colorimetric detection is performed, just as in the laboratory, but in situ under high pressure. Centimeter-scale changes in sulfide, iron, silica, or manganese concentrations can be determined in this way and have been correlated to temperature changes within animal aggregations (Johnson et al., 1988; Le Bris et al., 2006).

Recently, alternative methods based on miniaturized electrochemical sensors that are more rapid and less sensitive to clogging are enabling finer spatial and temporal characterization of the immediate environment of invertebrates at hydrothermal vents (Le Bris et al., 2001; Luther et al., 1999). For example, pH profiles with sub-centimeter resolution obtained using a 2-mm-diameter glass electrode show a marked difference between the inside and outside of a Pompeii worm tube, indicating that this tube was ventilated (Figure 2) (Le Bris et al., 2005). In situ voltammetry using gold-mercury microelectrodes has proven to be a powerful technique for simultaneous detection of oxygen and various

Figure 1. The in situ chemical analyzer "Alchemist" on the Alvin basket.  
Photo credit: Nadine Le Bris, Ifremer

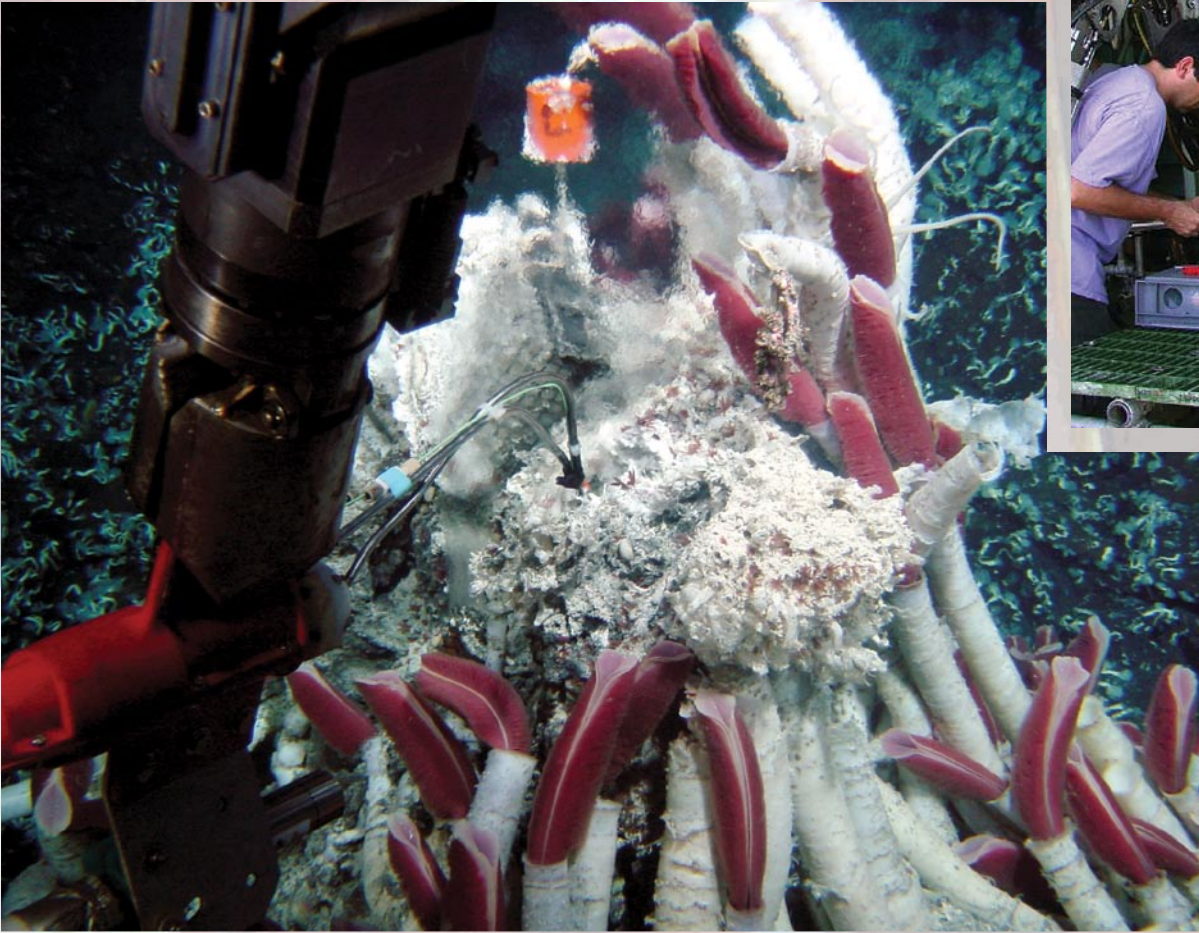
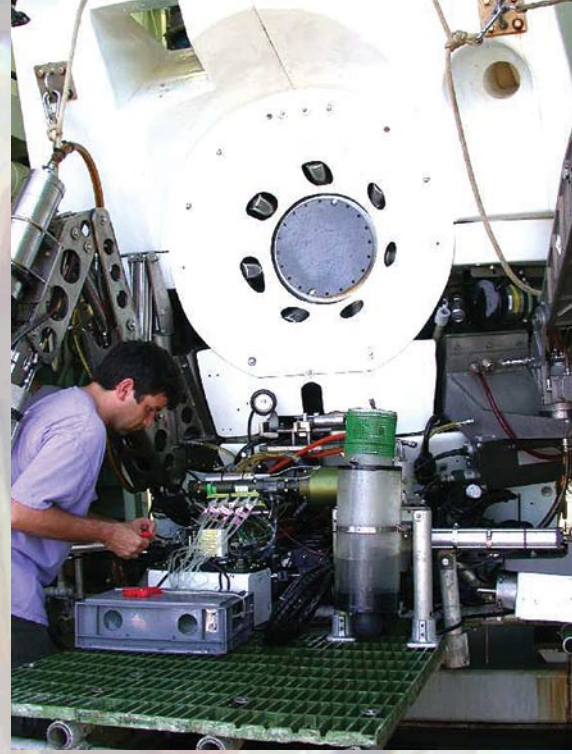


Figure 2. A pH-temperature probe and analyzer inlet close to worm tubes. Copyright Ifremer/PHARE2002

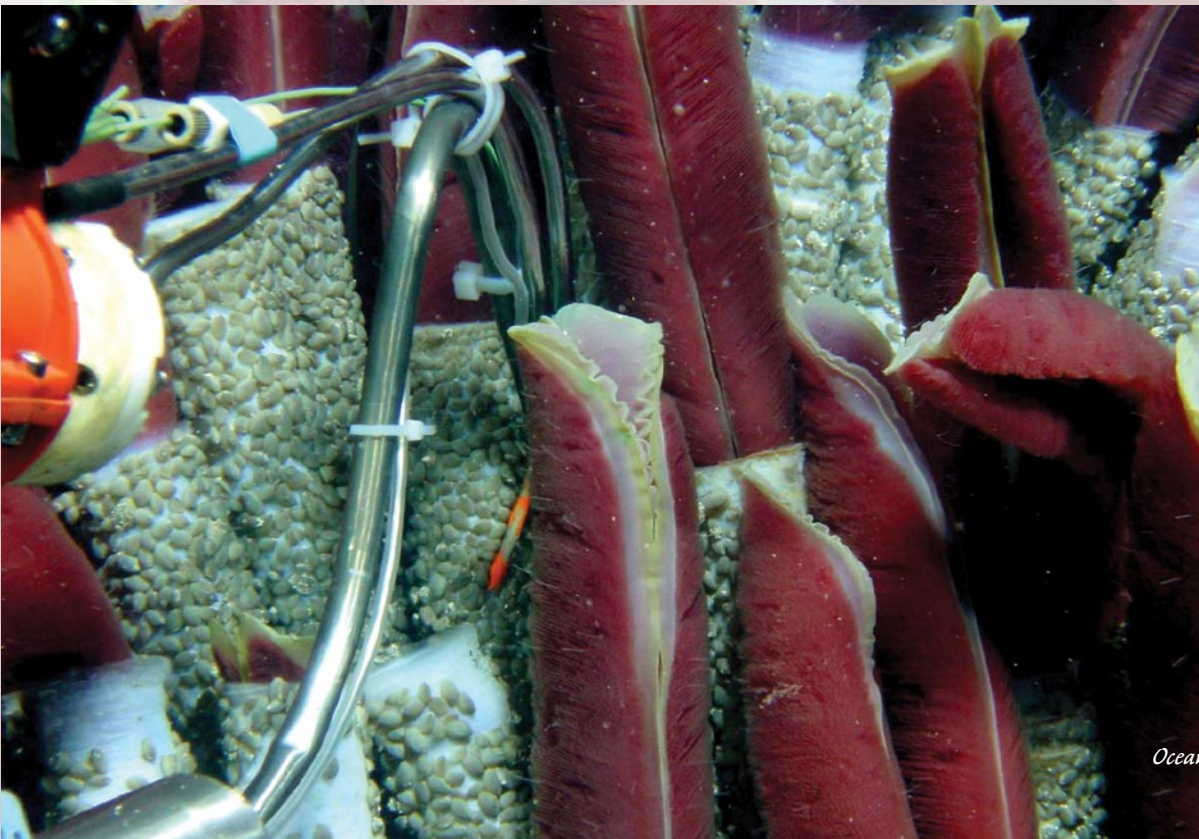




Figure 3. Pressurized aquaria for in vivo studies. From *Incubateurs Pressurisés pour l'Observation en Culture d'Animaux Marins Profonds (IPOCAMP)*. Reprinted with permission of F. Gaill, Université Pierre et Marie Curie-Paris6-CNRS


toxic and nontoxic sulfide forms in habitats (Luther et al., 1999, 2001). Miniaturization is a great technological challenge for these studies where centimeter-scale temperature gradients as high as 20°C indicate intense mixing of very different vent fluids (Le Bris et al., 2006). Further development of miniaturized sensors and their adaptation for autonomous use are eagerly awaited to improve monitoring of chemical conditions over longer time scales while minimizing disturbance to biological systems. Ultimately, deep-sea observatories could integrate instrumented platforms for in situ biological experimentation based on these sensors.

#### HIGH-PRESSURE VESSELS FOR BIOLOGICAL STUDIES ON VENT ANIMALS

An alternative way to study the tolerance of animal species for the thermal and chemical extremes of the hydrothermal environment is to use high-pressure devices in the laboratory to recreate the conditions of their natural environment. In some cases, such as the chimney worm *Alvinella pompejana*, the animals brought to the surface hardly survive the decrease in pressure before they can be placed in high-pressure storage. Very promising first trials await further optimization for maintaining them in aquaria for in vivo studies (Shillito et al., 2004). Many species do, however, cope better with the pressure decrease and can even be kept alive at atmospheric pressure, although they may then show abnormal physiology and behavior (Shillito et al., 2006), precluding their study under ambient atmospheric conditions on-board research vessels.

High-pressure aquaria were first developed in the early 1980s by Jim Childress's group at the University of California, Santa Barbara, to study the physiological requirements of newly discovered symbiotic organisms such as the giant tubeworm *Riftia pachyptila* (e.g., Goffredi et al., 1997a, 1997b; Scott et al., 1999). Use of these devices, combined with chemical regulation systems, enabled a complete characterization of the assimilation of mineral compounds by the organisms and their transfer to the bacterial symbionts. By combining a video-monitoring system with a high-pressure vessel (Figure 3) (Shillito et al., 2001), it was possible to determine the thermal tolerance and escape-behavior limits of several vent species. One of the surprising results of these studies was that many animals associated with extremely hot environments did not appear to have particularly high thermal tolerance (Ravaux et al., 2003). Using new high-pressure equipment, however, Girguis and Lee (2006) confirmed that at least one species living on Juan de Fuca Ridge chimneys is exceptionally tolerant of, and even prefers, temperatures as high as 40–50°C when placed in chemical and pressure conditions similar to its natural environment. To date, this is the only demonstration of thermophilic behavior in marine animals.

To investigate the specific requirements of embryos and larvae, Pradillon et al. (2001) developed new high-pressure incubators called PICCEL and PIRISM, with the latter being coupled to a microscopy imagery system. PIRISM enabled the development of *A. pompejana* embryos obtained by in vitro fertilization to be followed over

time in various temperature conditions. Using this approach, they showed that the early life stages were able to tolerate the conditions sustained in the environment of adults. Even if we still do not know where these embryos develop in nature, the habitats where they have a chance to survive can now be more precisely defined from their in vivo-determined sustainable thermal range. 

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