SELECTED MEIOFAUNA AROUND SHALLOW WATER HYDROTHERMAL VENTS OFF MILOS (GREECE): ECOLOGICAL AND ULTRASTRUCTURAL ASPECTS

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HYDROTHERMAL VENTS
MEDITERRANEAN SEA
MEIOFAUNA
NEMATODES
SULFIDE
DETOXIFICATION
ULTRASTRUCTURE

ABSTRACT – Gaseous emissions, caused by volcanic activity, occur in Paleohori Bay at the south coast of Milos (Aegean Sea, Greece). In June 1992 meiofauna was collected by SCUBA diving in a seep field in sandy sediments at 5-10 m water depth. Within an active vent area (site 1), the sediment temperatures ranged from 55-97°C and the sulfide concentrations were about 1000 µM. At the fringe of the area, temperatures decreased (25-30°C) as well as sulfide values (10-50 µM). Outside the active area, not directly influenced by the venting, the sediment temperature ranged between 20.0-20.7°C and no sulfide was detected. The sediment in direct vicinity of the emissions was devoid of meiofauna. Meiobenthic life was present only at a certain distance from the emissions at moderate temperature and sulfide conditions. Evaluating three transects from two sites it became apparent that meiofauna differed between the seeps, but also between seep and non-seep stations. This differentiated faunal distribution rendered generalizations of the seep meiofauna problematical. Overall, the meiofauna community was relatively poor consisting primarily of nematodes and a few harpacticoid copepods, turbellarians and ciliates. In the sulfidic sediments, meiofauna was dominated by some characteristic nematodes. The most frequent one, *Oncholaimus campylocercoides* (Oncholaimidae), occurred in abundances up to 300 ind. / 100 cm^3. Therefore, this characteristic species was selected for more detailed studies on structural adaptations to this hostile environment. Two different types of intracellular inclusions were found. Their possible function as sulfide detoxification mechanisms is discussed.

SOURCES HYDROTHERMALES
MÉDITERRANÉE
MÉIOFAUNE
NÉMATODES
SULFURES
DÉSINTOXICATION
ULTRASTRUCTURE

RÉSUMÉ – Des émissions de gaz dues à l'activité volcanique, se produisent dans la baie de Paleohoviri, sur la côte sud de Milos en mer Egée (Grèce). La méiofaune a été récoltée en plongée à une profondeur comprise entre 5 et 10 m dans les sédiments sableux d'une zone d'émissions hydrothermales en juin 1992. Dans une zone active, la température des sédiments varie entre 55 et 97°C et la concentration en sulfures est d'environ 1000 µM. En marge de cette zone la température décroît (25-30°C) ainsi que les valeurs en sulfures (10-50 µM). A l'extérieur de la zone active, la température des sédiments non directement influencés par les émissions s'établit à 20.0-20.7°C et les sulfures ne sont plus détectés. Les sédiments en relation directe avec les sites d'émissions ne contiennent pas de méiofaune. Celle-ci est présente seulement à une certaine distance des points de rejets dans des conditions modérées de température et de taux de sulfures. Trois transects sur 2 sites différents montrent que la méiofaune diffère entre les zones d'émissions mais aussi entre les sites et les zones hors sites. La distribution différentielle de la faune rend les généralisations à propos de la méiofaune des évents problématiques. Globalement, la communauté méiofaunique est relativement pauvre et se compose principalement de Nématodes et de quelques Copépodes Harpacticoïdes, Turbellariés et Ciliés. Dans les sédiments imprégnés de sulfures, dominent quelques Nématodes caractéristiques. Le plus fréquent est *Oncholaimus campylocercoides* (Oncholaimidae), avec une abondance pouvant atteindre 300 ind./100 cm^3. Cette espèce caractéristique a donc été sélectionnée en vue d'études détaillées à propos des adaptations des structures à cet environnement hostile. Deux types d'inclusions intracellulaires ont été trouvées. Leur fonction dans un mécanisme de désintoxication vis à vis des sulfures est discutée.

INTRODUCTION

There exist some reports on the soft bottom fauna in areas of cold gas, brine or oil seepage with considerable amounts of hydrogen sulfide (see Discussion). However, in the studies on sediment fauna from hydrothermal vents, the meiobenthos has been grossly neglected. In the recent paper by Kamenev et al. (1993) on the shallow water hydrothermal vents in the Bay of Plenty, New Zealand, the numbers of nematodes (e.g. Stilbonematinae) was reported to increase approaching the vent sites. Earlier, Meyl (1954) had investigated the nematode fauna from volcanic hydrothermal vents at the shore line of the Island of Ischia (Bay of Naples, Italy). In the above papers the steep gradients of the physicochemical regime typical for hydrothermal vents (sulfide, salinity, temperature) have not been evaluated in detail in relation to meiofaunal distribution. In the present study from shallow water volcanic hydrothermal vents off the island of Milos (Greece), meiofauna together with relevant physicochemical parameters were sampled by SCUBA diving following defined transects from areas not directly affected by the geothermal influence towards the vents. This allowed, on a relatively narrow scale, to relate the distributional pattern of dominant meiobenthic organisms to the impact of the hydrothermal regime. The present paper concentrates on the nematodes because of their numerical dominance and good preservation allowing for a detailed taxonomical evaluation. Additionally it was attempted to refer the prevalence of the dominating nematode species to characteristic structural features analysed by electron microscopy.

SAMPLING AREA, MATERIAL AND METHODS

Hydrothermal gaseous emissions, caused by volcanic activity, occur in large areas around the island of Milos (Greece), located in the South Aegean Sea on the Hellenic Arc (Dominico and Papastamatoki, 1975, Fytikas 1989). Paleohori Bay in the south of Milos was chosen for sampling (Fig. 1). The bay is approximately 1.4 km wide and has an average depth of approximately 12 m. The whole area is geothermally heated. The gaseous emissions consist predominantly of CO_2 and H_2S; CH_4 and H_2 were found in minor quantities (Dando, pers. comm.). The sediment in and around most seeps in the seep field was black which is probably due to iron sulfide. The black patch sampled was approximately 30 m² large and contained about 20 seeps. Here, the brownish surface sediment normally indicating oxidized conditions was lacking, the black sediment reached the surface (dark stippled area in Fig. 1, lower
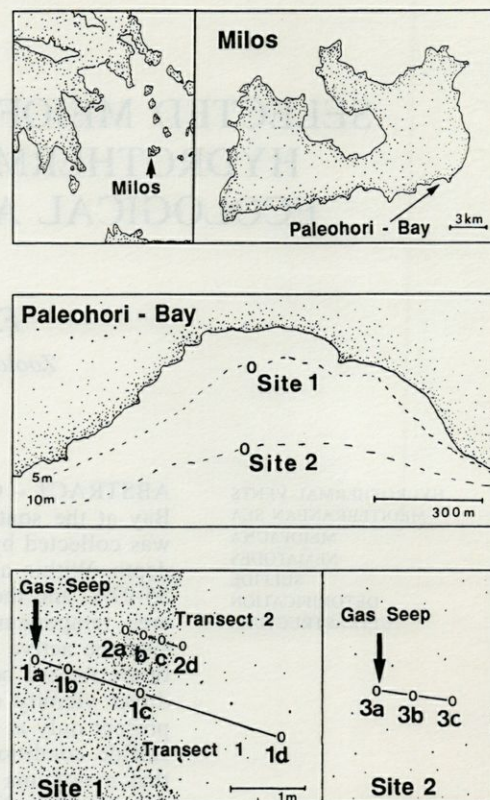

Fig. 1. – Study area and location of the sampling stations. Note black sulfidic patch indicated by stippled area at lower left.

left). The top of the black sediment was covered by a white mat consisting of bacteria and silicate (Dando and Gottschal, pers. comm.). During the sampling period the white mat became disturbed and suspended by wave action caused by a storm. It was observed to re-establish within 3 days. Outside the patch the sediment was yellowish-brown and the threshold layer towards the deeper, reduced horizons was in approximately 5 cm depth.

The position of sampling stations is mapped in Fig. 1. Samples from two different sites were taken by SCUBA diving in June 1992 at a seep field in 5-10 m water depth.

Site 1 was sampled in two transects consisting of four sampling stations each and covering different geochemical milieus and metric scales. Three replicates were taken at each station, the total number of samples amounted to 33.

Transect 1 – yielded a station line across the whole sampling area, beginning in the center of a seep, crossing the black "halo", and reaching the outside "reference" site. The overall length of this transect was 3.5 m and included 4 sampling stations :
Station 1a) at the seep inside the patch,
Station 1b) black sediment, covered by the white mat, approximately 50 cm next to the seep,
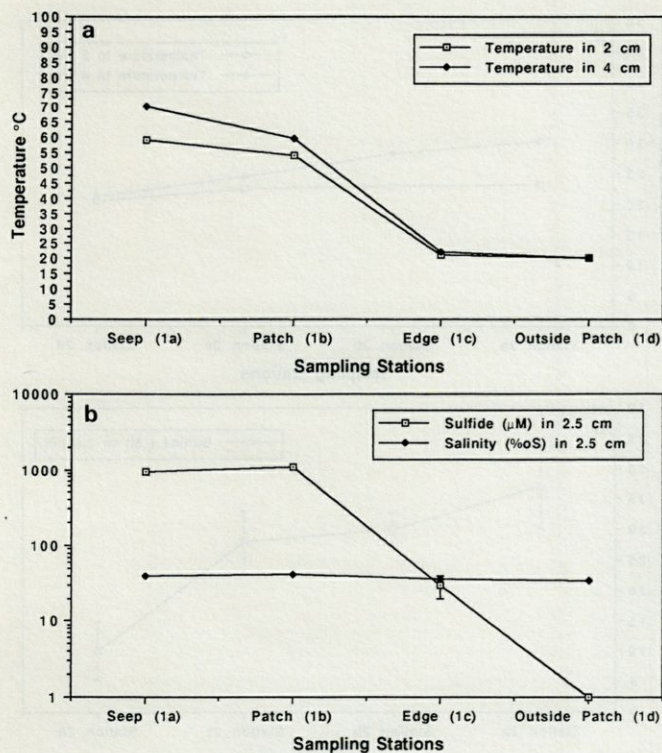
Fig. 2. – a, Transect 1 : Temperature conditions in 2 cm and 4 cm sediment depth. b, Transect 1 : Salinity and sulfide concentrations in 2.5 cm sediment depth.

Station 1c) the edge of the patch in 1 m distance to 1b
Station 1d) outside the patch in 2 m distance to 1c.

Transect 2 – analysed the edge of the patch in a more refined scale (overall length only 0.9 m). Samples were taken every 30 cm through this edge area, starting with
Station 2a) at its inner border, continuing with
Station 2b) and 2c) towards the outer edge area, and ending with
Station 2d) in the outer edge area.
Site 2, at 10 m depth, with the third sampling transect differed from the above through the absence both of a black halo and a white mat around the seep.
Station 3a) at the gas seep
Station 3b) in 50 cm distance from the seep
Station 3c) in 50 cm distance from 3b.

Physiographic parameters : Pore water samples for sulfide and salinity measurements were taken close to the fauna samples with special pore water suction corers (modified after Howes *et al*, 1985). Pushed into the sediment, 0.1-1.0 ml of water was obtained from 2.5 and 7.5 cm sediment depth. Immediately after retrieval and still under water, the pore water was injected into a septum-jar. For fixation of the sulfide, these sampling jars contained 2 ml of 0.12 M zinc acetate and 0.5 ml 1.5 M sodiumhydroxide. Three adjacent replicates were taken from each station. Water and sediment temperature were measured with a thermistor which was inserted into the sediment directly under water. Parallel to the fauna samples (see below), sediment samples were taken

with a perspex corer for analyses of grainsize distribution and organic carbon content. At transect 3, for technical reasons, no pore water samples could be taken.

The sulfide concentration was measured colorimetrically (Gilboa – Garber, 1971, modified by Howarth *et al.*, 1983) in the home laboratory ten days after sampling. Although fixed samples can be stored refrigerated for several days without loosing a significant amount of sulfide, our conservative interpretation leaves the option that the real sulfide concentrations might be slightly higher. Grain size distribution and the content of organic carbon were analysed according to common procedures (Buchanan, 1984, Giere *et al.*, 1988a). The salinity of the pore water was recorded using a hand refractometer.

The meiofauna samples were obtained using perspex tubes bevelled at the lower edge. The corers (5 cm inner diameter) were pushed into the sediment to the depth of 10 cm, closed with rubber-bungs and without further disturbance brought immediately to the Paleohori land base. Here, the cores were subdivided into two 5 cm-fractions, fixed and stored in 5-10 % formalin (final concentration).

Evaluation of fauna : The meiofauna was extracted from the sediment by the elutriation method (Pfannkuche & Thiel, 1988) through a 63 μm mesh sized sieve. The animals retained were sorted quantitatively under a dissecting microscope (magnification 25-50 x). Individuals of the large nematode species *Oncholaimus campylocercoides* (approximately 4000 x 80 μm) were
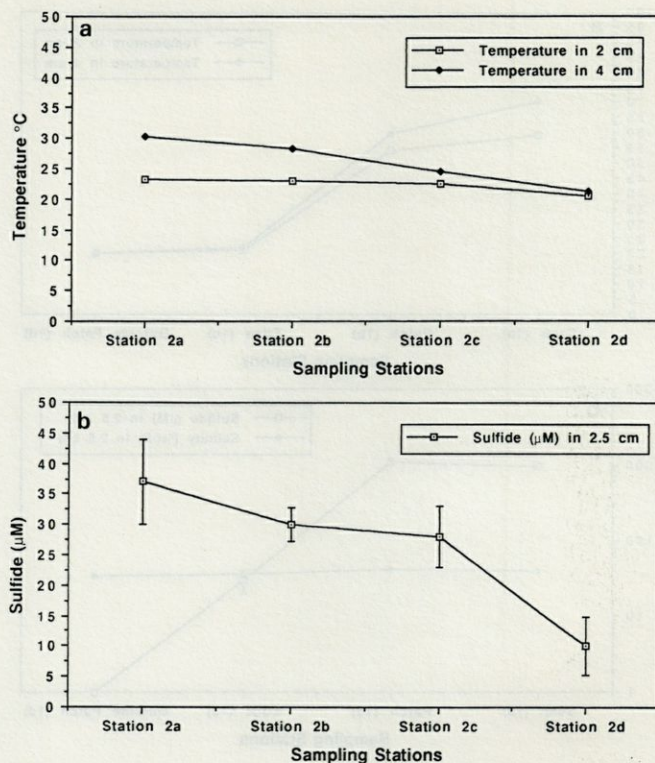
Fig. 3. – a, Transect 2 : Temperature conditions in 2 cm and 4 cm sediment depth. b, Transect 2 : Sulfide concentrations in 2.5 cm sediment depth, note different scale from Fig. 1b.

evaluated separately. After fixation (formalin 4 %) the animals were post-fixed in the home laboratory in Trump's fixative (McDowell and Trump, 1976). Selected specimens were contrasted in 1 % osmium tetroxide, followed by the conventional procedure for TEM preparations. Further investigation was done under the light and electron microscope (ZEISS EM 902A). Element analysis of precipitations in uncontrasted ultrathin sections was performed by Electron Enery Loss Spectrography (EELS) linked to the ZEISS electronmicroscope. This method allows exact location of chemical elements in ultrathin sections of tissues.

RESULTS

Distribution of environmental factors and meiofauna at site 1 (transects 1+2)

The sediment temperature (Figs. 2a, 3a) decreased from the seep to the area outside the black patch and from the sediment depth towards the surface. Directly at the seep, temperatures reached highest values in 10 cm sediment depth (97 °C). At the main sampling depth of 2-5 cm temperature was still between 50° and 60 °C. At the edge of the patch, temperatures in sampling depth varied between 21.3 °C and 30 °C. Outside the patch, the temperature was more or less constant (20.0-20.7 °C) from the sediment surface down to 10 cm depth. Water temperature directly above the sediment was 20 °C.

The salinity of the pore water (2.5 cm depth) increased in the patch from the periphery towards the seep site from 35‰ S to 45‰ S, but varied within stations at different depths.

Sulfide concentrations (Fig. 2b) were high within the seep (940 µM)) and inside the black patch (1080 µM). Vertically, contents at 5.5 cm sediment depth did not differ markedly from those in 2.5 cm as indicated in the graphs (Figs. 2b, 3b). At the edge of the patch, only moderate sulfide concentrations were measured (30-50 µM) both in 2.5 and 7.5 cm sediment depth (Fig. 3b). Outside the patch no sulfide was detected.

The sediment consisted of fine to medium sands (Md : 258-281 µm, QDI : 0.79-0.91) with the silt fraction increasing with depth at all sites. No significant differences were recorded between sites and stations along the transects. The organic carbon values ranged between 1290 and 985 µg/g sediment, with slightly increased values inside the black patch.

Distribution of meiofauna at site 1 (transects 1 and 2)

Overall, the meiofauna of site 1 was relatively poor consisting primarily of nematodes and a few harpacticoid copepods, turbellarians and ciliates. Also the reference samples outside the patch were scarcely inhabited, consisting of less than 400 nematodes and 75 "others" per 100 cm³ (Fig. 4). The bulk of the meiofauna was found in the upper 5 centimeters of the sediment, the 5-10 cm-fraction contained just a few specimens.
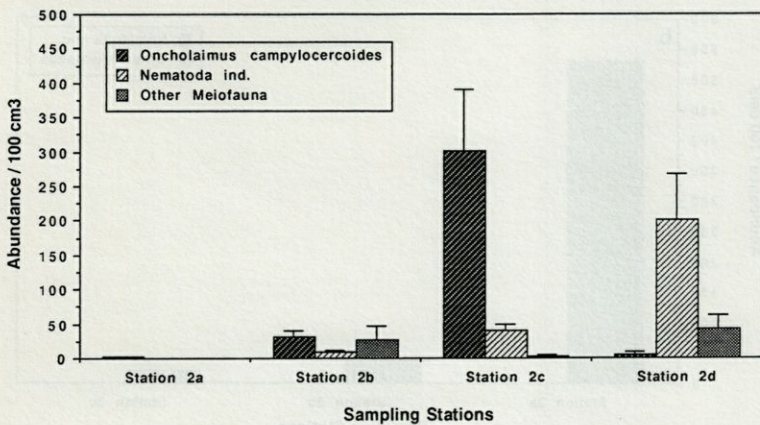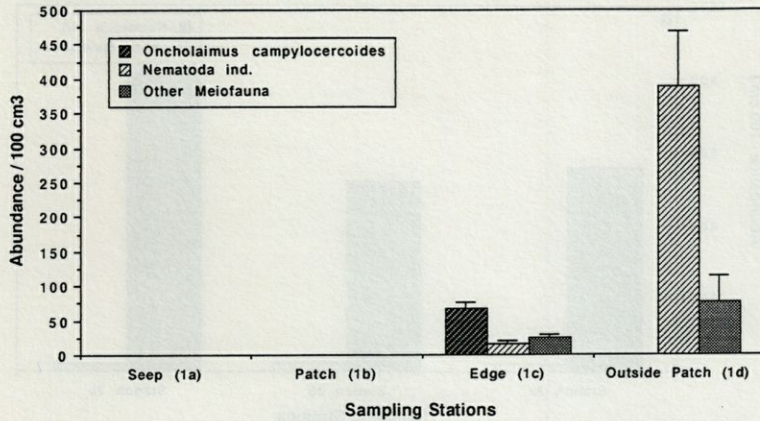
Fig. 4. – Transect 1 : Abundance of meiofauna in the 0-5 cm sample fraction (mean of three replicates).

Fig. 5. – Transect 2 : Abundance of meiofauna in the 0-5 cm sample fraction (mean of three replicates).

In transect 1, the most central sample station at the gas vents and in the black sediment patch around them was devoid of fauna (Fig. 4). The edge of the black patch (station 3) contained a rather monotonous meiofauna assemblage with the nematode *Oncholaimus campylocercoides* dominating the community (Fig. 5). From the edge area towards the outer stations of the transect, most meiofauna except *O. campylocercoides* increased in abundance.

Only *O. campylocercoides* had a different distribution, its small scale pattern was therefore studied in detail in transect 2. Most abundant (300 ind./100 cm^3) at station 2c, this species decreased numerically both towards the inner and outer stations of the transect (2a, b and d). At station 2c with the highest population density, sulfide concentrations (2.5 cm depth) were about 28 μM and the sediment temperatures (2-4 cm depth) varied between 22 and 24 °C.

To prove the consistency of the meiofauna distribution at various seep sites, transect 3 with the stations 3a-3c investigated another venting site in about 200 m distance from the above site. The different appearance of this vent was described earlier. Although covering a distance from directly at the vent to 1 m away from the seeping gas, all the three stations of this transect contained in the upper five centimeters a meiofauna with only minor differ-

ences both in abundance and composition (Fig. 6a). This assemblage largely conformed with that of the "control" station 1d of transect 1 outside the impact of the vent. This uniform situation changed going down to deeper sediment horizons. Contrasting to the usual decline of meiofauna populations with depth, at station 3a, directly at the gas outlet, the 5-10 cm horizon had an enhanced abundance of meiofauna (Fig. 6b). Here, representatives of long and slender nematode species, such as *Eubostrichus cf. parasitiferus, Siphonolaimus* sp. and *Chromaspirina* sp. prevailed. But this well defined "depth community" was restricted to station 3a only. In the corresponding layer of the more distant peripheral stations 3b and 3c, the typical decline of meiofauna abundance with depth was recorded. Contrasting to the other vent site described above (site 1), the formerly dominant *Oncholaimus campylocercoides*, was totally absent in this transect, even at a distance from the vent comparable to its peak occurrence at station 2c.

The numerical and ecological relevance of the nematode *Oncholaimus campylocercoides* (Oncholaimidae) in an area influenced by a hydrothermal vent led us to investigate this species in more detail. Main stress was laid on structural and histochemical aspects of possible adaptive significance enabling the species to thrive in a sulfidic environment.
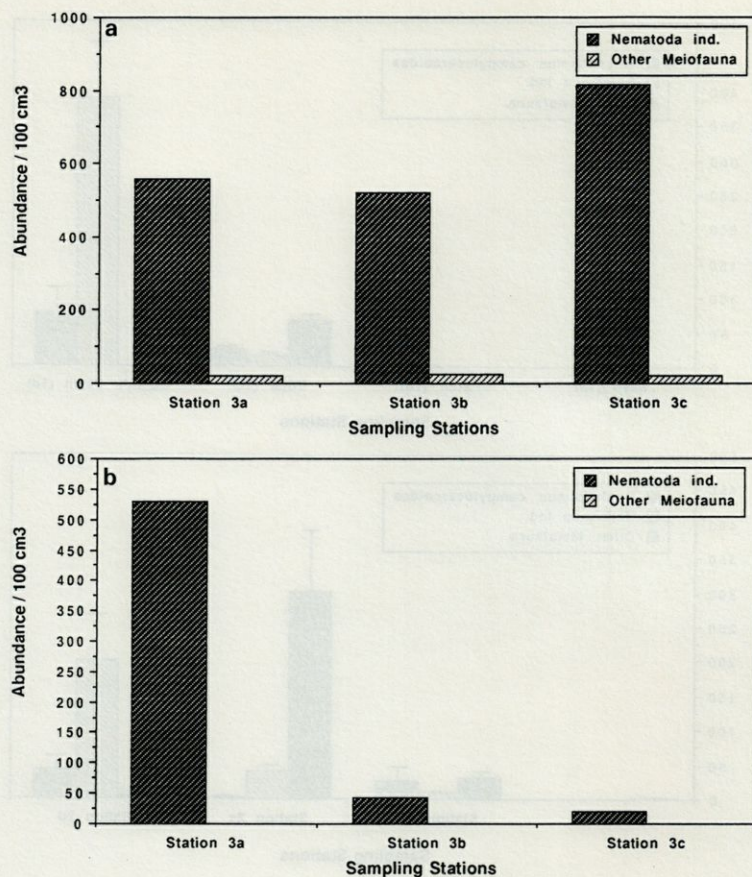
Fig. 6. – a, Transect 3 : Abundance of meiofauna in the 0-5 cm sample fraction (mean of two replicates). b, Transect 3 : Abundance of meiofauna in the 5-10 cm sample fraction (mean of two replicates).

As a characteristic feature of ultrathin sections through *O. campylocercoides,* inspected in the transmission electron microscope, the intestinal cells contained rich numbers of electron-dark, globular granules. Many of them showed a distinct concentric layering, while others appeared uniformly black (Figs. 7, 8, 9). In some of these granules, parts of the content seemed to be dissolved during the process of preparation leaving empty spaces (Fig. 8). Using the EELS method, in all of these globules iron and phosphorus could be detected (Fig. 11a, b).

O. campylocercoides was encountered both in live and fixed specimens in two different morphotypes. Yellowish pale specimens, corresponding to the normal appearance of oncholaimid nematodes, contrasted to shiny white individuals (reflected light). Under the light microscope this white appearance could be related to conspicuous vesicles distributed along the body of the worms in the epidermal layer. Their content lacked any internal structure and was apparently an oily substance (squeeze preparations). The transmission electron microscope showed these vesicles to be non membrane-bound containing an electron-lucent, amorphous mass, sometimes aggregated in several droplets of irregular size (Fig. 10). These bright inclusions were proven to contain rich amounts of sulfur (EELS ; Fig. 12).

DISCUSSION

The meiofauna from sediments in seeping areas characterized by a considerable amount of H_2S has been studied at several occasions. Powell and Bright (1981) and Jensen (1986) investigated brine seeps in 72 m depth studying a transect from high salinity and high sulfide concentrations to "normal" conditions. At the sulfide-impacted part of this gradient they found a distinct thiobiotic community (i.e. gnathostomulids and particular nematodes) which changed its character towards a normal, non-sulfidic community approaching to the peripheral oxidized bottom. A natural oil seep (gas and petroleum hydrocarbons) was studied by Montagna *et al.* (1989). These authors found increased abundances of sulfide oxidizing bacteria and of nematodes at the seep (see also Montagna and Spies, 1985, Montagna *et al.,* 1987). Dando *et al.* (1991) reported from a "pock mark" with methane seepage in the North Sea a rich population of the gutless nematode *Astomonema southwardorum* living in symbiosis with endosymbiotic bacteria (Austen *et al.,* 1993 ; Giere *et al.,* in press).

Not all studies discovered an overall increase of meiofauna in seep areas (Jensen *et al.,* 1992 ; Dando *et al.,* 1993). However, the metazoan meiofauna was mostly dominated by nematodes with typical representatives for sulfidic sediments, such
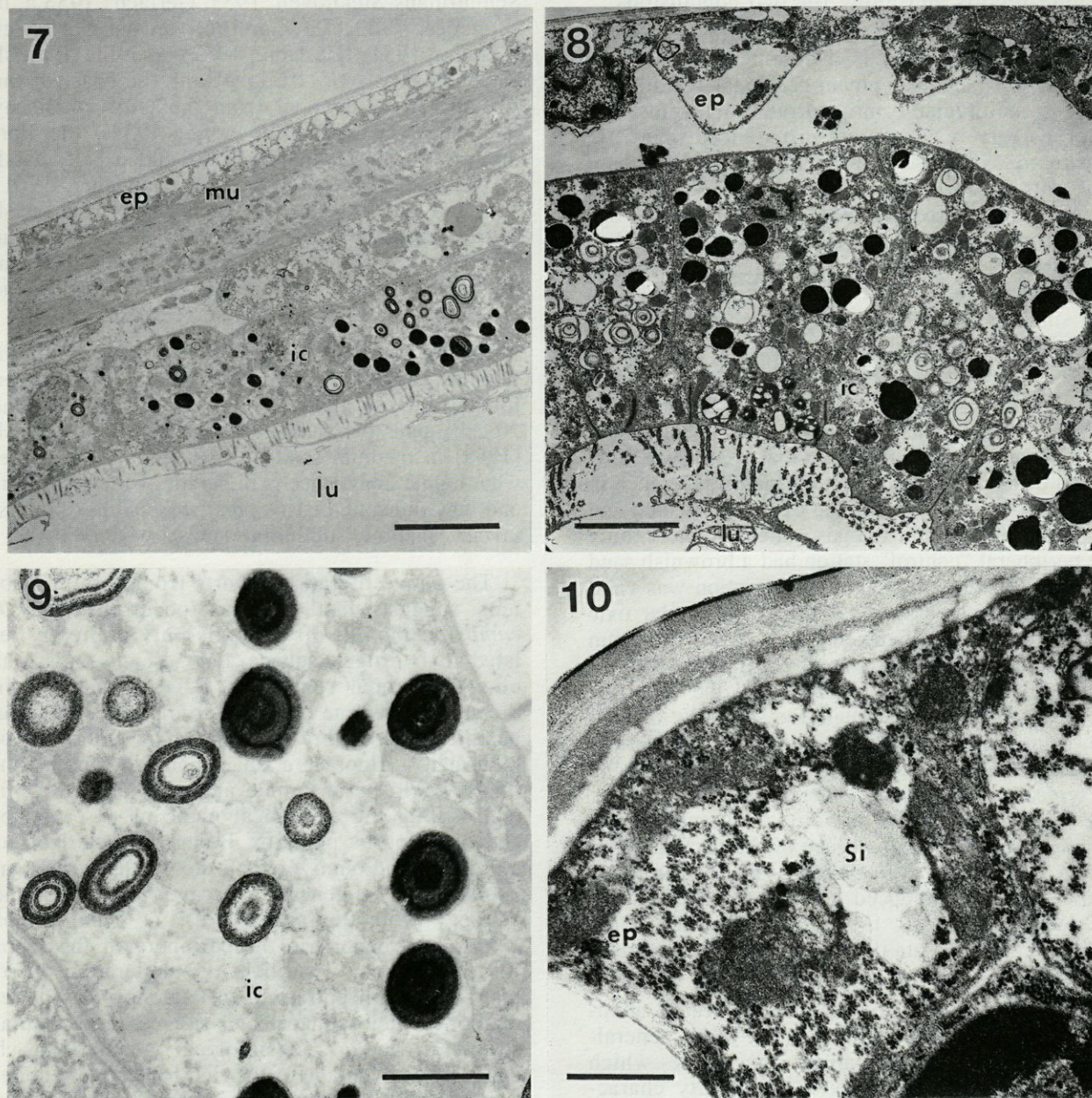
Fig. 7. – *Oncholaimus campylocercoides*, longitudinal section through mid-body region. Note electron-dark and concentric globules in the intestinal cells. Scale bar : 5 µm (electron micrograph). Fig. 8. – *Oncholaimus campylocercoides*, cross section through the mid body region. Note numerous electron-dark and concentric globules (partly empty) in the intestinal cells. Scale bar : 3.5 µm (electron micrograph). Fig. 9. – *Oncholaimus campylocercoides*, cross section through an intestinal cell. Note concentric structure of inclusions. Scale bar : 1.2 µm (electron micrograph). Fig. 10. – *Oncholaimus campylocercoides*, cross section through the epidermis and cuticle. Note electron-lucent, sulfur containing inclusion in the epidermis cell. Scale bar : 0.5 µm (electron micrograph). ep : epidermis ; ic : intestinal cells ; lu : gut lumen ; mu : muscle cells ; Si : sulfur inclusion.

as *Sabatieria longispinosa*, *S. punctata* and *Daptonema sp.* Contrastingly, at the deep sea bottom (Shirayama and Otha, 1990) did not find a specialized meiofauna community at a deep sea cold seep, though the abundance of meiofauna was slightly increased compared to the reference sites.

The same trend was recorded around shallow water hydrothermal vents in the study of Kamenev *et al.* (1993) from the Bay of Plenty, New Zealand, where the bacteria-symbiotic Stilbonematinae (Nematoda) were typical for the close vicinity of the vents.

The ecological results from the two vent sites off Milos essentially correspond to the above literature reports and allow for two conclusions :

(1) at different seep sites the distributional pattern of the meiofauna is different depending apparently on the local physiographical conditions. For the meiofauna from hydrothermal vents this heterogeneity is even more pronounced than it is known to exist also for macrofauna in the deep sea vents (Desbruyères *et al.*, 1994). These results suggest that more detailed studies on the hydrothermal vent (meio-)fauna within larger hydrothermal fields, i.e. differentiation between single seeps, will yield a much higher diversity and small-scale heterogeneity than anticipated so far. Therefore, generalizations on the fauna of larger hydrothermal areas on the basis of some few isolated samples are probably inadequate.

The meiobenthic populations at vent site 2 differed substantially from those at vent site 1. This faunal difference may refer to a major difference in the physiography, the absence of the black halo and the white mat around the gas outlet. At site 1, only the peripheral "control" station had a brownish sand surface. At site 2, this "normal" situation was valid for the whole area sampled. Hence, a regular oxic environment without development of a sulfuretum might represent a physiographic parallel between the surface of all the site 2 stations and the "control" of site 1 which would explain the similarity of their meiofauna populations. Only in the deeper layers of the seep outlet at site 2, the presence of a rich stock of typically thiobiotic, slender nematodes (Jensen, 1987 a) points to mild sulfidic conditions. This is also suggested by the presence of the bacteria-symbiotic nematode, *Eubostrichus*. Its bacteria are known to oxidize reduced sulfur compounds (Ott *et al.*, 1991; Polz *et al.*, 1992). In the absence of additional chemical data this hypothesis awaits further validation.

(2) the trend of an increase in meiofauna abundance towards the seep site is not to be generalized. But it holds often valid for nematodes which usually are represented by a community characterized by Jensen (1986, 1987a) as thiobiotic, i.e. especially adapted to life in sulfidic conditions.

The nematode species *Oncholaimus campylocercoides,* which in the present study lived in considerable numbers only in the vicinity of gas outlets under sulfidic conditions and moderate sediment temperatures, was in a single specimen found also by Meyl (1954) in geothermally heated sand (45 °C) close to volcanic hydrothermal vents at the shore line of the Island of Ischia (Italy). The distribution of *O. campylocercoides* at the Milos vent site 1 underlines not only its eurythermal nature, but also its euryhalinity with an occurrence at salinities up to 45‰ S. This euryoecious nature is also reflected by the widespread occurrence, not restricted to sulfidic areas

or normal oceanic salinity. It was regularly found in the Mediterranean Sea (De Coninck and Schuurmans-Stekhoven, 1933, Gerlach; 1953 a, b, c; Gadea, 1960; De Zio, 1966) in the North Sea (Schulz, 1951; Gerlach, 1952) and the English Channel (Warwick, 1971). Along the coast of the Atlantic it is recorded from the Gulf of Biscay (Delamare Debutteville *et al.*, 1954), the Portuguese coast (Galhano, 1970) and a salt marsh in Georgia, USA (Wieser and Kanwisher, 1961; Teal and Wieser, 1966). It occurs also in brackish water like the Baltic Sea (Gerlach, 1958) and the Black Sea (Filipjev, 1918; Gerlach, 1951) and tolerates even the hypersaline conditions in the Egyptian part of the Red Sea (Gerlach, 1964).

Most of the above reports refer to eulittoral samples, where only single specimens have been found. In the sublittoral (4-11 m water depth), some 30 specimens per sample were reported by Gerlach (1958) in medium sand of Kiel Bight. This somewhat higher abundance is in parallel with our results and may indicate, that *O. campylocercoides* prefers, in fact, shallow, sublittoral sands.

The above literature reports from varying habitats suggest, that the rich occurrence of *O. campylocercoides* off Milos is only indirectly related to hydrogen sulfide and gaseous emissions of hydrothermal vent areas. It is obvious that in the sulfidic regime of the hydrothermal vents, the benthic fauna is threatened by toxic emissions of hydrogen sulfide. Here, anaesthetized or killed animals would be an ample and easy prey for animals filling the ecological niche of a scavenger and omnivorous predator. Being characterized as a predator and omnivore and the whole family Oncholaimidae in general as scavengers (Teal and Wieser, 1966, Jensen, 1987b), *O. campylocercoides* may benefit in the periphery of the seeps for two reasons : it survives the moderate geothermal sulfide concentrations of Paleohori Bay better than other meiofauna species suggesting particular structural and/or physiological adaptations. It could take advantage of the rich food supply under low competitive stress provided in the hydrothermally-affected milieu.

A comparable ecological situation with irregular fatal events through development of sulfide is characteristic also for the occurrence of the closely related oncholaimid nematode *Pontonema vulgare.* Lorenzen *et al.* (1987) reported mass aggregations of this scavenger in sheltered bights of the Baltic Sea after the sediments turned sulfidic in periods of stagnant water, when most benthic animals were killed by low oxygen and sulfidic conditions. The ecological scenarios outlined above for *O. campylocercoides* and *P. vulgare* suggest that these species can be termed thiobiotic (Powell, 1989). Giere (1992) grouped species of this type of thiobiosis as sulfidophilic, oxitolerant.
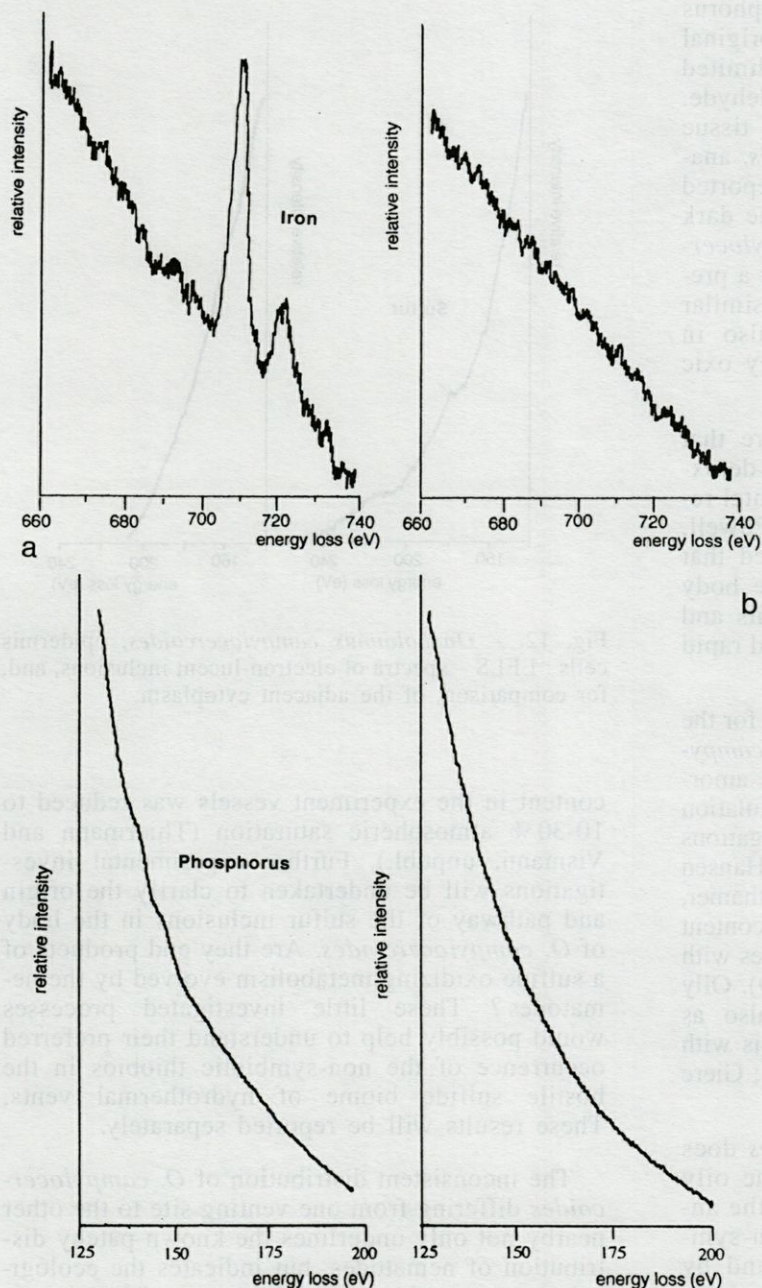
a

b

Fig. 11a, b. – *Oncholaimus campylocer-coides,* intestinal cells : EELS – spectra of granules and, for comparison, of the adjacent cytoplasm.

They utilize the sulfide niche, but not the sulfide proper.

An adaptation of *O. campylocercoides* to the impact of sulfidic conditions may be ultrastructurally indicated by the intracellular inclusions in those tissues most directly exposed to the ambient hydrogen sulfide (intestinal and epidermal cells). Unusual inclusions of various nature occur frequently in various invertebrates from sulfidic habitats (e.g. Jennings and Colam, 1970; Deutsch, 1978; Nuβ, 1984; Nuβ and Trimkowski, 1984; Giere *et al.*, 1988b). Particularly conspicuous and in their fine structure similar to those found in the present study are the dark, often multilayered intracellular globules in the intestinal cells of ne-

matode species typical for sulfidic muds (i.e. *Sabatieria wieseri, Terschellingia longicaudata, Sphaerolaimus papillatus, Siphonolaimus ewensis, Pontonema vulgare*). However, their significance is ambiguous and their adaptive value for a thiobiotic life rather disputed (see below). Element analyses of the globular content proved the presence of a multitude of elements such as Si, P, S, K, Ca and Fe (Nicholas *et al.*, 1987).

Particularly the globules found in the gut cells of *O. campylocercoides* resemble those in the related *Pontonema vulgare* (Oncholaimidae) (Jennings and Colam, 1970). The fact that in our analyses of globules in sections from formalinfixed *O. campylocercoides* sulfur was not detected

(iron, carbon, nitrogen, oxygen and phosphorus were recorded), does not preclude its original deposition in the worms considering the limited stability of sulfur compounds in formaldehyde. The crystalline inclusions in the muscular tissue of the freshwater nematode *Tobrilus gracilis,* analysed by Nuβ and Trimkowski (1984) and reported to contain sulfur, do not much resemble the dark globules found in the gut cells of *O. campylocercoides.* Moreover, their indicative value for a preference of sulfidic layers is arguable since similar crystalline structures have been found also in specimens of *Tobrilus gracilis* from clearly oxic horizons (Schmidt, unpublished).

The frequent statements in the literature that these globular structures serve as a sulfide detoxification mechanism need careful experimental reassessment in the light of recent studies (Powell, 1989; Somero *et al.*, 1989) who underlined that without an effective export mechanism the body would soon be flooded by globular deposits and then could not counteract the continuous and rapid penetration of H$_2$S.

The electron-lucent vesicles, characteristic for the epidermal cells of the white specimens of *O. campylocercoides,* contain sulfur, apparently in an amorphous, probably oily to viscous form. Accumulation of elemental sulfur in the form of oily aggregations is known from many sulfur bacteria (e.g. Hansen and van Gemerden, 1972; Oltman and Stouthamer, 1975; Pfennig, 1975). Their oily to viscous content has been found to consist of linear polysulfanes with their long –S-S– chains (Steudel, 1985, 1989). Oily sulfur compounds have been suggested also as deposits of sulfur bacteria living in symbiosis with animals (e.g. Cavanaugh, 1985; Vetter, 1985; Giere and Langheld, 1987; Fisher, 1990).

On the other hand, *O. campylocercoides* does not contain symbiotic bacteria. Hence, the oily sulfur compounds are to be derived from the animal's metabolism. Elemental sulfur in non-symbiotic thiobiotic meiofauna was also found by Powell *et al.* (1980) in thiobiotic turbellarians. They considered this as a widespread (detoxifying) mechanism in lower invertebrates living under sulfidic conditions. It might have parallels to the situation in sulfidic sea water, where the common spontaneous auto-oxidation of sulfide results in the formation of elemental sulfur (Almgren and Hagström, 1974). Related to thiobiotic animals this would allow the presence of elemental sulfur without necessarily a linkage to sulfur-oxidizing bacterial symbionts (Cavanaugh, 1985).

First experiments with *O. campylocercoides* support the above hypothesis : incubation in sulfidic conditions (100 μM H$_2$S concentration) resulted in formation of oily sulfur droplets in the epidermis of the worms as soon as the oxygen
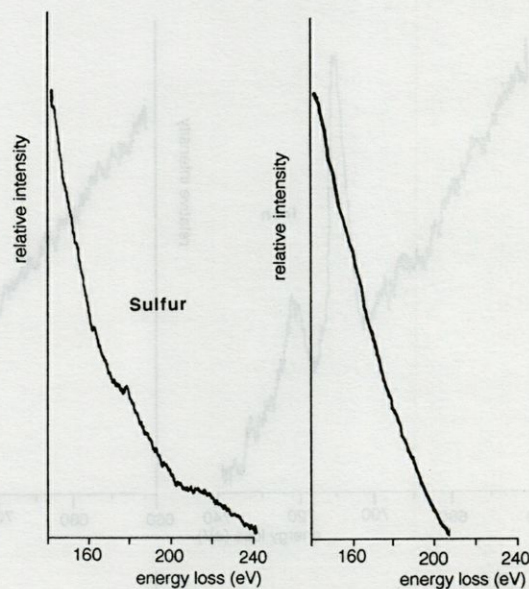

Fig. 12. – *Oncholaimus campylocercoides,* epidermis cells : EELS – spectra of electron-lucent inclusions, and, for comparison, of the adjacent cytoplasm.

content in the experiment vessels was reduced to 10-30 % atmospheric saturation (Thiermann and Vismann, unpubl.). Further experimental investigations will be undertaken to clarify the origin and pathway of the sulfur inclusions in the body of *O. campylocercoides.* Are they end products of a sulfide oxidizing metabolism evolved by the nematodes ? These little investigated processes would possibly help to understand their preferred occurrence of the non-symbiotic thiobios in the hostile sulfide biome of hydrothermal vents. These results will be reported separately.

The inconsistent distribution of *O. campylocercoides* differing from one venting site to the other nearby not only underlines the known patchy distribution of nematodes, but indicates the ecological heterogeneity even between singular gas outlets within one seep field. Further small-scale chemical and ecological studies are required to understand more thoroughly the complicated and fluctuating gradient pattern of abiotic and biotic factors in these shallow water hot vents.

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