

Trophic Structural Adaptations in Relation to the Bacterial Association of Bivalve Molluscs from Hydrothermal Vents and Subduction Zones

A. FIALA-MEDIONI¹ and M. LE PENNEC²

¹ *Université Paris VI, Laboratoire Arago, F-66650
Banyuls-Sur-Mer, France
Tel. 98-16-03-94 Telex ARAGOB 505020 F*

² *Université de Bretagne Occidentale, Laboratoire de Zoologie
F-29287 Brest Cedex, France
Tel. 98-16-03-94 Telex ARAGOB 505020 F*

Received February 13, 1987; Accepted April 12, 1987

Abstract

Ultrastructural observations on the gill of the bivalve molluscs found in dense populations at deep-sea hydrothermal vents of the East Pacific Rise, and in association with fluid emissions in subduction zones off Japan, show surprisingly high concentrations of endocellular bacteria probably involved in symbiotic relationships with the mollusc host.

In all species, the bacteria are highly integrated into the gill cells, but two types of association are found: in the mytilid, *Bathymodiolus thermophilus*, Kenk and Wilson (1985) the bacteria colonize only the apical part of the "lodging cells" which are not modified in their basal area. In the following Vesicomid species *Calyptogena magnifica*, Boss and Turner (1980), *C. phaseoliformis*, Métiver et al. (1986) and *C. laubieri*, Okutani and Métiver (1986), the cells colonized by the bacteria are completely transformed into bacteriocytes. These different types might reflect variable degrees of evolutionary change in the two families.

Two common features occur in both types and are probably related to the symbiotic relationships: the presence of lipid inclusions and the abundance of lysosomes.

Keywords: deep hydrothermal vents, subduction emissions, bivalve mollusc, trophic adaptations, bacterial symbiosis, gill ultrastructure

1. Introduction

Bivalve molluscs belonging mostly to the families Vesicomidae and Mytilidae are among the predominant organisms of luxuriant populations discovered on intense geological activity sites of the deep sea. First observed in 1976 in the Galapagos area (Corliss and Ballard, 1977) these populations were then found to characterise the hydrothermal vents of the East Pacific Rise and were studied especially in 21°N and 13°N sites (review in Hessler et al., 1985; Laubier et Desbruyère, 1984). They are also present in the Guayamas basin (Lonsdale, 1984), and more recently they were observed associated with the muddy sediments off the continental margin of Florida (Paull et al., 1984), Oregon (Suess et al., 1985) and as deep as 5960 m off Japan (Swinbanks, 1985a,b; Laubier et al., 1986). All the large sized new species develop surprisingly high biomasses in these oligotrophic zones, e.g. 10 kg.m²⁻¹ for *Bathymodiolus thermophilus* (Galapagos area, Hessler and Smithey, 1983), and 51 kg.m²⁻¹ for *Calyptogena phaseoliformis* (Japan subduction zones, Laubier et al., 1986). Ecological observations (Hessler and Smithey, 1983; Hessler et al., 1985; Laubier et Desbruyères, 1984; Laubier et al., 1986; Sibuet et al., 1986) show them to be strictly dependent upon the interstitial fluids rich in sulphur compounds, methane and different geothermally produced metals.

Biochemical adaptations related to the detection of chemoautotrophic processes (Felbeck et al., 1981, 1983; Felbeck and Somero, 1982; Fiala-Medioni et al., 1986) as well as the observations of endocellular bacteria associated with the gill of these species (Cavanaugh, 1983, 1985; Fiala-Medioni, 1984, 1986; Le Pennec and Hily, 1984; Fiala-Medioni and Métivier, 1986; Fiala-Medioni et al., 1986; Fiala-Medioni and Le Pennec, 1986) led to the hypothesis of a primordial trophic importance of symbiotic relationships between a mollusc and its bacteria. This association involves anatomical, structural and ultrastructural adaptations, which are reviewed here based on three examples: the Mytilidae *Bathymodiolus thermophilus*, Kenk and Wilson (1985) from 13°N in the East Pacific Rise, the Vesicomidae, *Calyptogena pacifica*, Boss and Turner (1980) from 21°N in the East Pacific Rise, and *Calyptogena phaseoliformis*, Métivier et al. (1986) from the subduction zones off Japan.

2. Results

All the species show evidence of reduction or simplification of the structures involved in particle feeding: reduced food groove along the ventral margin of the ctenidia, small labial palps, non plicate stomach and rectilinear in-

testine (Hily et al., 1986; Fiala-Medioni and Métiver, 1986; Fiala-Medioni and Le Pennec, 1986; Le Pennec and Fiala-Medioni, 1986). But the most remarkable modifications concern the highly vascularized, enlarged gill with the large majority of its cells containing abundant chemo-autotrophic gram-negative type bacteria. The structural aspect of the colonized cells as well as the type of bacteria vary with the species.

The mytilid, *Bathymodiolus thermophilus*, presents a gill composed of two ctenidia with descending and ascending lamellae fused on half of their height by interlamellar septa. The weight ratio between gill and other organs is 4 or 5 times higher compared to those of littoral mytilids (Le Pennec and Hily, 1984). SEM micrographs show the lamellae to be composed of homorabdic filaments. Thin transversal sections show the filament to be formed by a unicellular wall overlying a central blood lacuna with numerous hemocytes (Fig. 2A). Except for the small frontal and ab-frontal areas occupied by ciliary cells and mucous cells respectively, the wall is mainly composed of "lodging cells" that are perfectly integrated into the gill tissue and separated by thin bacteria-free cells (Fiala-Medioni, 1984; Le Pennec and Hily, 1984; Fiala-Medioni et al., 1986). In TEM observations (Fig. 1 type I, Fig. 2A,B), the bacteria (mean diameter of cross sections: 0.3 to 0.5 μm) which are concentrated in the apical part of the cell and are surrounded by peribacterial membranes, present a gram-negative type cell wall, a dense cytoplasm at their periphery and a well developed diffuse nucleoid (Fig. 2B). The cytoplasm of the basal part of the cell contains a large nucleus and the common organelles: endoplasmic reticulum, golgi vesicles and mitochondria; it is characterized by abundant inclusions of dense lipid droplets and numerous lysosomes (Fig. 2A). The result of pinocytotic processes can be seen between the peribacterial membranes and the cytoplasm of the host cell.

In both of the vesicomysids, the gill is also thick, large, and covering the entire visceral mass. *Calyptogena magnifica* presents two ctenidia, each composed of an outer and an inner demi-branch. Each demi-branch is formed by descending and ascending lamellae supported by interlamellar septa (Boss and Turner, 1980; Fiala-Medioni and Métiver, 1986). Transverse sections through the filament show a small ciliary part in the frontal area next to the lateral part which is well-developed and entirely composed of a single file row of bacteriocytes, separated by intercalary bacteria-free cells. The apical surface of both bacteriocytes and intercalary cells is covered by well developed microvilli. The bacteriocytes are completely filled with dense concentrations of bacteria (mean diameter 0.6–0.7 μm in cross section) in vac-

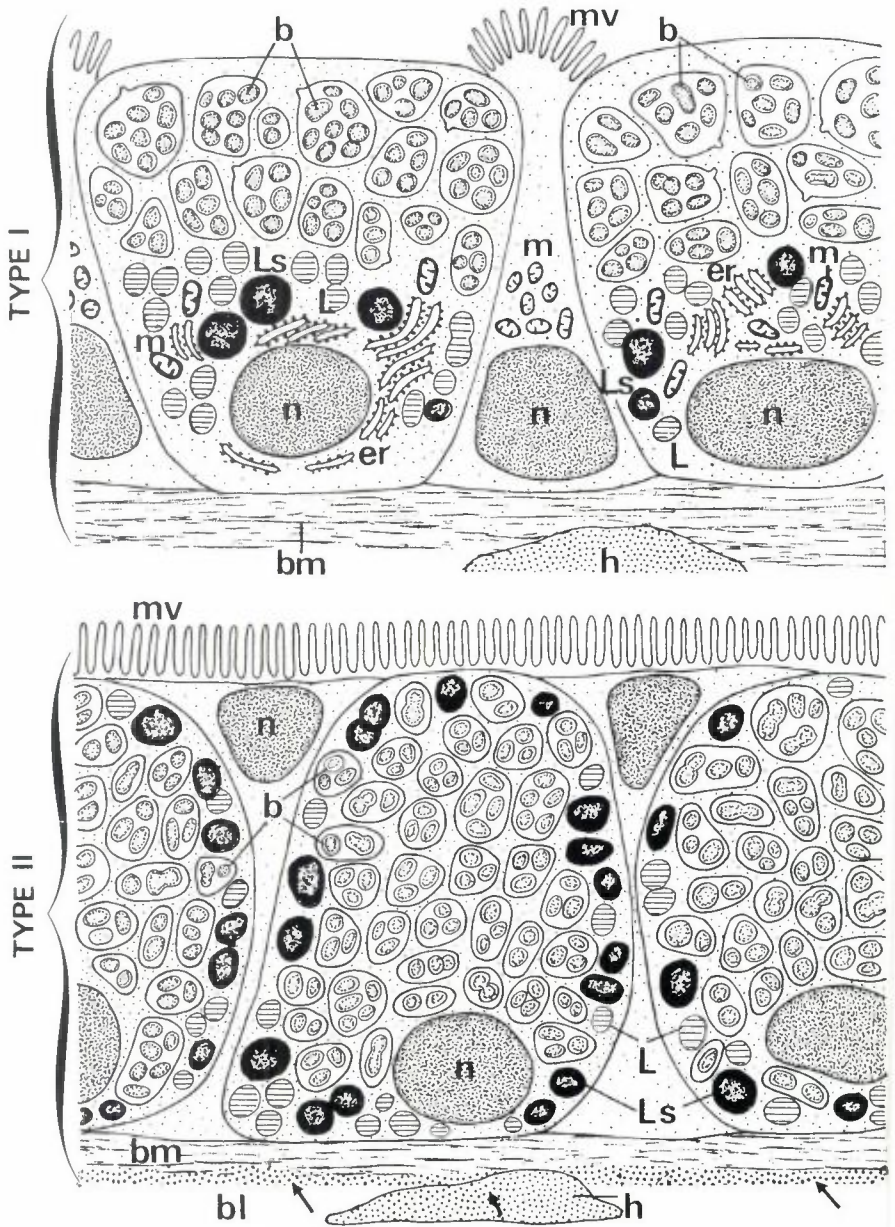


Figure 1. Graphic representation of the two types of bacterial associations; type I: mytilid type; type II: vesicomid type; b: bacteria; n: nucleus; m: mitochondria; er: endoplasmic reticulum; L: lipids; Ls: lysosomes; bm: basal membrane; bl: blood lacuna; h: hemocyte. Dense-electron grains are shown by arrows in type II. (Drawn by M.J. Bodiou).

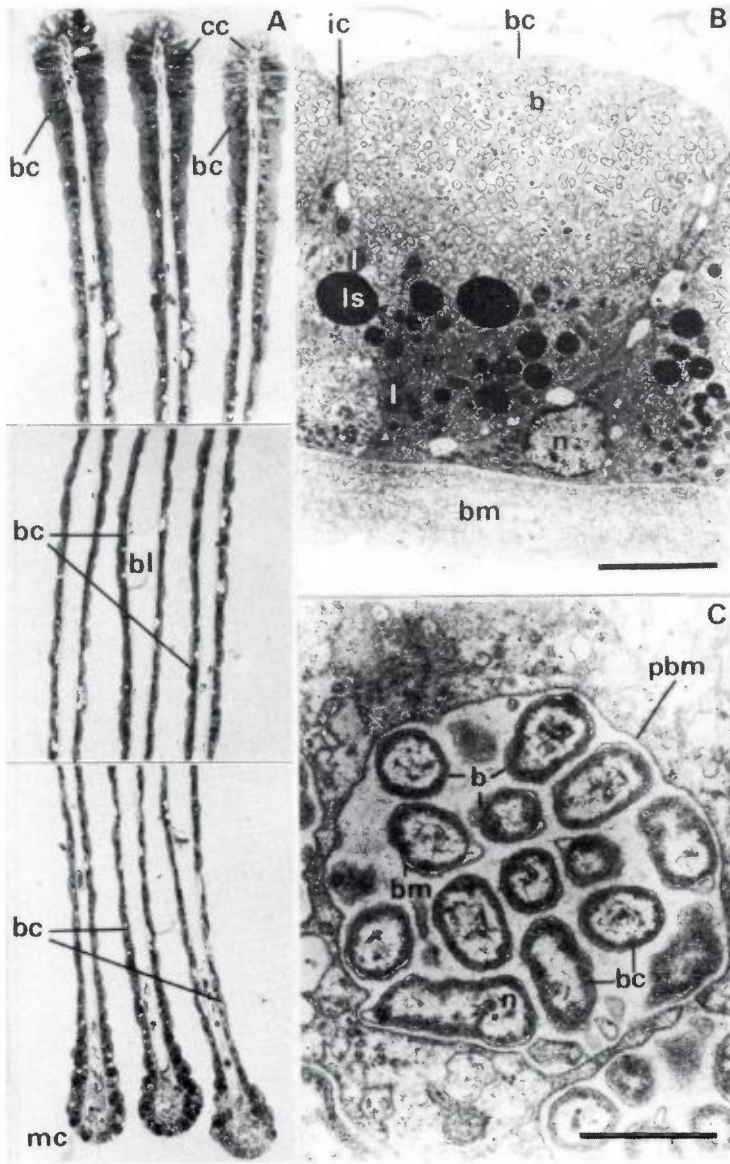


Figure 2. *Bathymodiolus thermophilus*. A, light micrograph of a thin cross section through three adjacent filaments; cc: ciliary cells of the frontal part; bc: bacterial cells; mc: mucous cell at the abfrontal part. B, electron micrograph of a cross section in the bacterial zone; bc: bacterial cell; ic: intercalary cell; b: bacteria; ls: lysosome; l: lipids; n: nucleus; bm: basal membrane. C, Magnification of a bacterial pouch; b: bacteria; bm: bacterial membrane; n: nucleoid; bc: bacterial cytoplasm (A, from Fiala-Medioni et al., 1986; B,C, from Fiala-Medioni, in press).

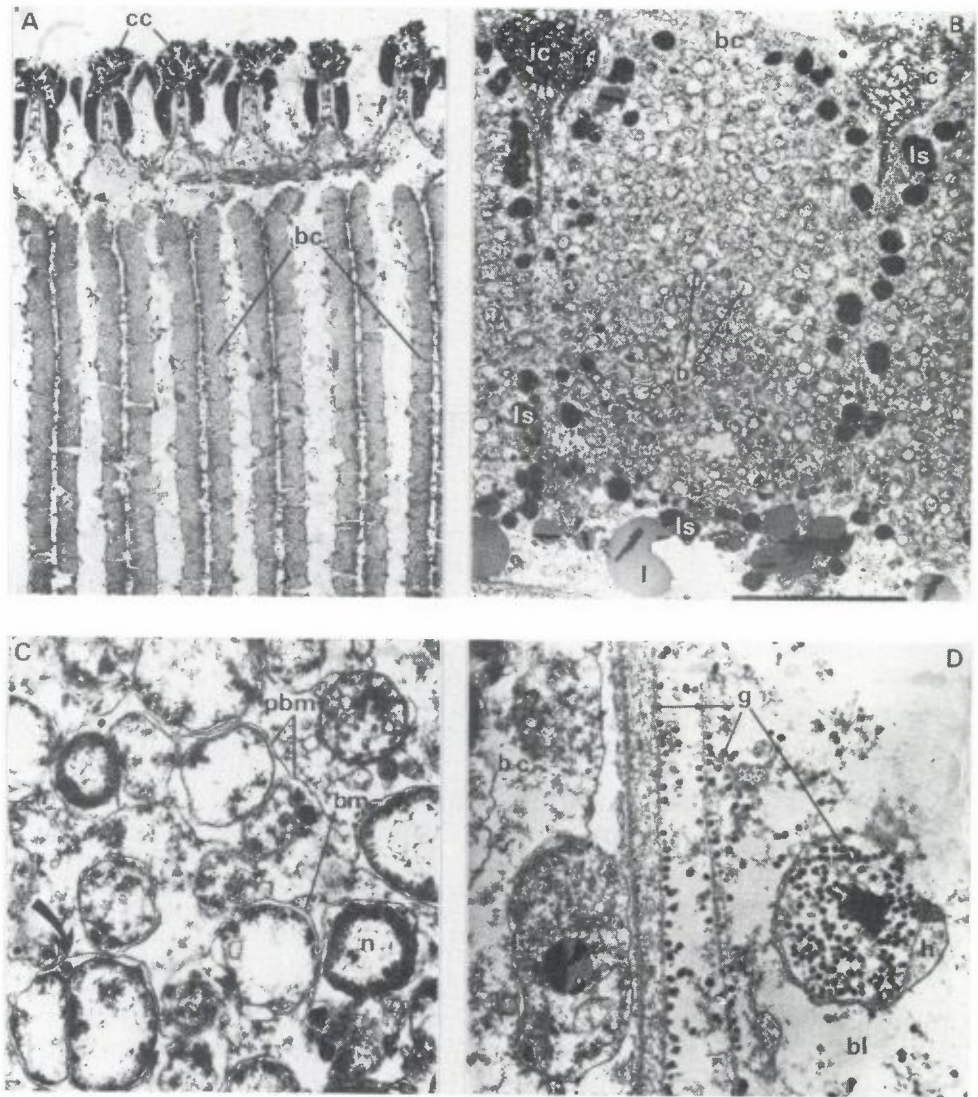


Figure 3. *Calyptogena phaseoliformis*. A, Light micrograph of a cross section through a lamella showing adjacent filaments with ciliated cells (cc) at the frontal part and bacteriocytes (bc); filaments are joined by interfilamentary septa (ifs). B, Electron micrograph of a transverse section through the bacterial part; bc: bacteriocytes; ic: intercalary cells; b: bacteria; ls: lysosomes; l: lipids. C, High magnification of bacteria showing dividing stage (arrow); bm: bacterial membrane; n: nucleoid; pm: pouch membrane; pl: phagolysosome. D, High magnification of the basal membrane (bm) zone; bc: bacteriocyte; bl: blood lacuna; h: hemocyte; g: electron-dense grains.

uoles surrounded by peribacterial membranes and presenting a gram-negative type cell wall, a dense cytoplasm and a diffuse nucleoid. The bacteria present numerous dividing stages. Few organelles are present except a large basal nucleus and some lysosomes. Small electron dense granules are abundant in the blood space near the membrane. In *C. phaseoliformis* the two ctenidia have only one branch with descending and ascending lamellae fused by interfilamentary septa. As for *C. magnifica* a small part on the frontal region of the filament carries ciliated cells; the major part of the filament is composed of bacteriocytes separated by very thin intercalary bacteria-free cells (Fig. 1 type II, Fig. 3A). In the blood lacuna the same electron dense granules are present near the basal membrane in the hemocytes and some inside the bacteriocytes (Fig. 3D).

The bacteriocytes are filled with dense concentrations of bacteria (mean diameter of cross sections: 0.5–1 μ m) with a gram-negative type cell wall and surrounded by peribacterial membranes (Fig. 3B,C). Abundant inclusions are present at the periphery of the bacteriocytes (Fig. 3B); clear ones show a positive reaction for lipids (coloration with Sudan B black, procedure from Ganter and Jolles, 1970). Lysosomes characterized by myelinic fibers are also abundant. In some bacteriocytes, mineral inclusions are enclosed in large membrane bound vacuoles, they seem to correspond to the yellow crystals visible in light microscopy, which have been shown to be elemental sulphur.

3. Conclusions and Discussion

The enlargement and the structural modifications of the gill clearly indicate that this is the main organ responsible for nutritional processes. The special adaptation related to the association with bacteria that are perfectly integrated into the gill tissue can explain the maintenance of abundant populations of such large bivalves in oligotrophic zones of the deep-sea abundantly supplied with chemical energy.

Two different modes of association are observed: in the first one (*Bathymodiolus thermophilus*), the bacteria colonize only the apical part of the cells, which present a normal structure in their basal part. Small sized as they are these bacteria would, according to their DNA composition, be related to the genus *Thiomicrospora*, using thiosulfate as primary source (Belkin et al., 1986). In the second one (*Vesicomysidae*), the contaminated cells are completely colonized and transformed into bacteriocytes. According to the description of Stetter and Zilling (1985), the bacteria might belong by their size, structure and mode of division to sulfur dependent Archaeobacteria.

They probably oxidize sulphur compounds for their metabolic requirements, according to the metabolic scheme described by Felbeck et al. (1983).

The source of energy used by the bacteria associated with the Vesicomidae of Japanese subduction zones remains to be elucidated. From a biochemical analysis Boulègue et al. (1986a) postulate the oxidation of methane present in abundance in the water emissions (Boulègue et al., 1986b). The bacteria which present a size, structure and mode of division close to that observed in bacteria of *C. magnifica*, do not show stacks of intracytoplasmic membranes as typical of type I methanotrophs found in the mussel off Louisiana (Childress et al., 1986). Furthermore the presence of abundant elemental sulphur crystals (Fiala-Medioni and Le Pennec, 1986) endorses the hypothesis of an active sulphur metabolism associated with the gill.

The differences observed in the two types might reflect variable degrees of evolutionary change in the two families. It is interesting that cells completely transformed into bacteriocytes (type II) are observed in the genus *Calyptogena* belonging to the deep-sea family Vesicomidae. All the species of this family found from 100 to over 2600 m (Boss and Turner, 1980) are probably strictly dependant on symbiotic relationships with chemoautotrophic endocellular bacteria as *C. magnifica*, *C. phaseoliformis* and *C. laubieri* showing a related simplification or great reduction of the organs involved in particle feeding (Boss and Turner, 1980; Fiala-Medioni and Métivier, 1986; Le Pennec and Fiala-Medioni, 1986). Type I is found in *Bathymodiolus thermophilus* belonging to the Mytilidae which are well-represented in shallow waters. *B. thermophilus* seems to have succeeded in the colonization of deep oligotrophic zones rich in chemical energy which is used through symbiotic relationships with endocellular chemoautotrophic bacteria. Beside this adaptation, this species has still conserved the ability to feed on particulate material (Le Pennec and Prieur, 1984; Fiala-Medioni et al., 1986; Hily et al., in press).

Despite the difference observed in the two models common features occur:

- In all species, the gram-negative-type coccoid bacteria are grouped in vacuoles surrounded by peribacterial membranes. These vacuoles tend to disappear in old bacteriocytes of *C. magnifica* (Fiala-Medioni and Métivier, 1986).
- Abundant lipid inclusions are observed in the "lodging cell" cell type (*Bathymodiolus thermophilus*) as well as in the "bacteriocyte type" (*Calyptogena phaseoliformis*). These inclusions might be related to the bacterial metabolism and represent some kind of reserve for the host.

- The lysosomes observed in the two types of association and obviously involved in lysosomic resorption of the bacteria (Fiala-Medioni, in press) may contribute to a trophic gain for the mollusc. Further experiments are needed to clarify the role of these inclusions in symbiotic relationships between the bacteria and their host.

This work relates to Department of Navy Grant N0014-87-G-033 issued by the Office of Naval Research. The United States Government has a royalty-free license throughout the world on all copyrightable material contained herein.

REFERENCES

- Belkin, S., Nelson, D.C., and Jannasch, H.W. 1986. Symbiotic assimilation of CO₂ in two hydrothermal vent animals, the mussel *Bathymodiolus thermophilus* and the tube worm *Riftia pachyptila*. *Biol. Bull.* **170**: 110-121.
- Boss, K.J. and Turner, R.D. 1980. The giant white clam from the Galapagos rift, *Calyptogena magnifica* species novum. *Malacologia* **20**(1): 161-194.
- Boulègue, J., Benedetto, E.L., Dron, D., Mariotti, A., and Letolle, R. 1986a. Biochemistry of *Calyptogena* sp. from subduction vents off Japan. *International Kaiko Conference on Subduction Zones, 10-15 November 1986*. Tokyo and Shimizu, p. 52.
- Boulègue, J., Charlou, J.L., de Kersabiec, A.M. 1986b. Fluids from subduction zones off Japan. *International Kaiko Conference on Subduction Zones, 10-15 November 1986*. Tokyo and Shimizu, pp. 37-38.
- Cavanaugh, C.M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrate form sulphide-rich habitats. *Nature* **302** (5903): 58-61.
- Cavanaugh, M. 1985. Symbiose of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. *Bull. Biol. Soc. Wash.* **6**: 373-388.
- Childress, J.J., Fisher, C.R., Brooks, J.M., Kennicutt, M.C., Bidigare, R., and Anderson, A.E. 1986. A methanotrophic marine molluscan (bivalvia, mytilidae) symbiosis: mussels fueled by gaz. *Science* **233**: 1306-1308.
- Corliss, J.B. and Ballard, R.D. 1977. Oases of life in the cold abyss. *Nat. Geogr.* **152**: 441-454.
- Felbeck, H. and Somero, G.N. 1982. Primary production in deep-sea hydrothermal vent organisms: roles of sulfide-oxidizing bacteria. *Trends Biochem. Sci.* **7**(6): 201-204.

- Felbeck, H., Childress, J.J., and Somero, G.N. 1981. Calvin-Benson Cycle and sulphide oxidation enzymes in animals from sulphide-rich habitats. *Nature* **293**: 291-293.
- Felbeck, H., Childress, J.J., and Somero, G.N. 1983. Biochemical interactions between molluscs and their algal and bacterial symbionts. In: *The Mollusca. Environ. Physiol. Biochem.* **2**: 331-358.
- Fiala-Medioni, A. 1984. Mise en évidence par microscopie électronique à transmission de l'abondance de bactéries symbiotiques dans la branchie de Mollusques bivalves de sources hydrothermales profondes. *C.R. Hebd. Seances Acad. Ser. Sci. Nat.* **298**(17): 487-492.
- Fiala-Medioni, A. 1986. Synthèse sur les adaptations structurales et fonctionnelles liées à la nutrition des Mollusques bivalves des sources hydrothermales profondes. *Oceanol. Acta* (in press).
- Fiala-Medioni, A. and Métivier, C. 1986. Ultrastructure of the gill of the hydrothermal vent bivalve *Calyptogena magnifica* with a discussion on its nutrition. *Mar. Biol.* **90**: 215-222.
- Fiala-Medioni, A. and Le Pennec, M. 1986. Structural adaptations related to the type of nutrition in the deep-sea bivalves of the subduction zones around Japan. *International Kaiko Conference on Subduction Zones, 10-15 November, 1986*. Tokyo and Shimizu, p. 56.
- Fiala-Medioni, A., Métivier, C., Herry, A., and Le Pennec, M. 1986a. Ultrastructure of the gill filament of an hydrothermal vent Mytilidae. *Mar. Biol.* **92**: 65-72.
- Ganter, P. and Jolles, G. 1970. Histochimie normale et pathologique. T2, Gauthier-Villars, Paris, pp. 927-1904.
- Hessler, R.R. and Smithey Jr., W.M. 1983. The distribution and community structure of megafauna at the Galapagos Rift hydrothermal vents. In: *Hydrothermal Processes at Sea Floor Spreading Centers*. P.A. Rona, K. Bostrom, L. Laubier, K.L. Smith, Jr., eds. NATO Conference Series IV. *Mar. Sci. (N.Y.)* **12**: 735-770.
- Hessler, R.R., Smithey, W.M., and Keller, C.H. 1985. Spatial and temporal variation of giant clams, tube worms and mussels at deep-sea hydrothermal vents. *Bull. Biol. Soc. Wash.* **6**: 411-428.
- Hily, A., Le Pennec, M., Prieur, D., and Fiala-Medioni, A. Anatomie et structure du tractus de *Bathymodiolus thermophilus*, Kenk et Wilson 1985 (*Mytilidae*) des sources hydrothermales profondes de la ride du Pacifique Oriental. *Cah. Biol. Mar.* (sous presse).

- Kenk, V.C. and Wilson, B.R. 1985. A new mussel (bivalvia Mytilidae) from hydrothermal vents in the galapagos rift zone. *Malacologia* **26**(1-2): 253-271.
- Laubier, L. et Desbruyères, D. 1984. Les oasis du fond des océans. *La Recherche* **15**: 1506-1517.
- Laubier, L., Ohta, S., et Sibuet, M. 1986. Découverte de communautés animales profondes durant la campagne franco-japonaise KAIKO de plongées dans les fosses de subduction autour du Japon. *C.R. Hebd. Seances Acad. Ser. Sci. Nat.* **303**(2): 25-29.
- Le Pennec, M. et Hily, A. 1984. Anatomie, structure et ultrastructure de la branchie d'un *Mytilidae* des sites hydrothermaux du Pacifique Oriental. *Oceanol. Acta* **7**: 517-523.
- Le Pennec, M. and Fiala-Medioni, A. 1986. Nutritional and reproductive strategies in the deep-sea bivalves of the subduction zone of Japan. *International Kaiko Conference on Subduction Zones, 10-15 November, 1986*. Tokyo and Shimizu, p. 55.
- Le Pennec, M. et Prieur, D. 1984. Observations sur la nutrition d'un mytilidae d'un site hydrothermal actif de la dorsale du Pacifique Oriental. *C.R. Hebd. Seances Acad. Ser. Sci. Nat.* **298**(III): 493-498.
- Lonsdale, P. 1984. Hot vents and hydrocarbon seeps in the sea of Cortez. Guaymas Basin phenomena differ from those of mid-ocean rises. *Oceanus* **27**(3): 21-24.
- Métivier, B., Okutani, T., and Ohta, S. 1986. *Calyptogena (Ectenagena) phaseoliformis* n.sp., an unusual Vesicomid bivalve collected by the submersible *Nautile* from abyssal depths of the Japan and Kurile Trenches. *Venus* **45**: 161-168.
- Okutani, T. and Métivier, B. 1986. Descriptions of three new species of Vesicomid bivalves collected by the submersible *Nautile* from abyssal depths off Honshu, Japan. *Venus* **45**: 147-160.
- Paull, C.K., Hecker, B., Commeau, R.P., Freeman-Lynde, C., Neumann, C., Corso, W.P., Golubic, S., Hook, J.E., Sikes, E., and Curray, J. 1984. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. *Science* **226**(4677): 965-966.

- Sibuet, M., Juniper, K., and Pautot, G. 1986. Structure and microdistribution of "cold seep" benthic communities in the Japan subduction zones (Kaiko program): modes and limiting factors of biological exploitation of sediment pore-water venting. *International Kaiko Conference on Subduction Zones, 10-15 November 1986*. Tokyo and Shimizu, p. 50.
- Stetter, K.O. and Zilling, W. 1985. Thermoplasma and the thermophilic sulfur. Dependent Archaeobacteria. In: *The Bacteria*, VIII. J.R. Sokatch, L.N. Ornaton, C.R. Woese and R.S. Wolfe, eds. Academic Press, New York, pp. 85-170.
- Suess, E., Carson, B., Ritger, S.D., Moore, J.C., Jones, M.J., Kulm, D., and Cochrane, G.R. 1985. Biological communities at vent sites along the subduction zone off Oregon. *Bull. Biol. Soc. Wash.* **6**: 475-484.
- Swinbanks, D. 1985a. Japan finds clams and trouble. *Nature* **315**: 624.
- Swinbanks, D. 1985b. New find near Japan's coast. *Nature* **316**: 475.