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IN SEDIMENTS OF COASTAL UPWELLING**

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Nitrate-storing sulfur bacteria in sediments of coastal upwelling.

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NITRATE-STORING SULFUR BACTERIA IN SEDIMENTS
OF COASTAL UPWELLING

DISSERTATION

zur

Erlangung des Grades eines
Doktors der Naturwissenschaften

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Heide Schulz

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Nitrate-storing sulfur bacteria in sediments of coastal upwelling

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1. Abstract

In three eutrophic coastal environments, the Chilean coast, the Namibian coast, and the Danish Limfjorden the abundance of nitrate-storing sulfur bacteria was investigated. During a 14 months period the marine *Thioploca* community off the Bay of Concepción (Chile) was studied with respect to changes in the population induced by seasonal variations in the upwelling intensity. The major results of this study were:

- In summer 1996, oxygen concentrations in the bottom water were near zero and the biomass was highest near the coast ($\leq 160 \text{ g m}^{-2}$ wet weight without sheaths).
- During winter, biomass declined at all stations due to higher oxygen concentrations under reduced upwelling intensity, but some filaments remained in deeper parts of the sediment.
- The depth distribution of thioplocas changed strongly with seasonal variations, but the community structure, e.g. species distribution, diameter of sheaths and number of trichomes per sheath, remained unchanged. These parameters were different at each station.
- In the Bay of Concepción, *Thioploca* spp. were found occasionally, but reached high biomass during summer.
- An undescribed morphological form of thioplocas with very short cells was discovered which is according to 16S rDNA sequences a close relative of the normal thioplocas but more diverse in its morphology and sequence.
- The short-cell morphotype of *Thioploca* occurs with highest frequency in near-shore sulfide-rich sediments, preferably at 5 - 10 cm depth in the sediment. Especially during growth phase these filaments populate deep parts of the sediment ($\leq 26 \text{ cm}$).

A survey for filamentous sulfur bacteria along the coast of Namibia resulted in the discovery of an unknown giant sulfur bacterium which was named *Thiomargarita namibiensis*. The characteristics of these bacteria are:

- *Thiomargarita* occurs as single round cells held together in a string by a common slime sheath. The cells are not motile.

- Most of the cells are 100 - 300 μm in diameter but frequently single cells with diameters of $\leq 750 \mu\text{m}$ occur. Thus, *Thiomargarita* is the largest known prokaryote.
- *Thiomargarita namibiensis* has internal sulfur globules and accumulates nitrate in a central vacuole in $\leq 800 \text{ mM}$ concentration.
- According to 16S r DNA sequences it is closely related to the large nitrate-storing *Thioploca* and *Beggiatoa* species.
- The population of *Thiomargarita* was found in very fluid sediments off Walvis Bay in biomass of $\leq 47 \text{ g m}^{-2}$.
- *Thiomargarita* cells are not sensitive to high oxygen or sulfide concentrations which is their special adaptation to survive under the changing conditions in the fluid diatom ooze off Walvis Bay.

In the Danish Limfjorden *Beggiatoa* filaments with a diameter of 12 μm contain internal nitrate accumulated to $\leq 240 \text{ mM}$ and occurred with highest frequency below the sediment surface where no free oxygen was available. Thus, the ability of using nitrate as electron acceptor is not restricted to the large sulfur bacteria abundant in upwelling areas.

In conclusion it was found that nitrate-storing sulfur bacteria are more abundant than previously assumed. Within this group of bacteria there were undescribed, morphologically distinct types that passed the attention in earlier studies.

2. Preface and Acknowledgment

The idea to carry out the following study arose in March 1994 on the last day of the "*Thioploca*-cruise" of the Max Planck Institute for marine Microbiology (Bremen). In many respect, this study is based on knowledge about thioplocas that originated in the combined information obtained by many different researchers that participated in this cruise in 1994.

The backbone of this study is an annual observation on the *Thioploca* community off the Bay of Concepción, Chile. From December 1995 to February 1997 four different stations were sampled during 14 cruises onboard the RV Kay Kay which belongs to the University of Concepción. The processing of the samples was done in the laboratories of the marine field station of the University of Concepción in Dichato. This part of the study which led to the two manuscripts "Population study of the filamentous sulfur bacteria *Thioploca* spp. off the Bay of Concepción, Chile" and "Two Morphotypes of marine *Thioploca* spp. observed off the coast of Chile" could only be realized with the enormous logistical help of Prof. Dr. Victor Ariel Gallardo. The cruises and the lab work were done in close collaboration with Bettina Strotmann who concurrently performed geochemical studies on the same stations. Both V. A. Gallardo and B. Strotmann are therefore co-authors on both manuscripts that resulted from this study. For the description of the new morphotype of *Thioploca*, a comparison of 16S rDNA sequences was important. For this samples of thioplocas were prepared which were processed for obtaining a sequence by Dr. Thorsten Brinkhoff. The phylogenetic tree was established by Dr. Ramón Rosselló-Mora. Therefore, both are co-authors on the second manuscript: "Two Morphotypes of marine *Thioploca* spp. observed off the coast of Chile". During twelve months of the stay in Chile the author was financed by the DAAD (German academic Exchange service). All other expenses were covered by the Max Planck Society.

In January and February 1997 Prof. Dr. Bo Barker Jørgensen, Prof. Dr. J. Gijs Kuenen, Dr. Lars Peter Nielsen, Sandra Otte, Thomas Kjær and Jakob Zopfi visited the marine field station in Dichato. Sandra Otte and Gijs Kuenen performed incubation experiments on *Thioploca* filaments that resulted in the

manuscript: "Ecophysiological studies on partially purified mixed cultures of *Thioploca* species" written by Sandra Otte. The authors contribution to this study was to give advises on how to treat *Thioploca* filaments and studies on the survival rates of filaments with different media.

In April 1997, the author participated in a cruise along the Namibian coast onboard the Russian RV Petr Kottsov. The results of this study are presented in the manuscript: "Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments". Dr. Timothy G. Ferdelman made it possible for to participate in this cruise by shipping equipment to Namibia. He was cruise leader during most of the cruise and helped obtain sediment samples. Dr. Thorsten Brinkhoff obtained the 16S rDNA sequences from the newly discovered *Thiomargarita* which Dr. Andreas Teske included into a phylogenetic tree. Mariona Hernández Mariné made transmission electron micrographs of *Thiomargarita*. For these contributions all of them are co-authors of the resulting manuscript.

In November 1997 the author took part in a study on *Beggiatoa* in the Danish Limfjorden together with Bettina Strotmann, Thomas Kjær, Dr. Lars Peter Nielsen and Prof. Dr. Bo Barker Jørgensen. The results of this project are described in the chapter "Nitrate storage by marine *Beggiatoa* spp. in Limfjorden, Denmark" and will ultimately be published by Bettina Strotmann. The authors contribution to this work was the description of the *Beggiatoa* population and measurements of internal nitrate concentrations.

Prof. Dr. Bo Barker Jørgensen is the last author on each manuscript included in this study, partly because he encouraged, supported and financed the projects. Furthermore, he invested much effort, time and patience in correcting and improving each manuscript.

I am very grateful for all the support, guidance and trust in my work that I received from my supervisor Bo B. Jørgensen. I would also like to thank Prof. Dr. W. Arntz for his efforts in evaluating this Ph.D. thesis, as well as the members of the committee: Prof. Dr. H. Willems, PD Dr. A. Mackensen, Dr. B. Donner and J. Klump.

Apart from those colleagues that contributed directly to my work and are co-authors on the manuscripts, there are many other friends and colleagues in Bremen and Concepción who helped me. Not all of them can be named, but I would like to express special thanks to Andrea Friedrich, Vanessa Madrid, Sabine Nienstedt, Michael Schrödl, Christoph Suppes, Bettina Strotmann, Paula Urrutia, Anyola Vega and Wiebke Ziebis for their help and friendship.

3. Current state of knowledge

3.1 The group of colorless sulfur bacteria

The sulfur-oxidizing bacteria are referred to as colorless sulfur bacteria to distinguish them from the purple sulfur bacteria which accumulate internal sulfur globules during anoxygenic photosynthesis. The colorless sulfur bacteria form two principle groups: one group oxidizes reduced sulfur species such as sulfide or thiosulfate without accumulating internal sulfur globules. The second group comprises all colorless bacteria that accumulate elemental sulfur in the presence of sulfide and gain energy by the oxidation of reduced sulfur species (see Table 3.1). These bacteria are generally rather large and characterized by a specific morphology, thus they are often referred to as "morphologically conspicuous sulfur bacteria". Although many of the genera in this group were described in the early days of microbiology because they were easy to describe, few strains have as yet been obtained in pure culture. Nevertheless, they are frequently observed in high numbers in marine and fresh water environments where sulfide and oxygen are present, and their significance for the sulfur cycle is widely recognized (KUENEN, 1989; LA RIVIÈRE and SCHMIDT, 1992). A third group of sulfur-oxidizing bacteria, that is normally not included in the group of colorless sulfur bacteria, is represented by the endosymbiotic sulfur oxidizing bacteria first discovered in *Riftia pachyptila* and other invertebrates living at deep sea hydrothermal vents (CAVANAUGH, 1981; FELBECK 1981).

The group of "morphologically conspicuous sulfur bacteria" harbors both filamentous and unicellular types (Fig. 3.1). The four filamentous genera form the family of *Beggiatoaceae*, named after the genus *Beggiatoa* (TREVISAN, 1842), which was the first genus to be described and is one of the most abundant. *Beggiatoa* filaments consist of rows of cells (trichomes) with single cells separated from one another by their cytoplasmic membrane and a peptidoglycan layer. *Thioploca* (LAUTERBORN, 1907) filaments resemble *Beggiatoa*, the main difference is that they usually do not occur as single trichomes, but as bundles within a common sheath. Both *Beggiatoa* and *Thioploca* species show a gliding movement accompanied by rotation.

"*Thiospirillopsis*" (UPHOF, 1927) looks like a *Beggiatoa* filament but is coiled in a spiral form, giving the impression of a corkscrew motion when gliding. *Beggiatoa* strains occasionally show the same morphology under certain culture conditions. The validity of the genus "*Thiospirillopsis*" is therefore uncertain. In contrast to these three genera, the filaments of *Thiothrix* (WINOGRADSKY, 1888) are attached at one end to a solid surface by a hold-fast structure. At the free end of the filament, single motile cells or homogonia of several cells can be released. Each filament of *Thiothrix* is surrounded by a slime sheath, and sometimes rosettes are formed. In the group of *Beggiatoaceae*, only *Beggiatoa* and *Thiothrix* strains have been isolated in pure culture. With the exception of marine *Beggiatoa*s, all of these strains grow chemolithoheterotrophically, gaining at least most of their energy via the oxidation of organic compounds.

Table 3.1 Genera of bacteria that oxidize reduced sulfur compounds and are generally known as the "colorless sulfur bacteria" (adapted from KUENEN, 1989).

with sulfur inclusion		without sulfur inclusion	
unicellular	filamentous	mesophil	thermophil
<i>Thiovulum</i>	<i>Beggiatoa</i>	<i>Thiobacillus</i>	<i>Sulfolobus</i>
<i>Thiospira</i>	<i>Thioploca</i>	<i>Thiomicrospira</i>	<i>Acidianus</i>
<i>Macromonas</i>	<i>Thiothrix</i>	<i>Thiodendron</i>	<i>Thermothrix</i>
<i>Achromatium</i>	" <i>Thiospirillopsis</i> "	<i>Thiospaera</i>	
<i>Thiobacterium</i>			
" <i>Bilophococcus</i> "			

The round to egg-shaped cells of *Thiovulum* (HINZE, 1913) are motile by means of peritrichous flagella. Cells are 5 - 25 µm in diameter. Their cytoplasm is generally concentrated at one end of the cell, with the rest of the remaining space occupied by a large vacuole (LA RIVIÈRE and KUENEN, 1989). They are often observed as a white veil on top of sulfidic marine sediments within the oxygen / sulfide interface (JØRGENSEN and REVSBECH, 1983). Although *Thiovulum* cells have not been isolated in pure culture, enrichment culture studies gave strong evidence for a lithoautotrophic metabolism based

on the oxidation of sulfide with oxygen and CO₂-fixation (WIRSEN and JANNASCH, 1978). Within a veil, the cells may induce advective transport of oxygen-rich water towards the sediment surface by a collective spinning movement, thereby increasing the oxygen availability (FENCHEL and GLUD, 1998). In decaying veils, "swarming cells" are frequently observed, which are slightly smaller than the usual *Thiovulum* cells and extremely fast swimming (WIRSEN and JANNASCH, 1978; GARCIA-PICHEL, 1989).

The genus *Thiospira* (WISLOUCH, 1914) is characterized by spirillum-formed, motile cells with polar flagella and sulfur inclusions. The cells of the type strain *T. winogradskyi* are maximally 50 µm long and 2.5 µm wide. *Thiospira* spp. are found in sulfurous marine and freshwater environments. The validity of the genus is uncertain, because some organotrophic strains of the genus *Spirillum* also accumulate sulfur in the presence of sulfide (LA RIVIÈRE and KUENEN, 1989). *Macromonas* cells (UTERMÖHL and KOPPE, 1924) are cylindrical to bean-shaped and filled with calcite and sulfur inclusions. The cells can be up to 40 µm long and are motile by a polar flagellum. All strains obtained in pure culture are organotrophs (LA RIVIÈRE et al., 1989). Like *Macromonas*, *Achromatium* cells (SCHEWIAKOFF, 1893) from freshwater habitats have many calcite inclusion and sulfur globules, but the form of the cells is more spherical to oval shaped, and they can be much larger in diameter (≥80 µm). On solid surfaces, a slow gliding type of movement is reported without apparent means of locomotion (LA RIEVIÈRE and SCHMIDT, 1992). No *Achromatium* strain could be isolated into pure culture, but ecophysiological studies indicate an enhanced oxidation of sulfide to sulfate when *Achromatium* cells are present (GRAY et al., 1997).

The genus *Thiobacterium* (LA RIVIÈRE and KUENEN, 1989) consists of a single species, *T. bovista*, that has not been successfully grown in pure or enrichment culture. The non-motile rod-shaped cells of up to 9 µm length have sulfur inclusions and are embedded in a gelatinous mass. These colonies tend to have a spherical form when free floating and a dendroid form when attached to a solid surface. *Thiobacterium* colonies have been found in fresh and brackish water, where sulfide is present and in thermal springs of up to 45 °C. Also represented by a single species is the genus "*Bilophococcus*" (MOENCH, 1988). "*B. magnetotacticus*" is the only known sulfur-accumulating bacterium which is magnetotactic. The coccoid cells of 1.4 -1.8 µm are motile

by means of two adjacent tufts of flagella. Near the flagella there are numerous magnetosomes, and each cell contains 1 - 3 sulfur globules. "*B. magnetotacticus*" was isolated magnetically from reconstructed waste water aeration basin environments. The abundance of the organism in fresh water or marine environments is unknown.

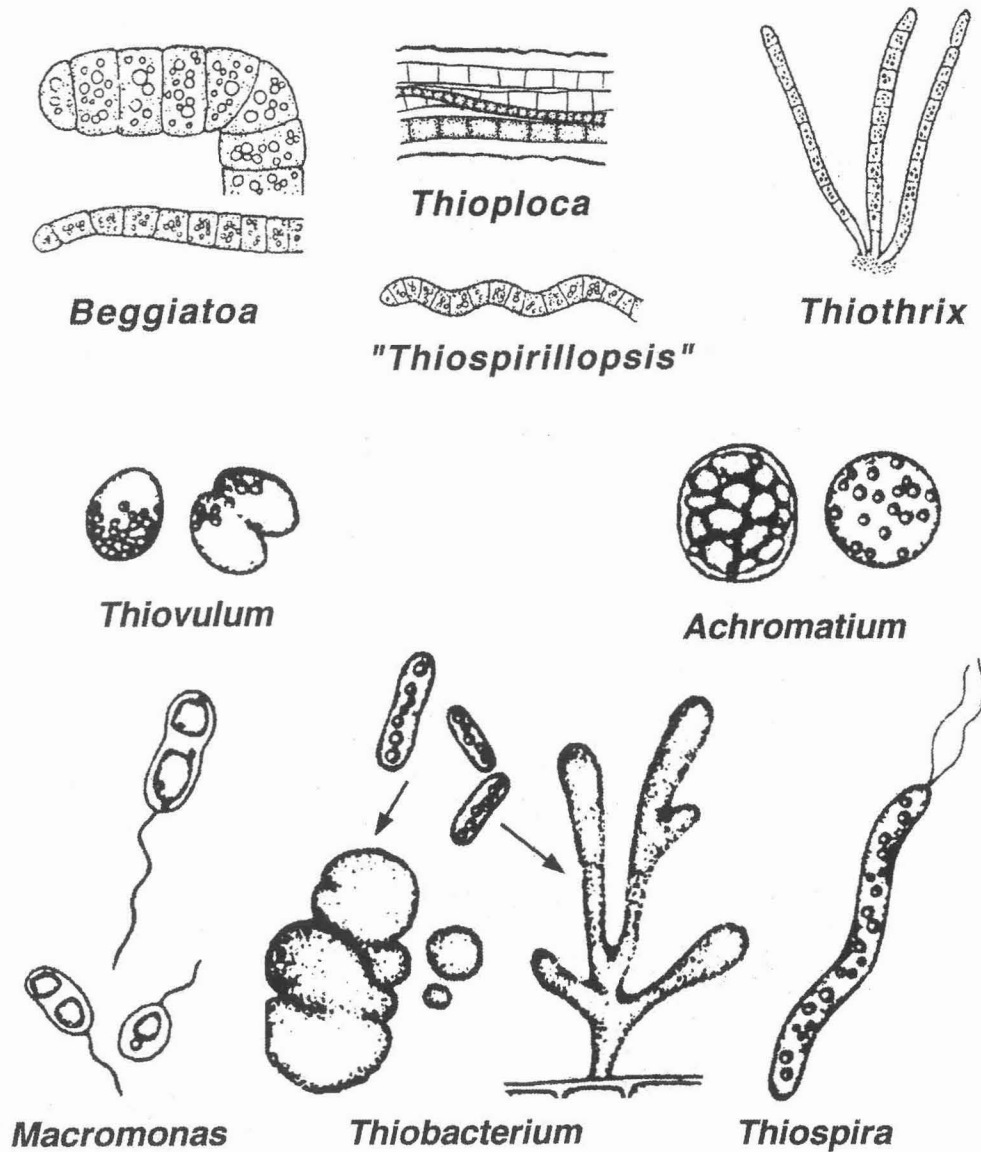


Fig. 3.1 Genera of bacteria that belong to the group of "morphologically conspicuous sulfur bacteria" (adapted from KUENEN, 1989 and SCHLEGEL, 1992).

The phylogenetic affiliation of "morphologically conspicuous sulfur bacteria", as determined by 16S rDNA sequences, is still incomplete because of the general lack of pure cultures in this group. However, some complete and incomplete sequences have been obtained for members of the genera *Beggiatoa*, *Thioploca*, *Thiothrix*, *Achromatium*, and *Thiovulum*. With the exception of *Thiovulum majus*, which is a member of the epsilon group of proteobacteria (LANE et al., 1992), all other genera belong to the gamma group of proteobacteria (TESKE et al., 1995; HEAD et al., 1996), which also harbors many sequences of endosymbiotic sulfur oxidizers and some strains of colorless sulfur bacteria without sulfur globules e.g. *Thiobacillus* and *Thiomicrospira* species. The sequences of *Beggiatoa* and *Thioploca* spp. together form a monophyletic branch in this cluster, although within these group the large, vacuolated *Beggiatoa* and *Thioploca* spp. seem to be closer related to each other than to the other species of both genera (AHMAD et al., 1999; JØRGENSEN et al., submitted). Both *Thiothrix* spp., which used to be included in the family of *Beggiatoaceae*, and *Achromatium oxaliferum* are more remotely affiliated to *Beggiatoa* and *Thioploca* species.

3.2 *Beggiatoa*

In the group of "morphologically conspicuous sulfur bacteria" the genus *Beggiatoa* was the first to be described (1804, as "*Oscillatoria alba*") and is today the most frequently investigated and well-known genus of sulfur-storing bacteria. Early observations of WINOGRADSKY on the appearance and disappearance of sulfur globules in *Beggiatoa* filaments led him to the conclusion that *Beggiatoa* spp. gain energy by oxidizing sulfide (1887). These studies are seen today as a major watershed in microbiology: for the first time, the concept of bacteria living on the oxidation of reduced inorganic compounds (lithotrophy) was introduced (ZAVARZIN, 1989). Ironically, the studies of *Beggiatoa* filaments led to a whole new emphasis in microbiology and subsequently many lithotrophic strains could be isolated with the exception of *Beggiatoa*.

For almost 100 years a basic discrepancy persisted: many field studies of *Beggiatoa* spp. suggested a lithotrophic physiology, but only organotrophic strains could be isolated into pure culture. Native *Beggiatoa* spp. were shown to form distinct mats of ~0.5 mm thickness exactly in the oxygen / sulfide

interface (JØRGENSEN and REVSBECH, 1983), and many details of their phobic reaction toward oxygen and light could be demonstrated (MØLLER et al., 1985; NELSON and CASTENHOLZ, 1982). In 1983, NELSON isolated a chemoautotrophic marine *Beggiatoa* strain using a gradient culture system (NELSON and JANNASCH, 1983) which finally ended the discussion of a principal possibility for beggiatoas to gain energy from the oxidation of sulfide. Several marine *Beggiatoa* isolates could be grown chemolithoautotrophically in gradient cultures, whereas all fresh water isolates were chemoorgano-heterotrophs (NELSON, 1992).

The discovery of dense populations of very large *Beggiatoa* filaments at a hydrothermal deep-sea vent site in Guaymas Basin (Gulf of California) led to a new interest in the genus *Beggiatoa* (JANNASCH et al., 1989). These beggiatoas form layers up to 3 cm thick on top of the sediments and up to 30 cm thick between vestimentiferan tube worms. The mats consist almost exclusively of *Beggiatoa* filaments of three size classes, with the largest filaments being 116-122 μm in diameter. Judging from their enzyme activities, the giant beggiatoas from this hot vent have a potential for lithoautotrophic growth (NELSON et al., 1989).

3.3 *Thioploca*

The genus *Thioploca* was established by LAUTERBORN in 1907 for a group of filamentous bacteria resembling beggiatoas that he discovered in Lake Constance. The main differences noted between *Beggiatoa* spp. and *Thioploca* was that *Thioploca* filaments occur as bundles in a common sheath and are often tapered at the ends. These two criteria remain the major distinguishing features of the two genera. The filaments of the type species *T. schmidlei* have diameters of 5 - 9 μm . LAUTERBORN found that in Lake Constance, thioplocas occurred in greater water depths than beggiatoas. He also observed that thioplocas protruded deep into sediments which did not smell of sulfide, whereas beggiatoas were found at the surface of sediments smelling strongly of sulfide. Since this first description, fresh water *Thioploca* spp. have been found in various lakes and springs in Germany, Russia, North America and Japan (KOLKWITZ, 1912; WISLOUCH, 1912; KOPPE, 1924; KOLKWITZ, 1955; MAIER and MURRAY 1965; MAIER AND PREISSNER, 1979; NAMSARAEV et al., 1994; NISHINO et al., 1998).

In 1965, MAIER described a population of *Thioploca ingrlica* (2 - 4.5 μm diameter) from Lake Erie that he compared with respect to ultra structure to a fresh water *Beggiatoa* species. Apart from many similarities, he found that *T. ingrlica* contained an additional wall component, and the cytoplasm of *Beggiatoa* sp. was richer in ribosomes but less dense than that of *T. ingrlica* (MAIER and MURRAY, 1965). This led him to conclude that in spite of their resemblance, the two genera were distinct. *T. ingrlica* was maintained in enrichment cultures in original sediment for several years and was recognized as a species (MAIER, 1984) described first by WISLOUCH (1912).

The first marine population of *Thioploca* spp. was found in the upwelling area off the Chilean coast (GALLARDO, 1977) and subsequently in Peruvian coastal sediments (ROSENBERG et al., 1983). The marine *Thioploca* communities are often high in biomass (up to 106 g wet weight per 0.1m² including sheath material), exceeding even the wet weight of benthic animals (GALLARDO, 1977). Two species of marine *Thioploca* have been described, *T. araucae* with filament diameters of 30 - 43 μm , and *T. chileae* with diameters of 12 - 20 μm (MAIER and GALLARDO, 1984). Apart from being larger and occurring at higher densities, the marine species differ from the fresh water thioplocas in ultra structure (MAIER et al., 1990). The cytoplasm is restricted to a thin outer layer of 1-2 μm , whereas the inner part of the cells is filled up by a large central vacuole that appears empty. This particular feature also occurs in the large marine *Beggiatoa* spp. from hydrothermal vents (NELSON et al., 1989).

3.4 Nitrate storing sulfur bacteria

Under natural conditions *Beggiatoa* spp. living in microoxic environments within the oxygen / sulfide interface should also be able to survive transient anaerobic conditions in these changing environments. Several electron acceptors have been proposed and tested as an alternative to oxygen for beggiatoas. NELSON and CASTENHOLZ (1981) showed that a chemorganotrophic *Beggiatoa* strain could reduce sulfur to sulfide under anaerobic conditions in the presence of acetate to support growth for at least five days. For *Beggiatoa alba* (VARGAS and STROHL, 1985) and several other marine and fresh water strains of *Beggiatoa* (NELSON et al., 1982), nitrate uptake could be

demonstrated, but these strains used nitrate only as nitrogen source and not as electron acceptor. SWEERTS et al. (1990) showed steep nitrate gradients into a mat of native fresh water *Beggiatoa* filaments that was placed on top of an agar. On the base of a 48 hour incubation with ^{15}N -labeled nitrate, they concluded that the *Beggiatoa* filaments were denitrifiers, reducing nitrate to nitrogen, in addition to using oxygen which was also consumed by the filaments. This study was later criticized because of the relatively long incubation time which could have allowed denitrifying contaminants to grow (MCHATTON et al., 1996).

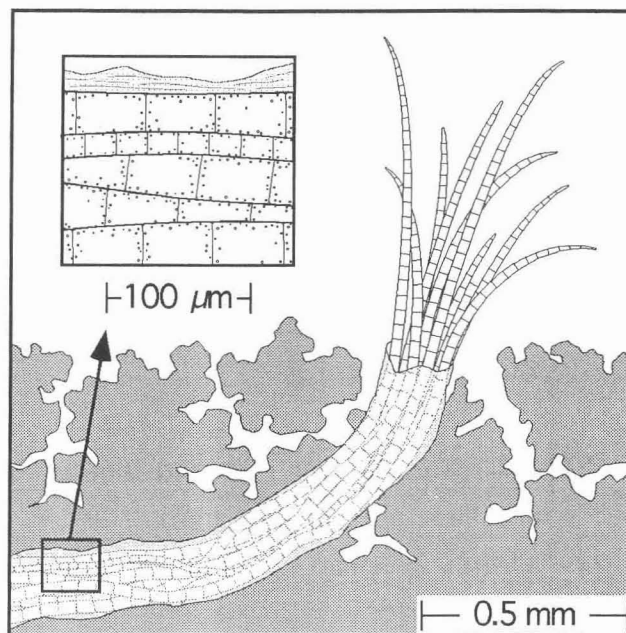


Fig. 3.2 Schematic picture of a sheath of marine thioplocas at the sediment surface with trichomes sticking up into the water. The insert shows the light-microscopic appearance.

During an expedition off the Chilean coast near Concepción in March 1994, NIELSEN measured internal nitrate concentrations of up to 500 mM in marine *Thioploca* filaments, a $\leq 20,000$ -fold concentration increase over ambient seawater (FOSSING et al., 1995). FOSSING et al. (1995) concluded that the purpose of the large, central vacuole of the marine thioplocas is not only to counteract a potential diffusion limitation (LARKIN and HENK, 1989), but also to serve as "anaerobic lungs" enabling the marine thioplocas to store their

electron acceptor in high concentrations (JØRGENSEN and GALLARDO, in press).

HUETTEL et al. (1996) investigated the chemotactic behavior of native *Thioploca* filaments in original sediment cores kept in a recirculating flume. Under anoxic conditions with addition of nitrate (25 μm), the terminal ends of the *Thioploca* filaments ascended from their sheaths and stretched up to 30 mm into the overlying seawater (Fig. 3.2), increasing the total nitrate uptake of the sediment. Oxygen concentrations of $\geq 15 \mu\text{m}$ caused the filaments to retreat to the sediment. Anoxic seawater without nitrate did not provoke an extension of filaments into the overlying water, whereas after 3 days of nitrate starvation, filaments extended from their sheaths when nitrate was added even with oxygen present at 160 μm concentration. Positive chemotaxis towards nitrate overruled the negative chemotaxis toward oxygen, strongly supporting the suggestion, that nitrate is the principal electron acceptor of the marine thioplocas. The chemotactic response of *Thioploca* filaments towards sulfide was found to be dependent on concentration. Below 150 μm , it caused an extension of filaments, whereas sulfide added at $\geq 150 \mu\text{m}$ concentration to the water led to a retreat of filaments.

Due to their ability to store nitrate and elemental sulfur and to positive chemotaxis for both nitrate and sulfide, marine thioplocas are not dependent on co-occurrence of their electron acceptor and donor. In March 1994, nitrate was only present in the bottom water and the few upper mm of the sediment, whereas sulfide was mostly $< 2\mu\text{M}$ in the upper 20 cm of the sediment inhabited by thioplocas (THAMDRUP and CANFIELD, 1996). An investigation of the community structure revealed that the orientation of *Thioploca* sheaths in the sediment was mainly vertical (Fig. 3.3), thus enabling the filaments to shuttle between sediment surface and deeper parts of the sediment in a directed manner (SCHULZ et al., 1996).

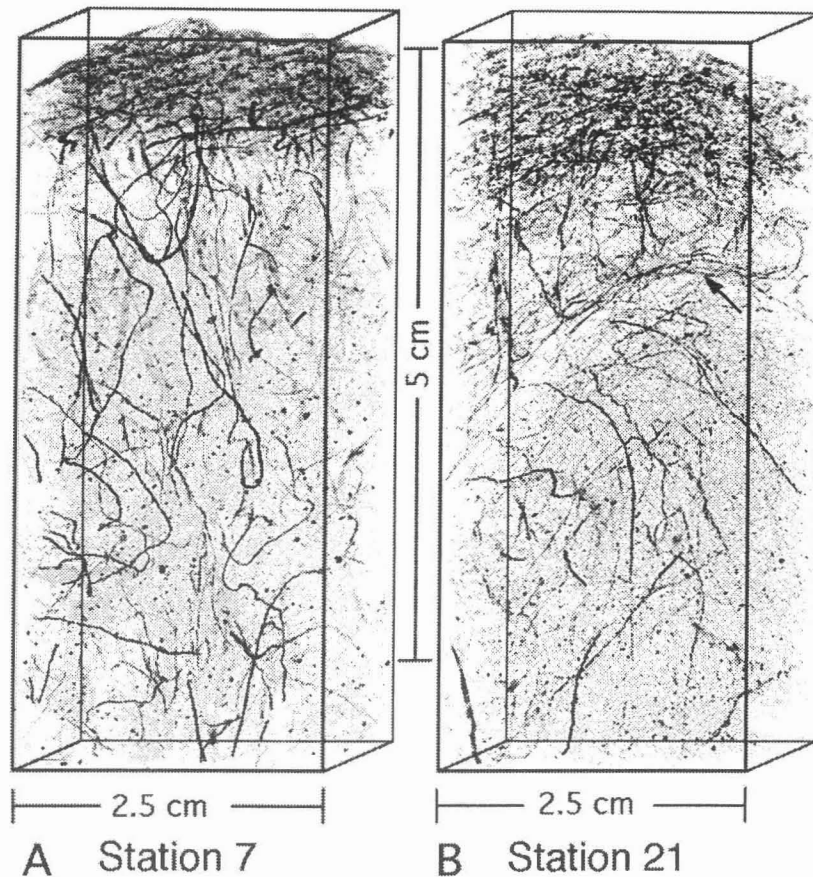


Fig. 3.3 Three-dimensional reconstruction of *Thioploca* sheaths (black) in a block of sediment (5 cm × 2.5 cm × 1 cm) from Station 7 (left panel), near the Bay of Concepción, and Station 21 (right panel), on the shelf. At the sediment surface there was a layer of most horizontally oriented sheaths. Underneath this the sheaths were oriented more or less vertically. The arrow in the right panel points to a second layer of more horizontally oriented filaments beneath the horizontal surface layer. The lower ends of the sheaths frequently bend back up towards the surface. At Station 7 there was a mat of *Beggiatoa* filaments on the sediment surface.

Parallel to the finding of nitrate storage by marine thioplocas, the large *Beggiatoa* spp. living at hydrothermal deep sea vents were also shown to store nitrate at 130 - 160 mM concentration (McHatton et al., 1996). Thus, the possession of a large central vacuole is consistent with accumulation of nitrate. Similar to thioplocas, hot vent beggiatoas occur in an oxygen-poor

environment, but the sulfide concentrations at the vents are higher than in Chilean sediments and the filaments do not protrude into the sediment or stretch into the overlying water. Possibly, they receive nitrate by small-scale hydrothermal fluid circulation suggested by GUNDERSEN et al. (1992).

The "morphologically conspicuous sulfur bacteria" are generally difficult to isolate in pure culture which is the base of classical microbiological studies. Nevertheless, these bacteria are abundant in many coastal sediments, where they can play an important role for the oxidation of sulfide. The recent finding of the use of nitrate as electron acceptor by large filamentous sulfur bacteria emphasizes the environmental importance of these bacteria, especially in areas where they occur in high density.

4. Aim of this study

The aim of this study was a quantitative analysis of the population dynamics and species distribution of nitrate-storing sulfur bacteria. These organisms couple the sedimentary sulfur and nitrogen cycle in a previously unknown manner. Thus, their distribution and biomass are a measure of the significance of their metabolism in coastal marine environments. Three regions, the Chilean coast, the Namibian coast, and the Danish Limfjorden were investigated with respect to abundance and morphology of these bacteria.

The principal focus of the study was on the *Thioploca* community off the Chilean coast, because this population of filamentous sulfur bacteria is extremely dense, occurs in a large area, and therefore is of great ecological importance. During a 14 months study, the population was analyzed and quantitative data on biomass and distribution of thioplocas were obtained in relation to bottom water oxygen and nitrate concentrations. The study was repeated in March 1998 to observe the influence of the "El Niño" phenomenon on the *Thioploca* community.

The hydrographic conditions off Namibia are comparable to the Chilean upwelling area, but no firm reports existed of dense populations of sulfur bacteria in the Benguela upwelling system. During a four week cruise along the coast of Namibia, sediment samples from 100 m water depth were taken and the biomass and depth distribution of nitrate-storing sulfur bacteria were investigated. The third part of the study comprised a more inconspicuous population of moderately sized beggiatoas (5-35 μm) that occur in the Danish Limfjorden. This population of filamentous sulfur bacteria was the only one whose seasonal variations had already been studied (JØRGENSEN, 1977a). In Limfjorden, the maximum density of *Beggiatoa* filaments was often observed in deeper parts of the sediment where no oxygen was available. Thus, this investigation focused on nitrate storage in these beggiatoas.

5. Results and Discussion

5.1 Population study of the filamentous sulfur bacteria *Thioploca* spp. off the Bay of Concepción, Chile

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5.1.1 Abstract

A community of filamentous sulfur bacteria, *Thioploca* spp., living in the Bay of Concepción, Chile and the adjoining shelf area was sampled from January 1996 to February 1997 at 4 -6 weeks intervals to investigate the influence of seasonal variations in upwelling intensity and oxygen concentrations on the population dynamics. The *Thioploca* community was described by its biomass, total number and diameter of sheaths, number of trichomes and species per sheath and abundance and depth distribution of different morphological forms, e.g. trichome diameters and ratios of cell-length to diameter. Bottom water concentrations of oxygen and nitrate were measured. The study was repeated in March 1998 to describe the influence of the "El Niño" Southern Oscillation on the *Thioploca* community. Throughout the summer 1996, oxygen concentrations in the bottom water were near zero, nitrate was 10 - 20 μM and the biomass was high, up to 160 g m^{-2} wet weight without sheaths. During winter, biomass declined due to higher oxygen concentrations under reduced upwelling intensity. The depth distribution of thioplocas changed strongly with seasonal variations, but the community structure, e.g. species distribution, diameter of sheaths and number of trichomes per sheath, remained unchanged. These parameters were different at each station. On the shelf the *Thioploca* community never vanished completely, although during "El Niño" the biomass was very low. In the Bay of Concepción, *Thioploca* spp. were only found occasionally, but reached high biomass during summer. Two populations of filamentous sulfur bacteria were observed in the Bay, filaments with short cells in sheaths, populating the upper 7 cm of the sediment, or filaments without sheaths living at the sediment surface.

5.1.2 Introduction

The filamentous sulfur bacteria of the genus *Thioploca* are abundant in the upwelling areas along the coast of Chile and Peru (GALLARDO, 1977; ROSENBERG, 1983). They are found in shelf sediments within the oxygen minimum zone and reach high biomass of up to 1 kg m^{-2} (including sheaths) (GALLARDO, 1977), being at times the most abundant benthic organism in the sediment. In contrast to the free-living, closely related sulfur bacteria,

Beggiatoa spp, *Thioploca* filaments live as bundles within a common sheath. The mainly vertically oriented sheaths may reach down many cm into the sediment (SCHULZ et al., 1996). Studies on partially purified mixed cultures of *Thioploca* spp. showed that they oxidize sulfide to sulfate while reducing nitrate to ammonia (OTTE et al., manuscript submitted). The bacteria store elemental sulfur as globules in the peripheral cytoplasmic layer and concentrate nitrate in a central vacuole in concentrations of up to 0.5 M (FOSSING et al., 1995). These and other observations suggest that the marine thioplocas commute in their sheaths between the sediment surface, where they take up nitrate from the overlying seawater, and deeper parts of the sediment, where free hydrogen sulfide is available.

Because of the high biomass of *Thioploca* communities, they may play an important role in controlling the biogeochemistry of sediments in the oxygen minimum zone off Chile and Peru (JØRGENSEN and GALLARDO, in press). In the sea floor off Concepción, the thioplocas transport large amounts of nitrate into the sediment, thereby increasing the total nitrate pool of the sediment up to 100-fold (THAMDRUP and CANFIELD, 1996). Different approaches have been used to estimate the proportion of sulfide production which is oxidized by thioplocas. Using the amount of elemental sulfur bound by *Thioploca* spp. and the average sulfate reduction rates, FERDELMAN et al. (1997) concluded, that 17 - 34 % of the sulfide produced in the sediment could be re-oxidized by thioplocas, whereas based on CO₂ uptake rates they calculated that *Thioploca* spp. could only account for approximately 18 % of the sulfide oxidation. Much lower values were suggested by THAMDRUP and CANFIELD (1996) estimating from depth-integrated nitrate consumption rates of bag-incubations, that 2-4 % of the sulfide was oxidized with nitrate, while FOSSING et al. (1995) concluded from areal nitrate uptake rates measured *in situ*, that up to 20 % of the sulfide produced in the sediment was oxidized by nitrate presumably consumed by *Thioploca* filaments. In the absence of oxygen during much of the year, it is not clear how the rest of the sulfide is oxidized.

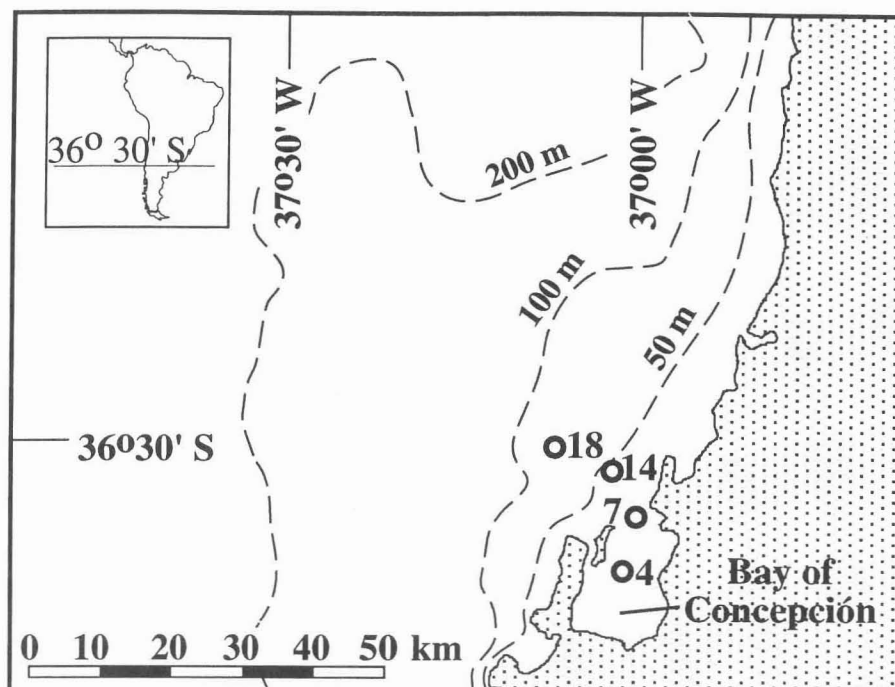


Fig. 5.1 Map of the sampling area (Chile) showing the Bay of Concepción and the adjoining shelf area. The four stations are marked with open circles. The dashed lines (isobaths) indicate water depths.

A general problem in estimating the significance of thioplocas for the sulfur and nitrogen cycling in Chilean and Peruvian sediments is the lack of knowledge concerning the population dynamics of thioplocas. Most earlier investigations on the abundance of *Thioploca* spp. did not provide accurate biomass data, as the principal method used for measuring biomass was sieving the sediment for thioploca-sheaths with 1.0, 0.5 or 0.25 mm sieves and taking the wet weight, dry weight or ash free dry weight without distinguishing between living trichomes and dead sheath material (GALLARDO, 1977; ROSENBERG et al., 1983; GALLARDO, 1985; ZAFRA et al., 1988; GALLARDO et al., 1995). As the sheaths account for ca 90 % of the wet weight (SCHULZ et al., 1996) but have no significance for the metabolic activity, the biomass including sheaths does not reflect the physiological potential of a *Thioploca* population.

For this study, the sediment was sampled with a small Rumohr gravity-corer or a Multi-corer, which preserves the sediment surface, where most of the thioplocas are situated (SCHULZ et al., 1996).. The biomass of *Thioploca* spp. was calculated using the biovolume of living trichomes. From January 1996 to February 1997, four stations were sampled at 4 - 6 weeks intervals. Bottom water nitrate and oxygen concentrations were measured and the *Thioploca* community was characterized in terms of biomass, total number of sheaths, abundance and depth distribution of different morphological forms, average trichome diameters and ratios of cell-length to diameter, number of sheaths inhabited by different species, diameters of sheaths and number of trichomes per sheath. In March 1998 the sampling program was repeated in order to characterize the *Thioploca* community under "El Niño" conditions. Thioplocas are much larger than normal bacteria and have distinct morphological characteristics. This enabled us to quantify and describe the population and its reaction to environmental changes directly, which is otherwise not possible for non-photosynthetic prokaryotes.

5.1.3 Materials and Methods

Sampling Four stations in the Bay of Concepción and the adjoining shelf area (Fig. 5.1) were sampled during 12 cruises onboard the Chilean research ship, Kay Kay, of the University of Concepción. Sediment and bottom water samples were obtained from Station 4 (36° 38' 8" S, 73° 02' 3" W), Station 7 (36° 36' 5" S, 73° 00' 6" W), Station 14 (36° 32' 1" S, 73° 03' 0" W), and Station 18 (36° 30' 8" S, 73° 07' 6" W). Water depths were 24 m, 32 m, 64 m and 88 m, respectively. Sediment samples were taken by a small Rumohr gravity-corer of 74 mm inner diameter and 1 m length. At each station, three cores were taken and immediately subsampled into Plexiglas tubes of 3.6 cm inner diameter and 30 cm length. Bottom water samples were taken with a 5 l Niskin bottle approximately 1 m above the sediment. Bottom water temperatures were 11 to 12 °C throughout the year. At the end of May and beginning of June only one sediment sample for each station could be taken.

Bottom water oxygen and nitrate Oxygen concentrations were measured by Winkler titration using 300 ml bottles. The reagents were added immediately after taking the samples and the bottles were stored in the dark until they were titrated the same day. Bottom water samples for later determination of nitrate (including nitrite) were frozen. The nitrate and nitrite in these samples were reduced to NO in 80 °C Vanadium(III) and detected by chemiluminescence (BRAMAN and HENDRIX, 1989).

Biomass and species distribution Sediment cores were stored in the laboratory at 5 °C for up to 10 days, open at the top and with 3 - 5 cm of bottom water over the sediment. The first core taken from each station was processed on the following day to be compared later with results from the other two cores processed within the following 10 days to check for changes in biomass or species distribution due to storage. Within 10 days no significant changes were observed. Sediment cores were extruded from the tubes and placed on a slightly tilted surface. The sediment around the sheaths was washed away carefully with sea water from a squirt bottle starting at the bottom of the core. One cm of sediment was consecutively washed away and the exposed sediment was examined for sheaths of *Thioploca* using a binocular microscope at 16 × magnification. At each depth interval the exposed sheaths were counted and five sheaths were randomly picked for observation under the microscope. For each of the five sheaths, the diameter of the sheath was measured, the number of filaments counted, and the diameter of one filament and the length of 5 to 10 cells of this filament were measured at 1000 × magnification. A preliminary investigation had shown that trichomes of one size class within a sheath have very similar diameters and cell lengths. In sheaths containing filaments of two or three different diameter classes the number of filaments, the diameter and the cell length were measured separately for each of those classes.

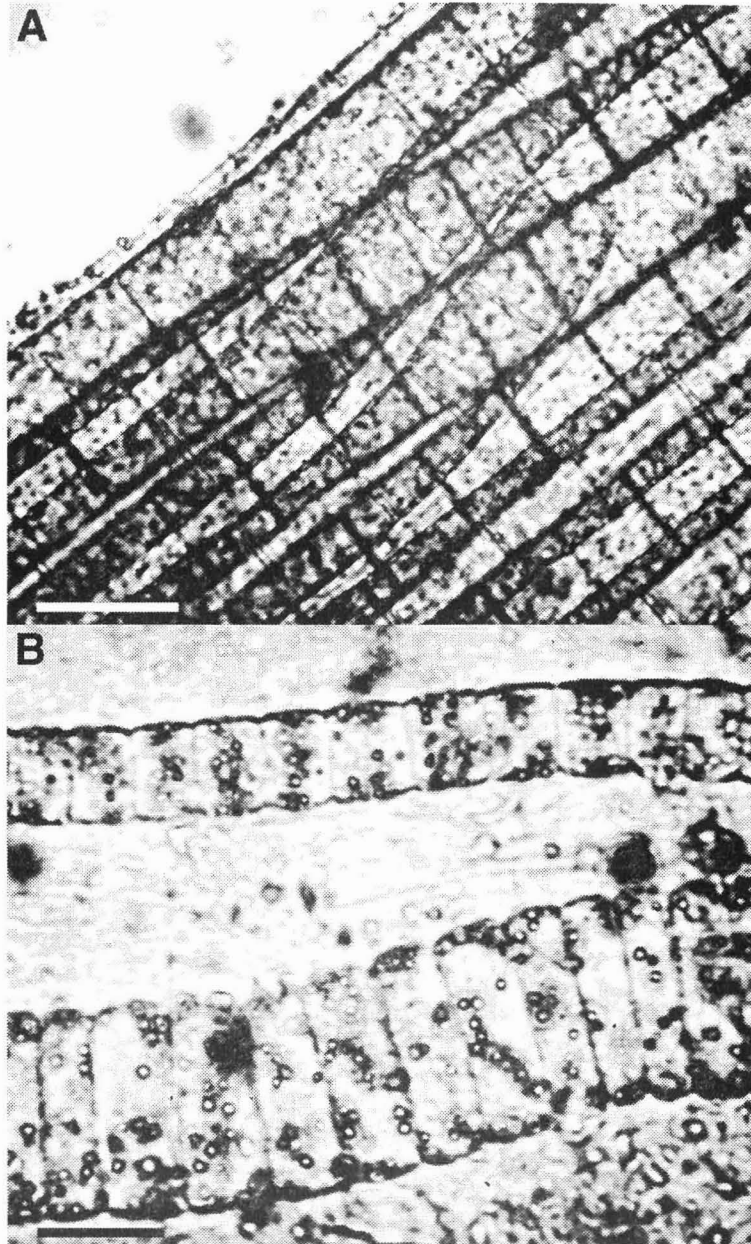


Fig. 5.2 Trichomes of *Thioploca* spp. in sheaths. (A) The long-cell morphotype, here *T. araucae*, with a normal ratio of cell-length to diameter. (B) Two short-cell trichomes with diameters of 35 μm and 75 μm living in the same sheath. Bars represent 50 μm .

Calculations At each depth interval for each of the five observed sheaths the volume of *Thioploca* trichomes was calculated using the diameter of the trichomes, the number of trichomes in the sheath, and the average length of sheaths in this particular sediment depth. The average length of sheaths at different sediment depths was determined in an earlier study where the three-dimensional position of the sheaths in the sediment was investigated (SCHULZ et al., 1996) and could be used for this study. The average biovolume of the five sheaths removed at each depth interval was multiplied by the number of sheaths per cm². The biovolumes at all depths were added up to give the total biovolume per unit surface area. The biomass of *Thioploca* filaments was calculated from biovolume assuming that the density of the trichomes is 1 g cm⁻³. The mean of three cores was calculated.

5.1.4 Results

Observations on different morphotypes During a preliminary study of the *Thioploca* community in December 1995 it was observed that, in addition to the usual morphological form of *Thioploca* trichomes with cylindrical or slightly barrel-shaped cells, there was an undescribed morphotype with much shorter cells and rounded sides (Fig. 5.2). To discriminate this morphotype from the known *Thioploca* spp. the cell-lengths of each trichome were measured and it became apparent that, using the ratio between average cell-length and trichome diameter, the population fell into two groups of ratios, one ≤ 0.48 and one > 0.48 . The diameters of short-cell filaments do not separate as clearly into different size classes as diameters of normal thioplocas, but peaks of frequency occur at 14 - 20 μm and 24 - 32 μm .

For a comparison of trichome and sheath parameters the data of all three cores per sampling station and time have been separated into two morphological groups of trichomes. One group included data of filaments with ratios of cell-length to trichome diameter of ≤ 0.48 , which was defined as the short-cell morphotype of *Thioploca* species. The second group contained long-cell morphotype filaments with ratios > 0.48 . This group contained *T. chileae* and *T. araucae*, which can be distinguished on the basis of their trichome diameters (MAIER and GALLARDO, 1984; TESKE et al., 1995; SCHULZ et al.,

1996). For all parameters the average, median and most frequent value at each time and station were calculated to evaluate whether the parameter followed a Gaussian distribution. For all parameters these 3 values yielded very similar results, except for the depth distribution of morphotypes, where the most frequent value was clearly higher than the average value for the short-cell morphotype and lower for *T. chileae* and *T. araucae*.

Bottom water parameters During the summer and fall months of January - June 1996 the bottom water overlying the sediment was almost oxygen free at all stations (Fig. 5.3 A). In winter (July - September 1996) the oxygen concentrations increased to values around 20 μm and decreased again to near zero in spring and early summer 1996 (October - December). During summer 1996 / 1997 oxygen concentrations were higher and more fluctuating than in the previous summer. Nitrate concentration were generally rather constant (10 - 30 μm). Only during winter 1996 lower values near zero were found (Fig. 5.3 B).

Biomass The total biomass of *Thioploca* filaments at all stations was highest in summer 1996 (January - March) and declined during autumn (Fig. 5.4 A and B). At Station 18, which was furthest offshore, the biomass tended to be lower during summer 1996. During winter 1996 biomass remained low at all stations, but the *Thioploca* population never disappeared completely. In spring and summer 1996 / 1997 only the biomass at Station 7 increased significantly but remained lower than observed in the previous summer.

Number of sheaths The total number of inhabited sheaths per cm^2 (Fig. 5.4 B) generally followed the pattern of the total biomass, but the fluctuations were not as pronounced as for the biomass (Fig. 5.4 A). At all stations the number of sheaths per cm^2 was high in summer 1996 and declined during the fall (April - May 1996). The number of sheaths remained low during winter and only in spring and summer 1996 / 1997 did the number again increased at Station 7, however remaining low at Station 14 and 18.

The percentage of observed sheaths of the different groups (Fig. 5.5) showed how the composition of the *Thioploca* communities changed with time and between stations. At Station 18, sheaths containing trichomes of the short-cell morphotype occurred rarely throughout the year, while they were mostly

abundant at Station 14 and even more abundant at Station 7. At Station 14 and 18 sheaths containing *T. chileae* were more frequent than *T. araucae* throughout the year, while at Station 7 sheaths containing *T. araucae* were more common.

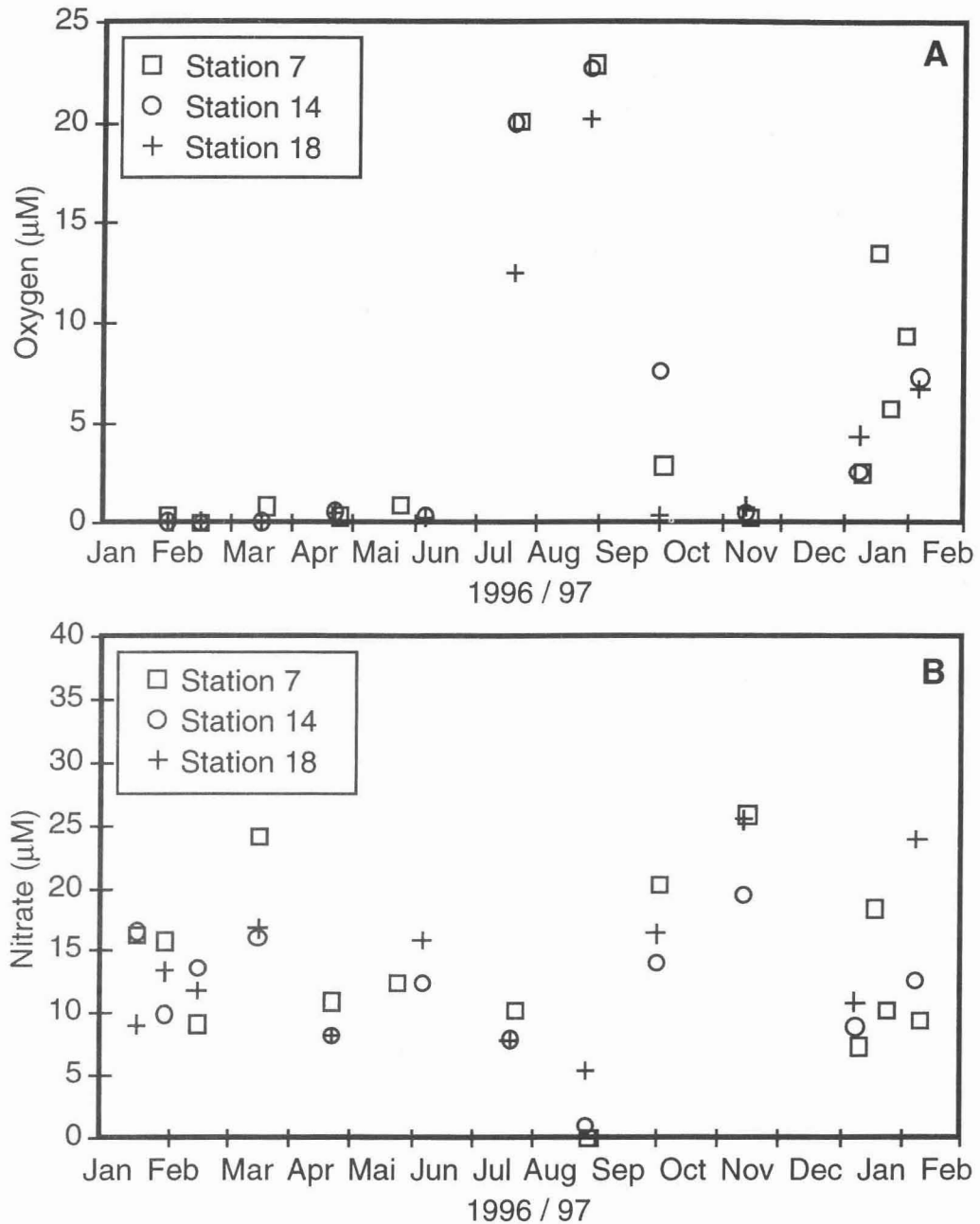


Fig. 5.3 Bottom water concentrations of oxygen (A) and nitrate (B) measured on the three shelf stations from January 1996 until February 1997.

Depth distribution To describe the depth distribution (Fig. 5.6), the most frequent depth of each type of sheath is given rather than the average depth, as the latter does not follow a Gaussian distribution. Sheaths containing more than one type of trichome have been counted for each type of trichome they contained. As a general tendency, sheaths containing the short-cell morphotype of *Thioploca* occurred mostly in deeper parts of the sediment, while *T. chileae* and *T. araucae* were more frequent at the sediment surface. The short-cell morphotype was most abundant at 5 - 8 cm depth at Station 7, while at Stations 14 and 18 the most frequent depth was more variable reaching depths of up to 15 cm in the sediment.

Stable trichome parameters The average diameter of trichomes (Fig. 5.7) as well as the average ratio of cell-length to trichome diameter (Fig. 5.8) were found to be remarkable stable throughout the year at each station. This was particularly true for *T. araucae*, which showed constant dimensions also between stations. For *T. chileae* ratios of cell-length to diameter were generally slightly higher at Station 14 and slightly lower at Station 7. The diameters of the short-cell morphotype showed more variability without a clear seasonal change, whereas their ratios of cell-length to diameter remained constant.

Stable sheath parameters The average percentage of mixed sheaths containing more than one morphological type of trichomes, as well as the average diameter of the different types of sheaths and the number of trichomes per sheath remained stable over the 14 months of observation, but were different between stations. Average values of all observations have been summarized in Table 5.1.1. Occasionally, the smallest *Thioploca* species, "*T. marina*" (2.5 - 5 μm), was observed in sheaths together with *T. chileae* or *T. araucae*, but their frequency could not be quantified, because the very small filaments are easily hidden by the larger filaments. In general, they appeared at all stations within the upper 3 cm of the sediment during most of the year.

The mixed sheaths were separated into 3 groups, one containing only trichomes of the long-cell morphotype (i.e. *T. araucae* and *T. chileae*), one containing only short-cell filaments of two groups with different diameter, and one containing long-cell and short-cell trichomes mixed. The last two groups occurred very rarely at all stations, never comprising more than 1 % of all sheaths observed, while sheaths containing *T. chileae* and *T. araucae* together were much more abundant. At Station 7 and 18 about 10 % of the sheaths were inhabited by mixed *T. chileae* and *T. araucae*, while at Station 14 it was only 3 %.

The diameters of sheaths inhabited by *T. araucae* were larger than of those inhabited by *T. chileae* or short-cell trichomes at all 3 stations. At Station 14 sheaths containing *T. chileae* or short-cell trichomes were smaller than at the other two stations. *T. chileae*-inhabited sheaths were larger at Station 7 than at the other two stations. The same was the case for sheaths containing short-cell trichomes at Station 18. In sheaths containing *T. araucae* the average number of trichomes per sheath was around 6 - 7 at all stations. At Station 14 and 18 there were about 8 trichomes of *T. chileae* per sheath, while at Station 7 there were only about 4 trichomes. The average number of short-cell trichomes per sheath was around 3 at all stations. Occasionally, maximal numbers of around 100 trichomes per sheath occurred especially for *T. chileae*, but the majority of sheaths contained much less trichomes, e.g. 95% of the sheaths had ≤ 7 trichomes for the short-cell morphotype, and ≤ 20 for *T. chileae* and *T. araucae*, respectively.

Maximal biomass densities and depths of filaments The highest biomass density was generally found in the upper 0.5 - 1 cm of the sediment (Fig. 5.9). In August 1996, this depth increased at Station 7 and 14, followed by Station 18 in September 1996. At Station 7 the depth of highest biomass density returned to 0.5 - 1 cm, while at Station 14 it remained at 1 cm depth and at Station 18 at 2 cm. During the 14 months of observation, the maximal depths at which *Thioploca* trichomes could be found changed considerably between 8 and 22 cm. This was mainly due to short-cell trichomes which penetrated deepest into the sediment at all stations during summer 1996 and summer 1997. The maximal depth to which long-cell trichomes could be found, 4 - 13 cm, was more stable at each station and decreased towards the coast.

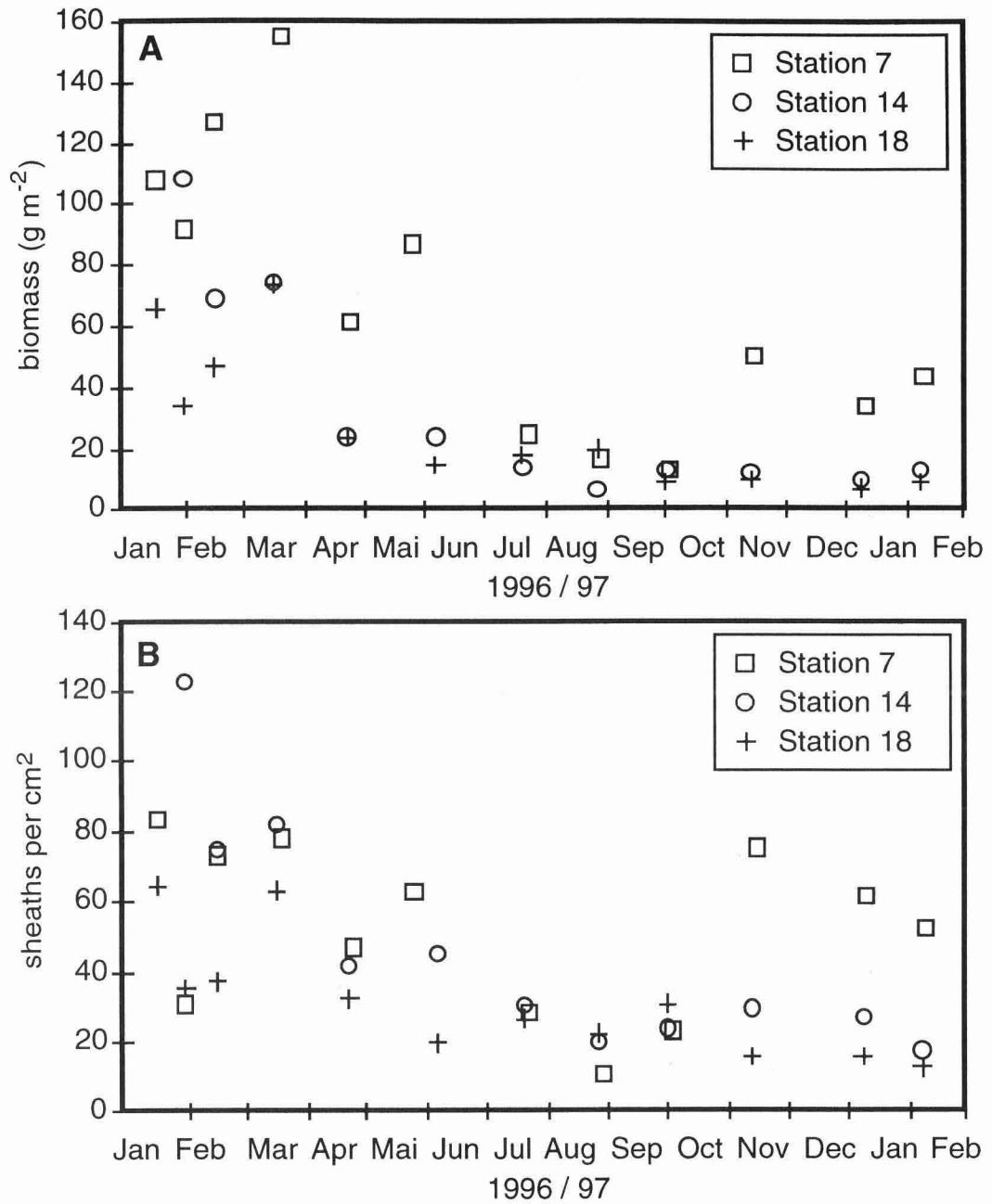


Fig. 5.4 Population size of *Thioploca* spp. on the three shelf stations during 14 months of investigation. (A) Total biomass without sheaths in g wet weight per m². (B) Total number of sheaths per cm².

Tab. 5.1.1 Stable sheath parameters (mean of 14 months \pm standard deviation) for each of the three shelf stations: percentage of mixed sheaths, occupied by trichomes of different diameter, with filaments of only long-cell morphotype, only short-cell morphotype or short-cell and long-cell morphotype living together. Mean diameter of sheaths inhabited by the short-cell morphotype or by one of the long-cell morphotype species, *T. chileae* or *T. araucae*. Mean number of trichomes in sheaths inhabited by trichomes of the short-cell morphotype or by one of the long-cell morphotype species, *T. chileae* or *T. araucae*.

Parameter	<i>Thioploca</i> type	Station 7	Station 14	Station 18
Mixed sheaths (%)	Long-cell morphotype	11.0 \pm 5.7	3.2 \pm 1.7	8.9 \pm 5.5
	Short-cell morphotype	0.4 \pm 0.7	0.6 \pm 1.0	0.2 \pm 0.4
	both morphotypes mixed	0.7 \pm 0.8	0.9 \pm 0.6	0.8 \pm 0.7
Diameter of sheaths (μm)	Short-cell morphotype	108 \pm 12	99 \pm 22	137 \pm 29
	<i>T. chileae</i>	159 \pm 31	93 \pm 11	113 \pm 13
	<i>T. araucae</i>	176 \pm 25	187 \pm 22	167 \pm 19
Number of trichomes per sheath	Short-cell morphotype	2.5 \pm 0.6	3.2 \pm 1.0	2.9 \pm 0.9
	<i>T. chileae</i>	3.8 \pm 1.5	8.1 \pm 1.7	7.6 \pm 1.7
	<i>T. araucae</i>	6.1 \pm 1.9	6.9 \pm 2.6	5.8 \pm 2.0

The Bay of Concepción Station 4, in the middle of the Bay of Concepción, was not constantly populated by typical thioplocas and the type of community was different from the shelf stations. In February 1996 a population of short-cell *Thioploca* living in sheaths with a total biomass of 40 g m⁻² was found in the upper 7 cm of the sediment. From July 1996 to February 1997 this short-cell community was also observed, but with very low biomass (< 1 g m⁻²). In January and February 1997, sheathless filaments (per definition beggiatoas) with diameters typical for *T. araucae* were found on top of the sediment with a biomass of 15 and 5 g m⁻², respectively. Similar populations were observed in February, July, August and October 1996, but with very low biomass. The bottom water oxygen and nitrate concentrations at Station 4 were similar to the shelf stations.

Observations during "El Niño" in March 1998 At Station 18 the oxygen concentrations measured in the bottom water in March 1998 were high compared to winter 1996 (16 μm), and even higher at Station 4, 7 and 14 (30 - 35 μm). Nitrate concentrations were 16 - 28 μm . The total biomass of *Thioploca* spp. was very low at Stations 7, 14 and 18 (3, 1 and 5 g m^{-2}), even lower than in winter 1996. The total number of sheaths was correspondingly low. The average sediment depth of highest biomass density was at 2 cm at Station 14 and 18, and at 3 cm at Station 7. The downward extension of the population was 6 -10 cm at Station 7, as much as 8 -11 cm at Station 14 and 8 - 9 cm at Station 18. Average trichome diameters and ratios of cell-length to diameter of *Thioploca* trichomes remained unchanged on the shelf stations, except for the short-cell trichomes which decreased in diameter. Sheath diameters were slightly greater at Station 7 and 18, but the number of trichomes per sheath was unchanged at all stations. The number of mixed sheaths with trichomes of different size classes remained the same at Station 7 and 18, but at Station 14 no mixed sheaths were found. The proportion of different morphological types remained generally the same, except for Station 14, where *T. chileae* decreased to 22 %, while the short-cell trichomes increased to 64 %. Generally, replicate samples showed larger heterogeneity than before with respect to distribution of morphotypes and total biomass. At Station 4, a population of sheathless trichomes with diameters typical for *T. araucae* was found at the sediment surface at a biomass of 2 g m^{-2} .

5.1.5 Discussion

Seasonally changing parameters The variations in biomass (Fig. 5.4 A) corresponded well to the changes in bottom water concentrations of oxygen and nitrate (Fig. 5.3), which are dependent on the hydrographic conditions. Under normal spring and summer conditions, long periods of strong southerly winds induce coastal upwelling, and equatorial subsurface water rich in nutrients and depleted in oxygen covers much of the continental shelf. During winter or "El Niño" conditions, northerly winds dominate, which reduce the upwelling, and the shelf is covered by subantarctic water of lower salinity and higher oxygen concentrations (STRUB et al., 1998). The seasonal upwelling off the coast of Concepción was most pronounced during summer

1995 / 1996, but in the summer 1996 / 1997 upwelling was less stable. Consequently, the oxygen content of the bottom water fluctuated more and the biomass of thioplocas was significantly lower than the previous summer.

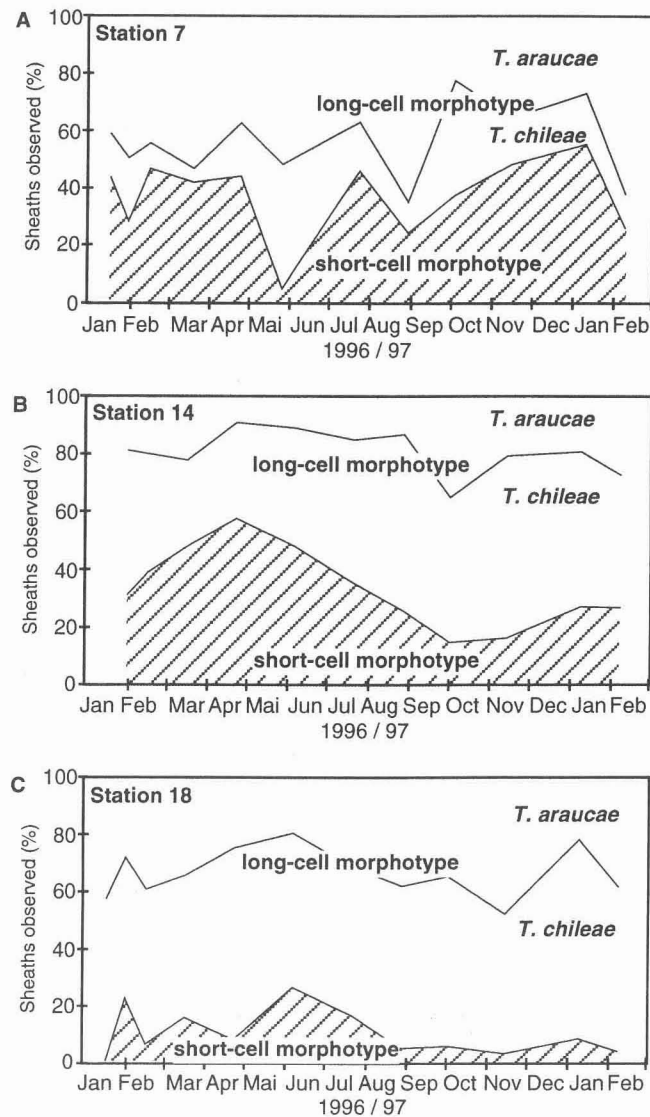


Fig. 5.5 Relative abundance of sheaths of short-cell morphotype of *Thioploca* spp (hatched area) and the two species of the long-cell morphotype (white area) *T. chileae* and *T. araucae*. on each of the three shelf stations during a study of 14 months.

The increase in bottom water oxygen seems to have been the main reason for a decline in *Thioploca* biomass under winter conditions. Trichomes in the upper 1 cm of the sediment, where most of the biomass is located during summer (SCHULZ et al., 1996), disappeared and, consequently, the highest biomass density was found deeper in the sediment (Fig. 5.9). This is consistent with observations from the Peruvian upwelling areas, where highest biomasses of *Thioploca* spp. were clearly associated with the lowest bottom water oxygen concentrations (ZAFRA, 1988). However, in contrast to what was observed by GALLARDO (1985; 1995) and ZAFRA (1988), the decrease in biomass was not accompanied by a decrease in the average number of trichomes per sheath (Table 5.1.1), but was mainly correlated with a decrease in the number of sheaths (Fig. 5.4 B and 5.10), especially at Stations 14 ($r^2 = 0.9$) and 18 ($r^2 = 0.8$). At Station 7, this correlation was less pronounced ($r^2 = 0.5$) and a much higher biomass was observed with a comparably low number of sheaths. During the decline of the biomass, a high proportion of empty sheaths was occasionally found at the sediment surface, but these sheaths decomposed rapidly and disappeared within the following month.

The depth extension of the *Thioploca* community was also affected by seasonal changes. During summer 1996, filaments of the short-cell morphotype were found deep down in the sediment (Fig. 5.9) but disappeared in autumn and winter 1996, leaving behind empty sheaths which were often stained black from iron sulfide. In summer 1996 / 1997, the short-cell filaments again colonized deeper parts of the sediment at Station 7, but did not expand significantly at Station 14 and 18 (Fig. 5.9). Thus, colonization of deeper parts of the sediment by short-cell filaments generally co-occurred with times of high biomass (Fig. 5.4 A).

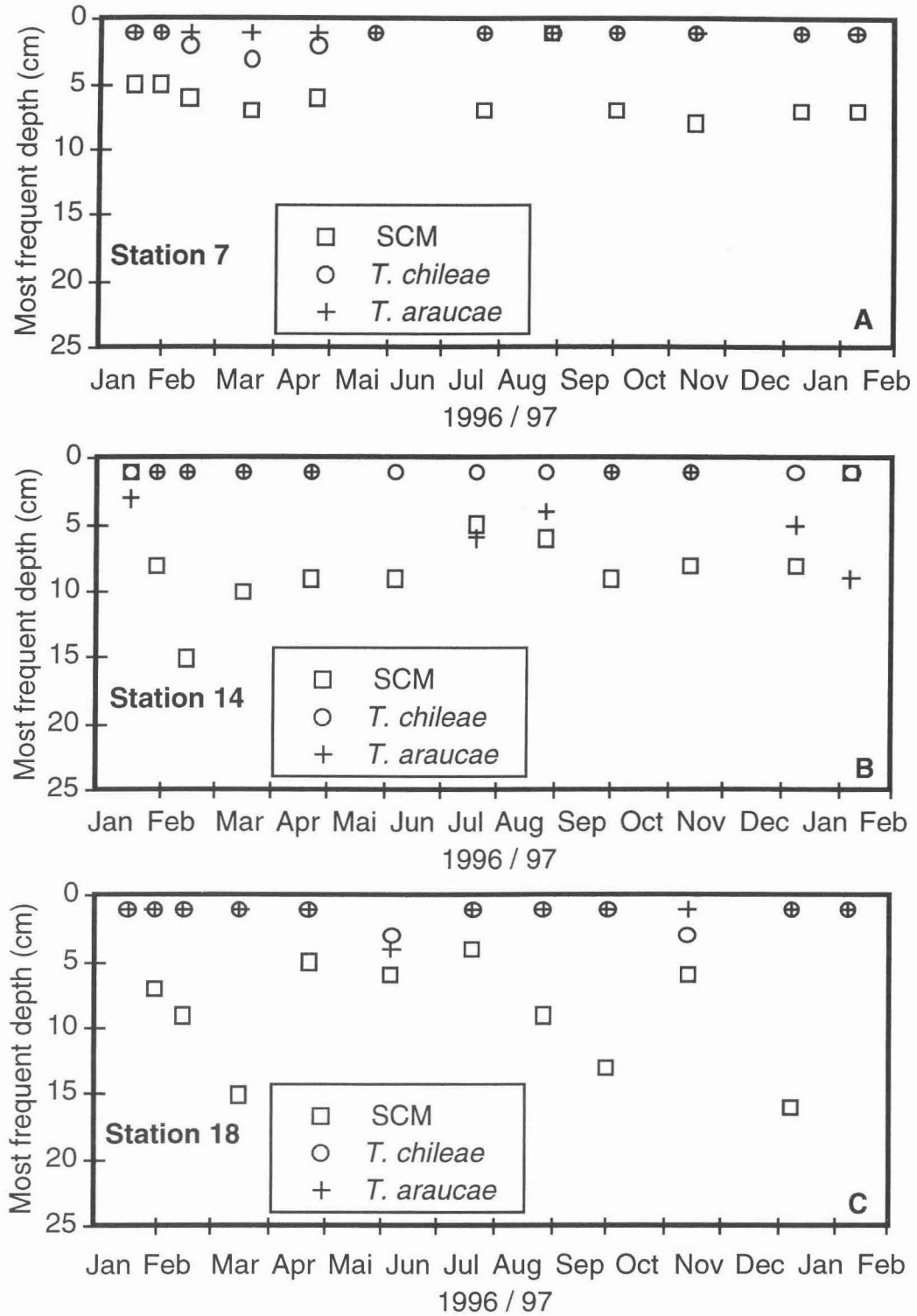


Fig. 5.6 Most frequent depth of sheaths of the short-cell trichomes and the two long-cell species *T. chileae* and *T. araucae* on each of the three shelf stations during 14 months of investigation.

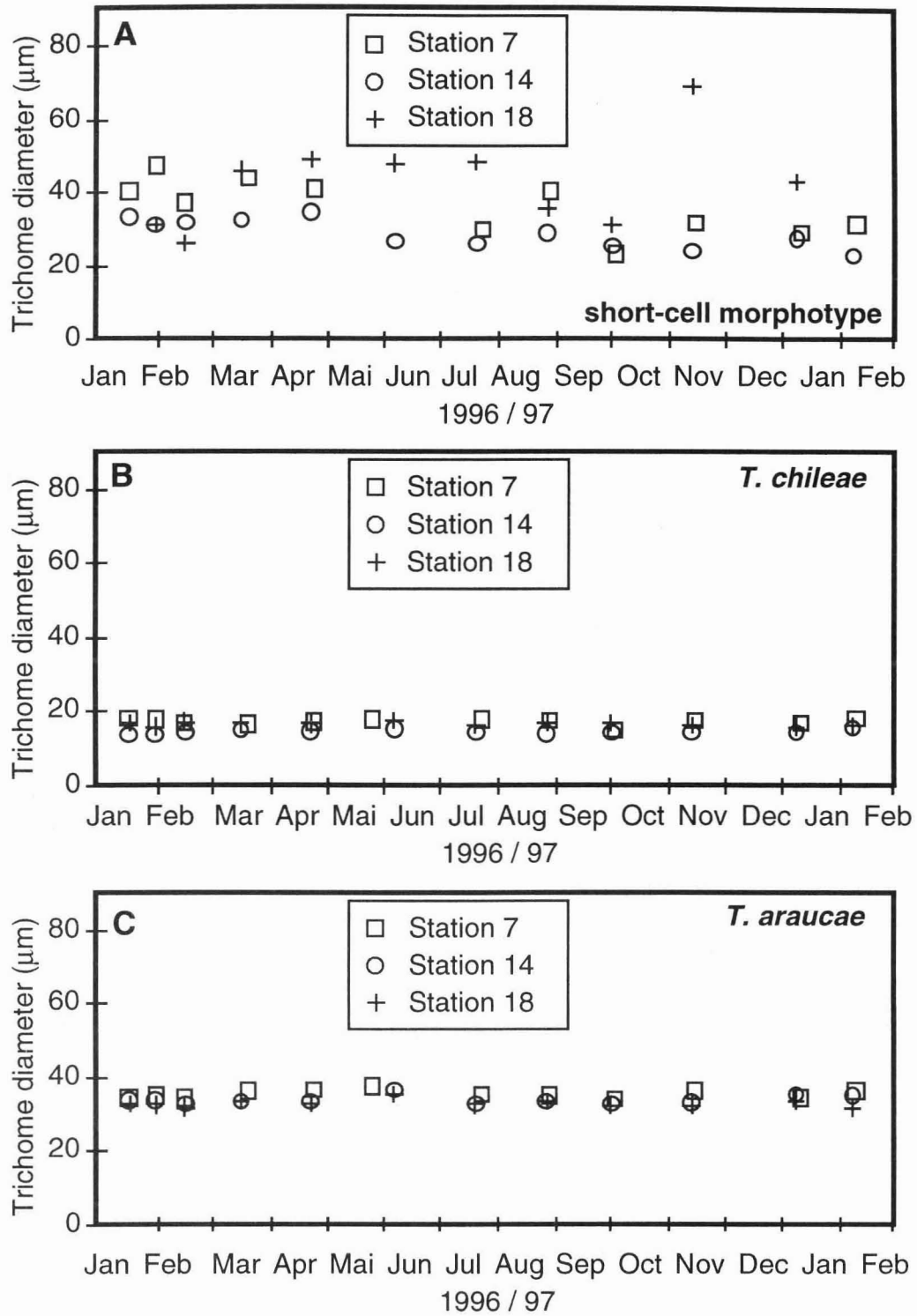


Fig. 5.7 Average diameter of trichomes of (A) short-cell morphotype, (B) *T. chileae* and (C) *T. araucae* on each of the three shelf stations from summer 1996 until summer 1997.

Population dynamics From the increases in biomass between subsequent observations it is possible to assume minimum doubling times of *Thioploca* spp. under natural conditions. At Station 7 the highest rates of increase were found in February and October 1996 and corresponded to doubling times of 23 and 22 days, respectively. The mean doubling time of biomass during periods of biomass increase was 38 days at Station 7, compared to 56 and 55 days at Station 14 and 18. At Station 14, the shortest doubling time of biomass, 35 days, was observed in September 1996. However, this is probably not the fastest growth rate since the *Thioploca* population at Station 14 was already decreasing at the beginning of the study and did not reach a similarly high biomass the following summer. At Station 18 the greatest increase in biomass was found in February 1996 and corresponded to a doubling time of 24 days. Thus, it can be assumed that the maximum growth rates of *Thioploca* spp. under the environmental conditions correspond to a doubling time of approximately 3 weeks, which compares well to the 26-52 days doubling time of filaments growing on acetate estimated from laboratory experiments with thioplocas from Station 7 (OTTE et al., manuscript submitted).

At Station 4 the population of sheathless trichomes at the sediment surface increased 14-fold during September 1996, which corresponds to a doubling time of 5 days. These filaments were found to be identical to *T. araucae* according to their 16S rDNA sequence (TESKE et al., in press). As the population of sulfur bacteria at Station 4 was generally more patchy in distribution and fluctuated more than at the three shelf stations, this doubling time is only a rough estimate. Compared to the thioplocas living in shelf sediments, the value appears to be high. However, compared to chemo-autotrophic marine *Beggiatoa* strains, which have doubling times of 1 - 4 days (NELSON and JANNASCH, 1983; NELSON et al., 1986a) the number might still be realistic, although the *Beggiatoa* filaments were much smaller (4 - 5 μm in diameter) and should therefore grow faster. Nevertheless, the distribution of filamentous sulfur bacteria seems to be more heterogeneous within the Bay, thus a more precise estimation of growth rate would demand a higher number of samples.

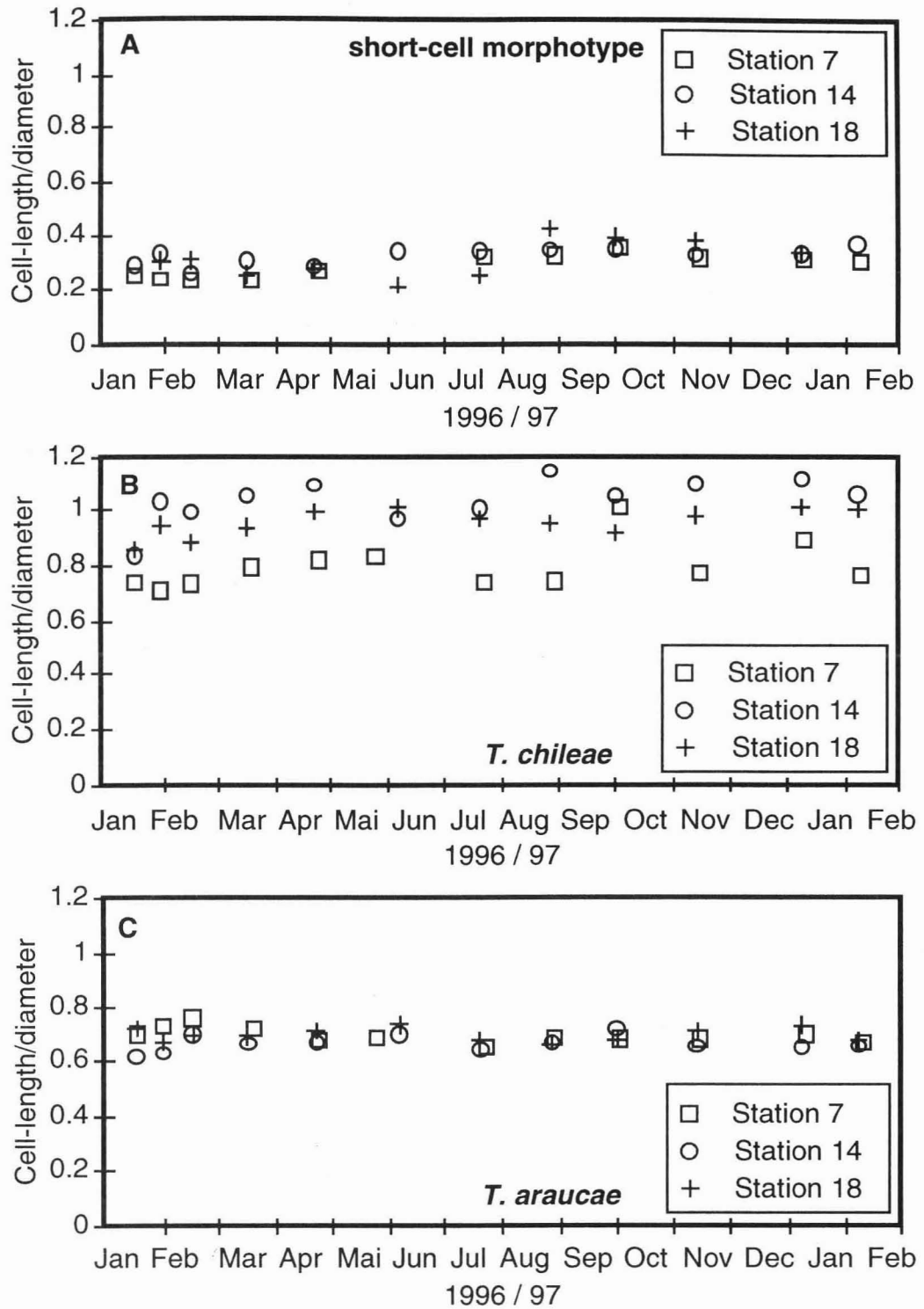


Fig. 5.8 Average ratio of cell-length to diameter of, (A) short-cell morphotype, (B) *T. chileae*, and (C) *T. araucae* on each of the three shelf stations from January 1996 until February 1997.

Parameters unaffected by seasonal changes The average trichome diameters of *T. chileae* and *T. araucae* remained constant at each station throughout the period of observation (Fig. 5.7). Thus, they can be regarded as stable morphological features of the species, unaffected by varying environmental conditions. The diameter of the trichomes is probably genetically determined, which is also indicated by 16S rDNA sequences (TESKE et al., 1995). A similar, remarkable invariance was observed for the ratios of cell-length to diameter for these two species (Fig. 5.8). Thus, cell division during growth apparently does not lead to cells that are in average shorter than those during stationary phase. Although the average ratios of cell-length to diameter remained unchanged over the year, they were different between stations for *T. chileae*, whereas for *T. araucae* no differences between stations were observed. This may indicate that each station was populated by a slightly different type of *T. chileae*. The average diameters of the short-cell filaments were less constant than observed for *T. chileae* and *T. araucae*, which is probably because they comprise different species with different diameters (see chapter 5.2). The variance in diameters did not correspond to seasonal changes of the biomass. Furthermore, the average ratios of cell-length to diameter were almost as stable as observed for *T. chileae* and *T. araucae* and, thus the average cell-lengths are stable parameters for each station unaffected by seasonal changes.

The variations in the relative abundance of the short-cell morphotype of *Thioploca* over the period of observation (Fig. 5.5) seem to correlate with the seasonal changes in biomass (Fig. 5.4 A). For *T. araucae* and *T. chileae*, the differences in relative abundance between the three shelf-stations were greater than the seasonal variations at each station. Thus, it appears that the species composition of the *Thioploca* community is not affected greatly by seasonal changes but is influenced rather by conditions that are characteristic for each station. This could be the sulfate reduction rates, which were increasing significantly towards the coast but expressed a less pronounced seasonality (STROTMANN et al., in prep.).

The percentage of mixed sheaths, occupied by different morphotypes or species of *Thioploca*, as well as the diameter of sheaths and the number of trichomes per sheath, varied between sampling times without showing a general trend or a correlation with changes in biomass (Table 5.1.1). Again,

differences between stations were more pronounced than temporal variations. Sheaths containing short-cell trichomes of different diameters ($> 5 \mu\text{m}$ difference) and sheaths inhabited by short-cell trichomes together with trichomes of *T. araucae* or *T. chileae* occurred rarely at all stations ($< 1 \%$) while sheaths containing *T. chileae* together with *T. araucae* trichomes were much more abundant (3-11%). An earlier study showed that in 85 % of mixed sheaths *T. araucae* was the dominant species. This led to the hypothesis that mixed sheaths form when trichomes, penetrating into the bottom water, retreat into their sheaths and draw other trichomes with them, which can happen more easily in the larger sheaths of *T. araucae* (SCHULZ et al., 1996). This hypothesis is supported by the observation that mixed sheaths were most abundant at Station 7, where *T. araucae* dominated, and least abundant at Station 14, where *T. araucae* was rarest.

The average diameter of sheaths and the average number of trichomes per sheath for *T. araucae* were quite similar at all stations, while for sheaths of *T. chileae* clear differences were found. At Station 14 sheaths of *T. chileae* were on average smaller than at any other station while the average number of trichomes in these sheaths was the highest. The *T. chileae* sheaths at Station 7 were the largest of this group and contained the lowest number of trichomes. For the short-cell morphotype the number of trichomes per sheath was low at all stations, while the diameter of sheaths were quite distinct. Thus, an increasing diameter of sheaths is not necessarily proportional to higher numbers of trichomes. Rather, these two parameters vary independently, and do not correlate with the growth state of the population. Comparison of the number of trichomes per sheath at the different stations indicates that at Station 7 the number of trichomes per sheath is much higher for *T. araucae* sheaths than for the other sheath types, while at Stations 14 and 18 *T. chileae* sheaths have the highest number of trichomes. Thus, at each station the dominating species had the highest number of trichomes per sheath irrespective of seasonal changes. Since all sheaths were on average below 200 μm thick and many even below 100 μm (Table 5.1.1), sieving with 1.0, 0.5 or even 0.25 mm sieves will lead to loss of sheaths. The resulting error is probably variable, as average sheath diameters differed among stations.

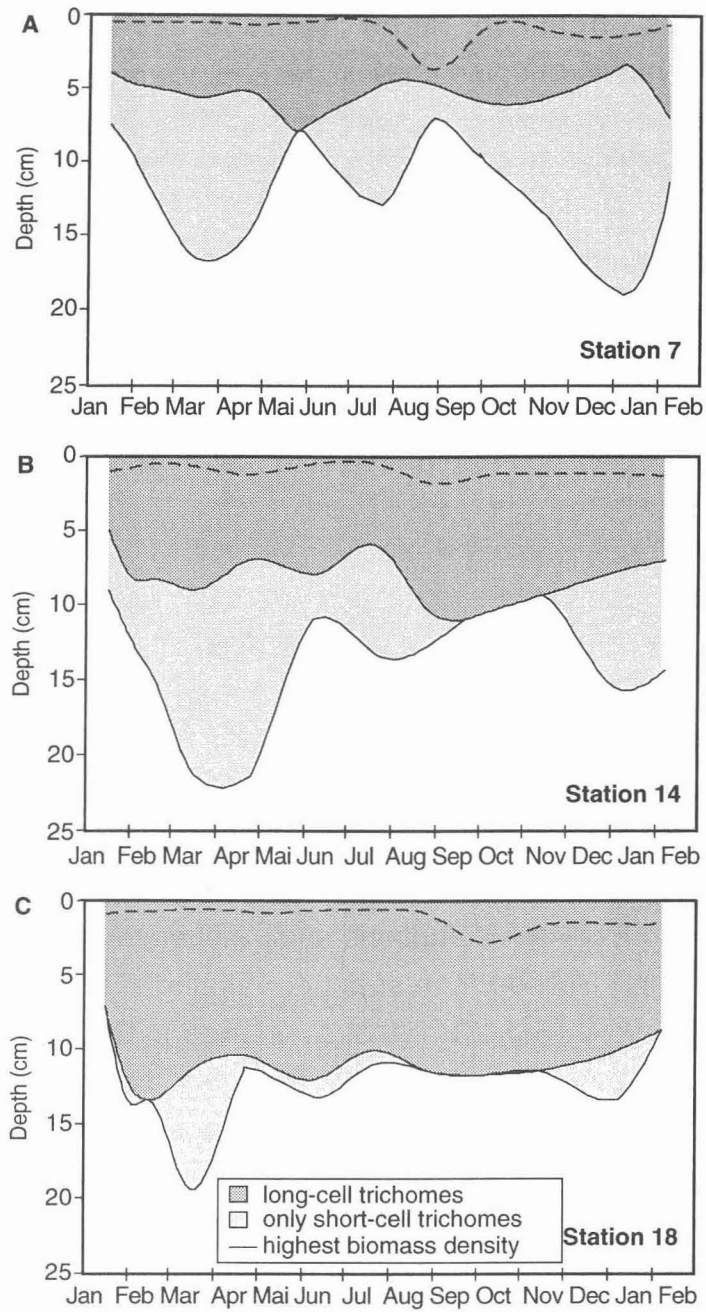


Fig. 5.9 Maximum downward extension of long-cell trichomes (*T. araucae* and *T. chileae*) (dark gray) and short-cell trichomes (light gray) and the average depth of highest biomass density (dashed line) for each of the three shelf stations during 14 months of observation.

Differences between shelf stations As a general trend, the biomass of the *Thioploca* community increased towards the coast and was accompanied by an increased number of short-cell trichomes that reached deeper into the sediment during growth phases. Concurrent with this, the highest biomass of thioplocas reported to date, 106 g per 0.1 m², including sheaths, was found near Station 7 (GALLARDO, 1977). The changes in the community across the shelf coincided with sulfate reduction rates that were higher towards the coast (FERDELMAN et al., 1997; STROTMANN et al., in prep.). With the increasing sulfide production the energy available from sulfide oxidation increases, which apparently leads to higher populations of thioplocas near the coast.

Some parameters, that were independent of seasonal changes, such as the relative proportion of *T. chileae* and *T. araucae* (Fig. 5.5) and the maximal depth to which these two species occur (Fig. 5.9), showed increasing fluctuations when approaching the coast. A reason for this might be that in more shallow waters fluctuations in the intensity of upwelling and thus in the primary production have a stronger effect on the sediment. Since a smaller fraction of the primary production is degraded in the shallower water column, also short-term productivity fluctuations may influence the processes in the sediment more, which is seen from stronger variations in the sulfate reduction rates on coastal stations (STROTMANN et al., in prep.). The most frequent depth for short-cell trichomes (Fig. 5.6) was the only parameter showing less fluctuations towards the coast. At the same time, these filaments were more abundant in coastal sediments. Thus, the stronger variations of depth might have been a result of lower numbers of observation at Station 14 and especially at Station 18.

Station 14 can be considered intermediate between Station 18 and 7 in terms of biomass and stability. Nevertheless, in some respects this station was exceptional. The relative proportion of *T. araucae* filaments was the lowest (Fig. 5.5) and they occurred more consistently in deeper parts of the sediment than at other stations (Fig. 5.6). The *T. chileae* filaments at this station had generally shorter cells (Fig. 5.8), and their sheaths were much smaller in diameter and contained more trichomes than at Stations 7 and 18 (Table 5.1.1). Comparably high biomass was accompanied by highest numbers of sheaths of all stations (Fig. 5.10), and the number of mixed sheaths was on

average 3 times lower at Station 14 (Table 5.1.1). Altogether, it seems that the *T. chileae* population, dominating at Station 14, is different from the *T. chileae* populations at the other two shelf stations.

The Bay of Concepción The areal sulfate reduction rates reported for Station 4 are 3 - 4 times higher than for the shelf stations and, other than in the shelf sediments, free hydrogen sulfide regularly accumulates in the pore water up to the sediment surface (FERDELMAN et al., 1997; STROTMANN et al., in prep.). Probably as a result of this, Station 4 was not continuously populated by thioplocas and, occasionally, two distinct types of populations occurred. In the summer of early 1996, short-cell filaments populated the upper 7 cm at Station 4 in high biomass (40 g m^{-2}). At this time oxygen was not detectable in the bottom water, nitrate was present in $11 \mu\text{M}$ concentration and the sediment smelled strongly of hydrogen sulfide. During summer 1997, sheathless filaments populated the surface sediment with a biomass of up to 15 g m^{-2} . Except for the absence of a sheath, these filaments looked identical to *T. araucae* and they had the same 16S rDNA sequence (TESKE et al., in press). Consequently, they may be considered sheathless thioplocas. However, recent studies indicate that the phylogenetic affinity of the genera *Thioploca* and *Beggiatoa* needs to be reconsidered (AHMAD et al., 1999; JØRGENSEN et al., submitted). Although the biomass of filamentous sulfur bacteria seems low compared to the biomass of *Thioploca* spp. on the shelf ($10 - 160 \text{ g m}^{-2}$), it is in the same range as the biomass of *Beggiatoa* spp. encountered in a Danish fjord ($5 - 20 \text{ g m}^{-2}$) (JØRGENSEN, 1977a). In March 1997 oxygen concentrations as high as $12 \mu\text{M}$ were found in the bottom water and nitrate was present at $1 - 6 \mu\text{M}$. Thus, it is not clear whether the sheathless filaments accumulated at the top because they switched to an oxic respiration, or whether they still oxidized sulfide with the nitrate present in the bottom water, but without penetrating into the sediment. The latter seems more likely, since the filaments still possessed a central vacuole used for storing nitrate. Both types of populations seem to be able to endure higher sulfide concentrations than the type of *Thioploca* community found in shelf sediments. Nevertheless, the conditions in the Bay of Concepción do not support a stable population of sulfur bacteria. The general trend of higher fluctuations in the community towards the coast and higher proportions of *T. araucae* and short-cell filaments continues into the bay.

Effect of "El Niño" 1998 Effects of "El Niño" conditions on the total biomass of thioplocas including sheaths have been described for off Chile (GALLARDO et al., 1995), and off Peru (ARNTZ et al., 1985; TARAZONA et al., 1988, 1996; ZAFRA et al., 1988). In many respects the changes induced by the "El Niño" were similar to the changes observed in winter only more pronounced. Due to the prolonged absence of upwelling and the higher oxygen concentrations in the bottom water the *Thioploca* community on the shelf was strongly reduced, but on all stations these bacteria could still be found in low numbers. The highest biomass was found deeper in the sediment, as also observed in winter 1996. The highest biomass was found at Station 18 which was least affected by seasonal changes during the previous year. Some of the parameters that were independent of seasonal changes remained the same under "El Niño" conditions, e.g. the average diameters and cell-lengths and the number of trichomes per sheath, but the species composition at Station 14 changed significantly as well as the mean downward extension of the sheaths at Stations 7 and 14. Whereas the maximal sediment depth of *T. chileae* and *T. araucae* decreased continuously towards the coast in 1996 / 1997 (Fig. 5.9), the population extended down to about 10 cm at all stations in March 1998 and the average diameters of sheaths at Stations 7 and 14 increased slightly. Again, these parameters could be a consequence of the sulfate reduction rates, that were equally low at all shelf stations ($5 - 10 \text{ mmol m}^{-2} \text{ d}^{-1}$) in March 1998 during the "El Niño" (STROTMANN et al., in prep.). At Station 4, with bottom water oxygen and nitrate concentrations comparable to the previous summer, a population of sheathless filaments was found again on the sediment surface, similar to the population found in summer 1996 / 1997 but lower in biomass.

Summary The *Thioploca* community in the shelf sediments thrived best under intense upwelling conditions during summer 1996, which led to high biomasses increasing towards the coast and which was accompanied by a much deeper extension of the population. The high biomass was mainly comprised by *T. chileae* and *T. araucae* filaments living directly at the sediment surface, whereas filaments with short cells colonized the deeper parts of the sediment. Under winter and "El Niño" conditions, the *T. araucae* and *T. chileae* filaments living at the sediment surface disappeared, probably due to higher oxygen concentrations, and the population of short-cell filaments

in the deeper parts of the sediment also vanished. Each of the three shelf stations was distinct in its community composition, which was not greatly influenced by seasonal variations but did change under "El Niño" conditions. The average diameters and cell lengths remained stable at each station. In the Bay of Concepción thioplocas could not always be found, but in each of the three summers one of two different types of populations was found, either: a population comprised exclusively of short-cell filaments living in the upper 7 cm of the sediment, or a population consisting of sheathless filaments living on top of the sediment.

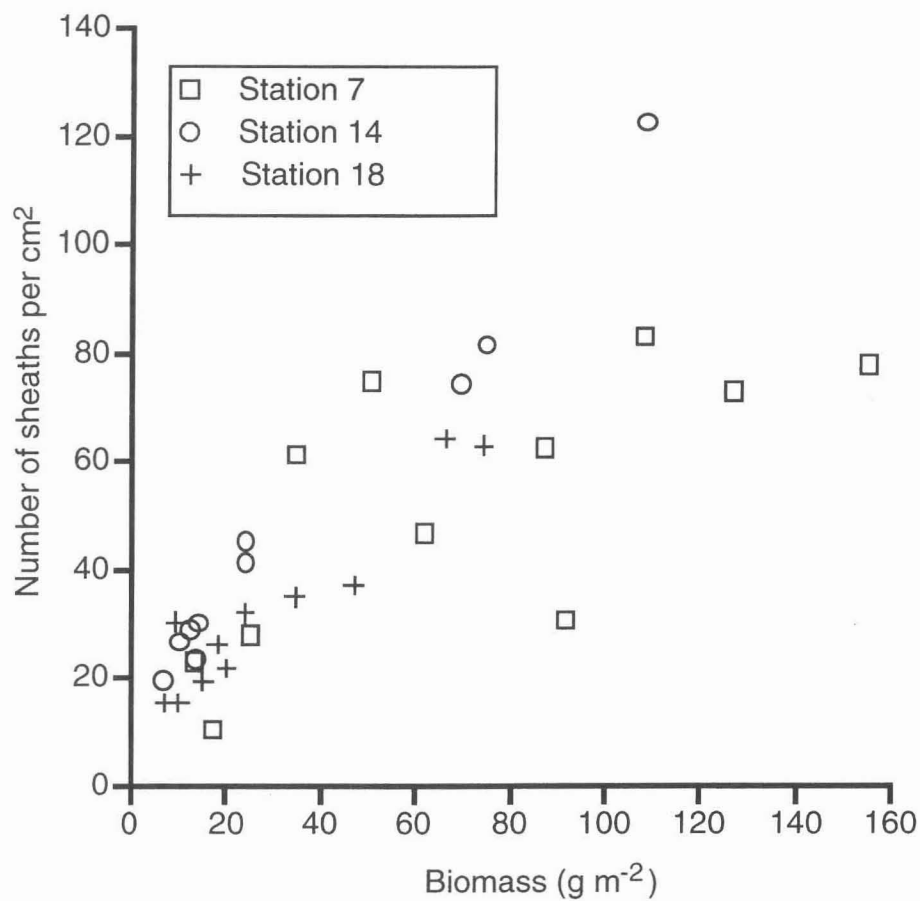


Fig. 5.10 Relation between the total number of *Thioploca* sheaths per cm² (y-axis) and total biomass of *Thioploca* spp. in g wet weight m⁻² (x-axis) for the three shelf stations.

5.1.6 Acknowledgments

We would like to thank the Captain of the RV Kay Kay, Miguel Monné, and the crew, Sergio Marileo and José Camaño, for their cooperation and helpfulness onboard, as well as the staff and colleagues at the University of Concepción and the Station for Marine Biology in Dichato, especially Miguel Torres, Mario Baltazar, Jaime Henríquez and Gonzalo Cid. Special thanks are owed to Paula Urrutia, Vanessa Madrid and Anyola Vega for uncountable favors. We also thank Volker Brüchert for valuable comments on the manuscript. Observations during "El Niño" were partly supported by FONDECYT Project # 1971336 and CONICYT's FONDAP-HUMBOLDT program. The study was supported by the Max Planck Society, Germany, the University of Concepción, and the German Academic Exchange Service (DAAD).

5.2 Two Morphotypes of marine *Thioploca* spp. observed off the coast of Chile

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5.2.1 Abstract

Filamentous sulfur bacteria, *Thioploca*, occur at high biomass in continental shelf sediments along Chile and Peru. They oxidize sulfide with nitrate, accumulated in a central vacuole and form bundles of trichomes in mainly vertically oriented sheaths of several centimeters length. During a seasonal study of the *Thioploca* community off the coast of Chile, an undescribed morphological form of thioplocas with very short cells was frequently observed. The ratio of trichome diameter to cell length separated the new morphotype from the known thioplocas, which have more cylindrical cells. A comparison of ca 800 bp partial 16S rDNA sequences of both morphotypes revealed that the short-cell trichomes comprise separate phylotypes distinguished from, but also clearly related to, the more homogeneous group of known *Thioploca* species. From 10 % to 95 % of the observed *Thioploca* trichomes were of this unusual morphology, the highest frequency was found in near-shore sulfide-rich sediments. They live preferably at 5 - 10 cm depth in the sediment, in contrast to the long cell thioplocas, which have highest density at the sediment surface. This suggests a difference in physiology between the short-cell and long-cell morphotypes.

5.2.2 Introduction

In 1972, marine *Thioploca* spp. were discovered along the coast of Chile and Peru (GALLARDO, 1977; ROSENBERG et al., 1983). These populations occurred at high biomasses and are the dominant benthic organisms in the Bay of Concepción and the adjoining shelf area (GALLARDO, 1977). Two marine species of *Thioploca* are recognized: *T. araucae* with filament diameters of 30 to 43 μm and *T. chileae* with diameters of 12 to 20 μm (MAIER and GALLARDO, 1984). Morphologically, *Thioploca* spp. are very similar to *Beggiatoa* spp., as both form multicellular filaments (trichomes) that shine white due to elemental sulfur inclusions. The main criteria to distinguish the two genera are that *Beggiatoa* spp. are free-living, whereas *Thioploca* spp. form bundles of filaments in a common sheath (LAUTERBORN, 1907). The morphological similarity of the two genera is consistent with a close phylogenetic relationship according to 16S rDNA sequences (TESKE et al., 1995). Attempts to isolate *Thioploca* spp. into pure culture have failed so far.

The marine *Thioploca* spp. concentrate nitrate to 0.5 M (FOSSING et al., 1995) in a central vacuole, that occupies > 90 % of the cell (MAIER et al., 1990). They appear to gain energy by the oxidation of sulfide to sulfur and sulfate with nitrate (FOSSING et al., 1995), which is reduced to ammonium (OTTE et al., manuscript submitted). They incorporate ^{14}C -labeled CO_2 and acetate and may be facultative autotrophs capable of mixotrophic growth (OTTE et al., manuscript submitted).

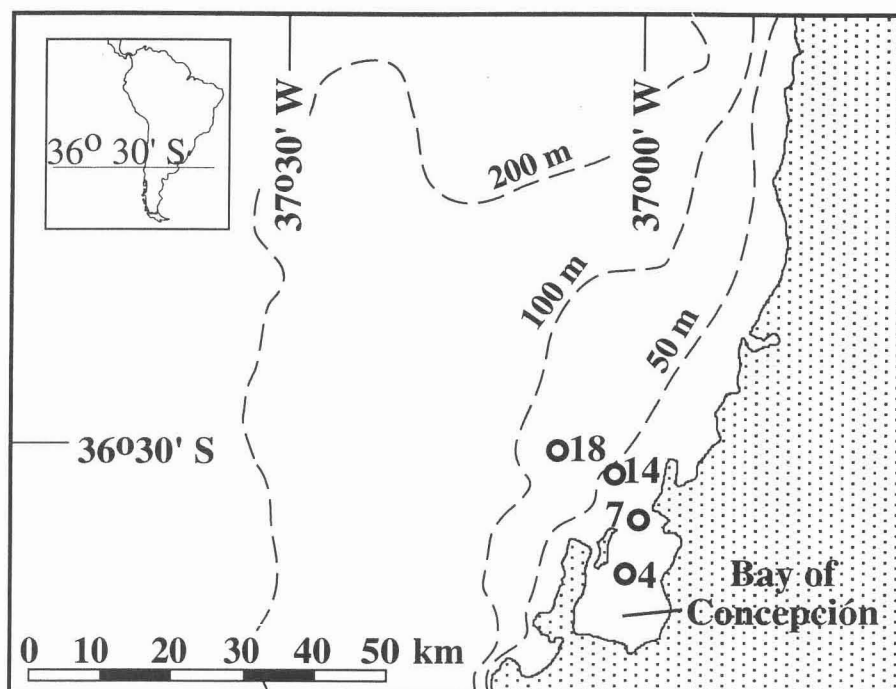


Fig. 5.11 Map of the sampling area showing the Bay of Concepción (Chile) and the adjoining shelf area. The four stations are marked with open circles. The dashed lines (isobaths) indicate water depths.

The marine *Thioploca* spp. seem to outcompete unicellular sulfide oxidizers by using nitrate as the only available electron acceptor, which they spatially separate from the sulfide. As the sheaths of *Thioploca* spp. are oriented mainly vertically (FOSSING et al., 1995; SCHULZ et al., 1996), they enable the filaments to commute between the sediment surface where nitrate is available in the overlying seawater and deeper parts of the sediment where

high sulfate reduction rates produce abundant hydrogen sulfide. Thus, by moving in an oriented manner within the sediment *Thioploca* spp. are able to take up and store both electron acceptor and electron donor (JØRGENSEN et al., in press; JØRGENSEN et al., submitted).

The typical morphology of *Thioploca* cells is cylindrical or slightly barrel shaped. During a seasonal study of the *Thioploca* community off the Bay of Concepción, Chile, we observed another morphotype with much shorter and rounded cells. These *Thioploca* filaments also seemed to occupy another ecological niche than the normal morphotype with longer cells.

5.2.3 Materials and Methods

Sampling Sediment samples for measuring filament diameters and cell lengths were obtained on four stations during 12 cruises between January 1996 and February 1997 onboard the Chilean research ship, Kay Kay, of the University of Concepción. Station 4 (36° 38' 8" S, 73° 02' 3" W) and Station 7 (36° 36' 5" S, 73° 00' 6" W) were within the Bay of Concepción at 24 and 32 m water depth, and Station 14 (36° 32' 1" S, 73° 03' 0" W) and Station 18 (36° 30' 8" S, 73° 07' 6" W) in the adjoining shelf area at 64 and 88 m water depth (Fig. 5.11). At each station, three cores of 8 cm diameter were taken by a small Rumohr gravity corer. During most of the study, oxygen concentrations in the bottom water were extremely low ($< 2 \mu\text{m}$), but in January 97 and during the winter months, July - September 96, oxygen concentrations rose to $25 \mu\text{m}$. Bottom water temperatures were 11° to 12 °C.

Processing of samples Subsamples were taken from the gravity-cores onboard the ship with Plexiglas tubes of 3.6 cm inner diameter and 30 cm length. They were stored in the laboratory at 5 °C for up to 7 days with the upper stopper removed. Sediment cores were extruded from the tubes and placed on a slightly tilted surface. The sediment around the sheaths was washed away carefully with sea water from a squirt bottle starting at the bottom of the core. One cm of sediment was consecutively washed away and the exposed sediment was searched for sheaths of *Thioploca* using a binocular microscope at 16 × magnification. At each depth interval, five sheaths were randomly picked for observation under the microscope. In each sheath, the

diameter of one filament and the length of 5 to 10 cells of this filament were measured at 1000 × magnification.

Sequencing Sheaths containing filaments of *Thioploca* spp. were taken from sediment sampled in March 1998 as described before. These cores were kept in a tank with 300 l of oxygen-poor sea water at 12 °C with addition of nitrate and slow circulation of the water. Due to this treatment, the filaments of *Thioploca* could be kept viable and growing until they were processed four months later. The diameter and the length of the cells were measured as described above and the sheaths were washed in DNA free water. Each sample prepared for sequencing contained a single sheath with filaments of similar diameter and cell morphology. The sheaths were placed in 10 to 20 µl of DNA free water. As the filaments could not be separated completely from the bacteria attached to the sheaths, the sequence of an in situ hybridization probe, specific for *Thioploca* and *Beggiatoa* (probe 829) (TESKE et al., 1995) was used as primer in the PCR reaction. By this method, only sequences belonging to the genus *Thioploca* or *Beggiatoa* were amplified in the PCR reaction and the products were clean enough to be sequenced directly without further cloning.

Comparative sequence analysis The new partial sequences of 805 bp were added to an alignment of about 5,300 homologous bacterial 16S rRNA primary structures (MAIDAK et al., 1999) by using the aligning tool of the ARB program package (STRUNK and LUDWIG, 1998). Aligned sequences were inserted within a stable tree by using the parsimony ARB tool (STRUNK and LUDWIG, 1998) that enables a reliable positioning of new sequences without allowing changes of the overall tree topology (LUDWIG et al., 1998).

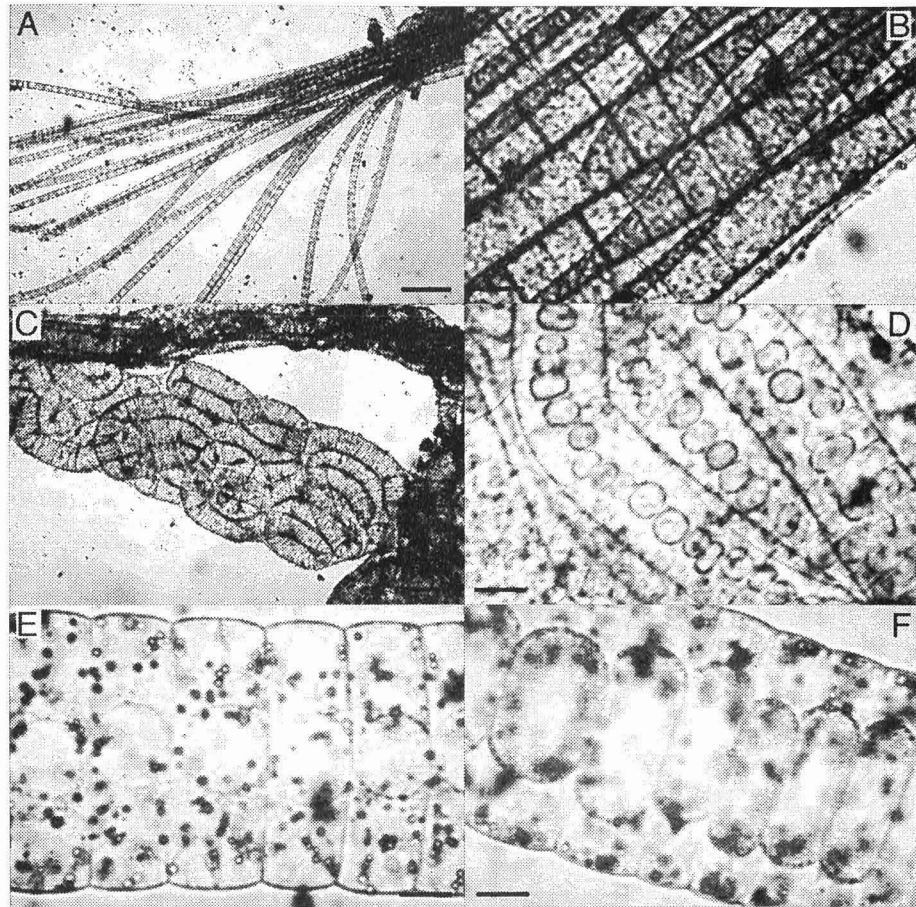


Fig. 5.12 Light micrographs of *Thioploca* filaments. (A) Bundle of *T. araucae* filaments with normal long cell morphology protruding from a sheath. (B) Trichomes of the long-cell morphotype, showing the cylindrical cells. (C) Sheath filled with trichomes of the short-cell morphotype, with some of them turning around within the sheath (D) Bundle of trichomes of the short-cell morphotype showing a similar roundish structure in each cell. (E) and (F) Single trichomes of the short-cell morphotype with barrel-shaped cells and internal round structures of different sizes. The Scale bars in (A) and (C) represents 100 μm , in (B) and (D) 25 μm and in (E) and (F) 10 μm .

5.2.4 Results

Differences in morphology The classical morphotype of *Thioploca* has straight filaments of cylindrical cells that appear square in the light microscope (Fig. 5.12 A and B). The new morphotype, in contrast, is much more flexible, protruding trichomes are not as straight and they have short cells with rounded sides (Fig. 5.12 C - F). In the large filaments of this short-cell morphotype, round structures of various sizes were frequently observed within the cells (Fig. 5.12 D - F). To the naked eye, inhabited sheaths of the short-cell morphotype appeared less white than those of the long-cell morphotype, although in the microscope the trichomes did not seem to contain less internal sulfur globules.

At station 7, 14 and 18 very large trichomes (84-217 μm diameter) were occasionally observed deeper in the sediment (5-21 cm depth). All of these trichomes were sheathless and had ratios of cell length to diameter below 0.3. In some of these filaments several of the round inner structures could be observed in each cell.

Cell lengths and diameters The diameter and cell length of nearly 5,000 filaments were measured during 14 months. Their ratios of cell-length to filament diameter separated into two groups in accordance with two distinct morphologies, one with shorter cells (Fig. 5.13, hatched bars) and one with longer cells (Fig. 5.13, white bars). The short-cell morphotype had a ratio of cell length to diameter of ≤ 0.48 , i.e. the diameter of the filament was more than twice the cell length, whereas the long-cells morphotype showed ratios > 0.48 , thus appearing more square in shape.

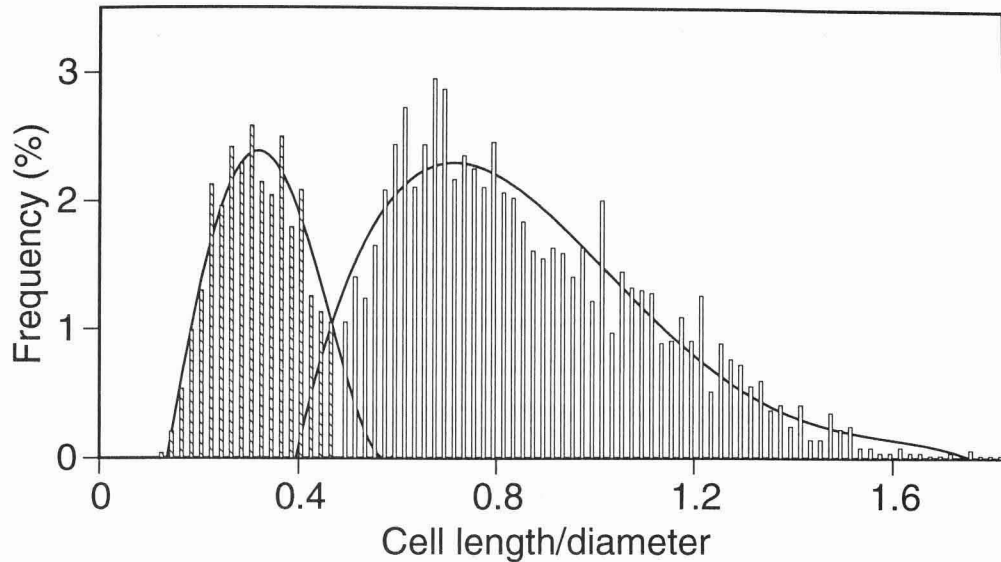


Fig. 5.13 Ratio of cell length to trichome diameter measured in 4848 randomly chosen filaments. The frequency of the ratio per class (0.02) is expressed in percentages of all measured ratios. Hatched bars represent short-cell filaments with a length : diameter ratio ≤ 0.48 and white bars normal filaments with a ratio > 0.48 . The black lines show curves fitted for the values of each group extrapolating into the area of overlap.

Diameter distribution The frequency of diameters in the long-cell morphotype (Fig. 5.14 A) showed three separated groups, that correspond to the three marine species of *Thioploca* (MAIER and GALLARDO, 1984; TESKE et al., 1995; SCHULZ et al., 1996): "*T. marina*" (3-6 μm), *T. chileae* (12-22 μm), and *T. araucae* (28-42 μm). The group of *T. chileae* did not show a Gaussian distribution, but had two peaks, one at 16-18 μm and a smaller at 20-22 μm , while *T. araucae* had one peak at 34-36 μm . "*T. marina*" occurred less frequently and usually in mixed bundles together with one of the two larger species. Also the short-cell morphotype separated into different groups of diameter (Fig. 5.14 B), but these were not clearly separated. Peaks occurred at 16-18 μm and 26-28 μm . Many filaments were $>36 \mu\text{m}$ diameter gradually tailing off at larger diameters.

16S rDNA sequences Twelve partial 16S rDNA sequences were obtained for seven *Thioploca* of the long-cell morphotype and five of the short-cell morphotype and inserted into a phylogenetic tree (Fig. 5.15). All *Thioploca* sequences affiliated with the single nearly complete 16S rRNA gene sequence of *T. ingrica*. Within the group of *Thioploca* and other nitrate-storing sulfur bacteria two phylogenetic branches appeared. One comprised *T. chileae*, *T. araucae* plus all sequences of the long-cell morphotype (and one sequence of the short-cell morphotype with a trichome diameter of 21 μm) and a sheathless filament from the Bay of Concepción (TESKE et al., in press). Most of the long-cell morphotypes with diameters between 15 and 22 μm (L15-1, L15-2, L19, L21 and L22) shared nearly identical sequences with *T. chileae* while a trichome of 34 μm (L34) and a trichome of 17 μm (L17) was closely related to *T. araucae* as well as the sheathless trichomes from the Bay of Concepción. The second branch was comprised of the large (84 μm) *Beggiatoa* sp. of Monterey Canyon (AHMAD et al., 1999), *Thiomargarita namibiensis* (see chapter 5.4) and the short-cell morphotype sequences.

Spatial distribution The two morphotypes were differently distributed in the sediment (Fig. 5.16). In the group of the long-cell morphotype, almost 30 % of the observed filaments were found at the sediment surface. The frequency of filaments declined exponentially with depth down to 18 cm within the sediment and 97 % of the filaments were found in the upper 10 cm (Fig. 5.16 A). The filaments of the short-cell morphotype were found most frequently at 6-7 cm depth and occurred down to 24 cm in the sediment (Fig. 5.16 B) and > 30 % of the filaments were found below 10 cm.

About 25 % of all filaments observed were of the short cell morphotype, however their abundance differed among the stations (Fig. 5.17). At Station 4, in the Bay of Concepción, > 90 % of the filaments in sheaths were of the short-cell morphotype, while at Station 18, some 20 km off the coast, only 10 % were of the short-cell type. At Station 7 and 14, near the mouth of the bay (Fig. 5.11), one third of thioplocas belonged to the short-cell group. Thus, the frequency of the short-cell morphotype declined with increasing water depth.

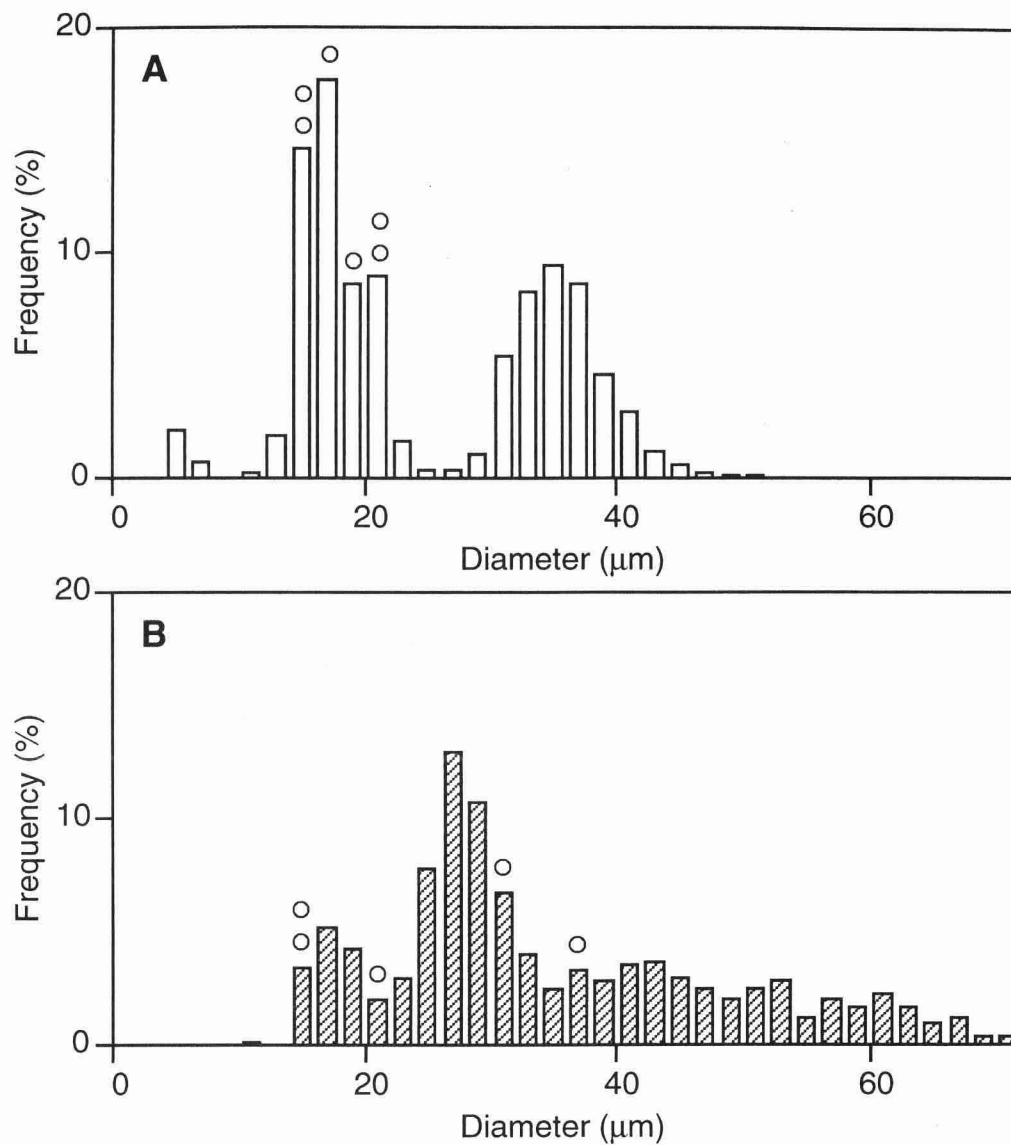


Fig. 5.14 Distribution of filament diameters for long-cell (A) and short-cell (B) *Thioploca* trichomes expressed as percent frequency per class (2 μm). The circles indicate the diameters of filaments used for 16S rDNA sequencing. The total number of observation was 3461 for long-cell and 1387 for short-cell trichomes.

5.2.5 Discussion

Morphological differences The most obvious feature of the new morphotype of *Thioploca*, is that the cells are much shorter than previously described for that genus. The ratios of cell length to filament diameter, clearly separates the marine thioplocas into two morphotypes (Fig. 5.13). Thus, there is not a continuous transition between the two morphological forms. However, there is an area of overlap between the two groups (ca. 0.40 - 0.56) where a clear classification is not possible based on cell length : diameter ratio. Cells of the new morphotype also have roundish sides. Although new for thioplocas, this particular morphology occurs regularly in *Beggiatoa*, especially in the wide forms (NELSON et al., 1989; LARKIN and HENK, 1996). This confirms the close affiliation of the two genera (TESKE et al., 1995).

The single trichomes of the short-cell morphotype appeared less rigid when outside of their sheath than those of the long-cell morphotype (Fig. 5.12 A). This observation and the presence of intracellular spheres of different sizes in the short-cell morphotype (Fig. 5.12 D-F), could indicate that the vacuole in the short-cell morphotype is under development or that the filaments have lower turgor pressure, e.g. due to low internal nitrate concentrations. However, the large vacuolated *Beggiatoa* spp., found at hydrothermal vents of the Guaymas basin, resemble in morphology the short-cell *Thioploca* spp. (NELSON et al., 1989), yet they store nitrate in concentrations comparable to the long-cell morphotype (MCHATTON et al., 1996). Internal nitrate was also measured in filaments of the short-cell morphotype, although in lower concentrations, than in the long cell morphotype of *Thioploca* spp. (J. ZOPFI, pers. com.) Therefore, a high internal nitrate concentration does not necessarily result in the rigid morphological form of the long-cell morphotype. It seems more likely that the short-cell morphotype has more flexibility due to the rounded cells and the higher proportion of joints in a filament. As a result of their larger flexibility, the filaments of the short-cell morphotype are even able to turn around within the sheath (Fig. 5.12 C), whereas filaments of the long-cell morphotype are always unidirectional and can only reverse the direction of their gliding movement.

The long-cell morphotype of *Thioploca* separated into three groups of diameters (Fig. 5.14 A), which corresponds to the three species previously observed on in the Chilean shelf (MAIER and GALLARDO, 1984; TESKE et al., 1995; SCHULZ et al., 1996). The trichomes defined as *T. chileae* (14-22 μm) did not show a Gaussian distribution, although the high number of observations should suffice statistically to reveal a bell-shaped curve, as was found for the *T. araucae* group (30-44 μm) (Fig. 5.14 A). This could indicate, that there are two slightly different groups of *T. chileae* overlapping in their trichome diameter, although this was not revealed by 16S rDNA sequencing (Fig. 5.15). A clear distinction of diameter groups was not possible for the short-cell morphotype (Fig. 5.14 B). Thus, the trichome diameter is a less useful character to distinguish species of the short-cell morphotype.

Phylogenetic relation The analysis of the 16S rDNA sequences showed that the short-cell morphotype of *Thioploca* is phylogenetically distinct from, but closely related to the known long-cell *Thioploca*. Two of the short-cell morphotype sequences we investigated were closely related to beeggiatoas living at hydrothermal vents (AHMAD et al., 1999), while others affiliated with the recently discovered *Thiomargarita* from Namibia (SCHULZ et al., in press, chapter 5.4.) or the smaller *Thioploca ingrlica* (3 - 4 μm) from Randersfjord in Denmark (TESKE et al., 1995) (Fig. 5.15). It seems that only the long-cell morphotype of *Thioploca* is a monophyletic branch with very similar sequences congruent with a stable morphology, while the rest of the nitrate-storing marine sulfur bacteria: short-cell thioplocas, *Thiomargarita* and large vacuolated *Beggiatoa* spp. are more diverse in morphological appearance and 16S rDNA sequence. The sequences of the long-cell morphotype had a high similarity and formed two groups clustering around *T. araucae* and *T. chileae*. The sequence data, thus, confirmed the validity of these two species defined by their trichome diameter. The sequences of the short-cell morphotype were more diverse, which is in accordance with the large variation in diameters. The higher genetic diversity in this group is also demonstrated by the occurrence of single very large trichomes of more than 100 μm in diameter. Yet, one of the short cell bundles possessed a sequence identical with the long cell *T. chileae*. As this occurred only once it might have been an error or an example of a filament of the overlapping zone of the two morphotypes, where a clear separation using the ratio of cell length to diameter is not possible (Fig. 5.13).

Spatial distribution From the spatial distribution of motile bacteria it is possible to gain information about their preferred environment and, thus, their physiological requirements. The bacteria often show a positive chemotaxis towards chemical compounds required for their growth and a negative chemotaxis against harmful conditions. Among the sulfur bacteria, such movements have been demonstrated for *Beggiatoa* (JØRGENSEN and REVSBECH, 1983; NELSON and CASTENHOLZ, 1982; NELSON et al., 1986b), *Thiovulum* (GARCIA-PICHEL, 1989; FENCHEL and GLUD, 1998) and *Thioploca* (HUETTEL et al., 1996). The long-cell morphotype of *Thioploca* is found most abundantly at the sediment surface (SCHULZ et al., 1996) and the density decreases exponentially with sediment depth (Fig. 5.16 A). The long-cell *Thioploca* have a positive chemotactic response towards nitrate and low sulfide concentrations (< 100 μm) and a negative response to oxygen and higher sulfide concentrations (HUETTEL et al., 1996). As nitrate is available at the sediment surface (THAMDRUP and CANFIELD, 1996), their vertical distribution may indicate, that the filaments are drawn by a positive chemotactic movement towards the sediment surface, or that the filaments avoid high sulfide concentrations in the sediment. The latter seems unlikely, as the sulfide concentrations measured in the sediments did not exceed 100 μm (FERDELMAN et al., 1997) (B. STROTMANN, in prep.). In the vertical distribution of short cell trichomes there is a small peak of frequency at the sediment surface and a very pronounced peak at a sediment depths of 5 -10 cm (Fig. 5.16 B). As the short-cell filaments are motile, it can be assumed, that they are attracted by certain chemical conditions at this depth, implicating a difference in physiology towards the long-cell filaments, that accumulate at the sediment surface.

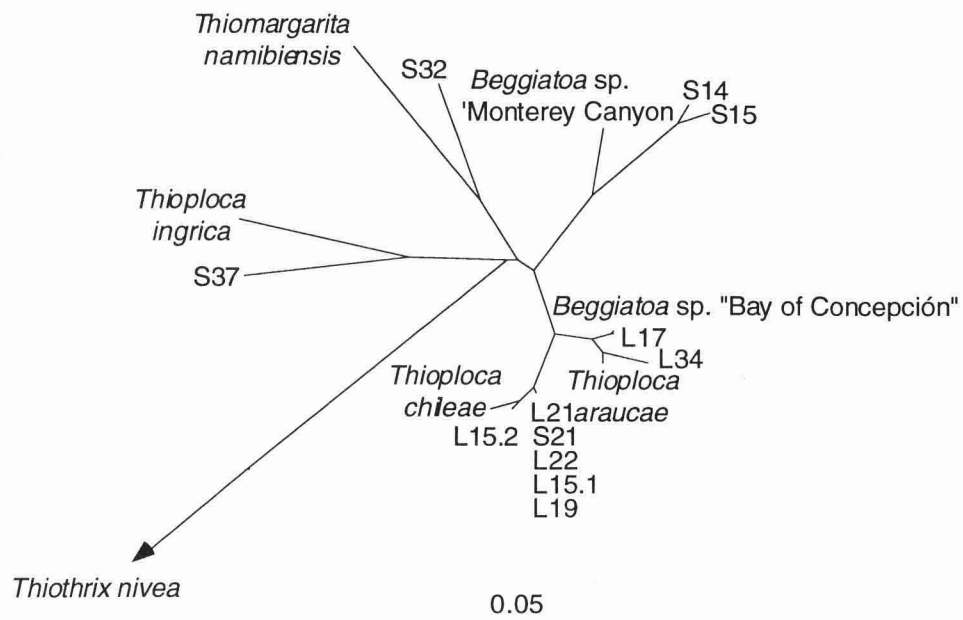


Fig. 5.15 16S rDNA phylogenetic tree showing the affiliation of marine *Thioploca* spp., *Thiomargarita* and *Beggiatoa* sp. from Monterey Canyon based on sequenced fragments of 805 bp (*E. coli* positions from 21 to 825) (BROSIUS et al., 1981). The tree is based on a parsimony analysis including only complete or almost complete 16S rDNA sequences of representative bacteria (MAIDAK et al., 1999). Phylogenetic position of the fragments resulted from the insertion of the aligned sequences into the tree by using the parsimony ARB tool (STRUNK and LUDWIG, 1998) without modifying its topology during the sequence positioning. Partial sequences of *T. chileae* and *T. araucae* (Teske, unpublished sequences) were treated identically as the newly obtained sequences. The bar indicates 10 % estimated sequence divergence.

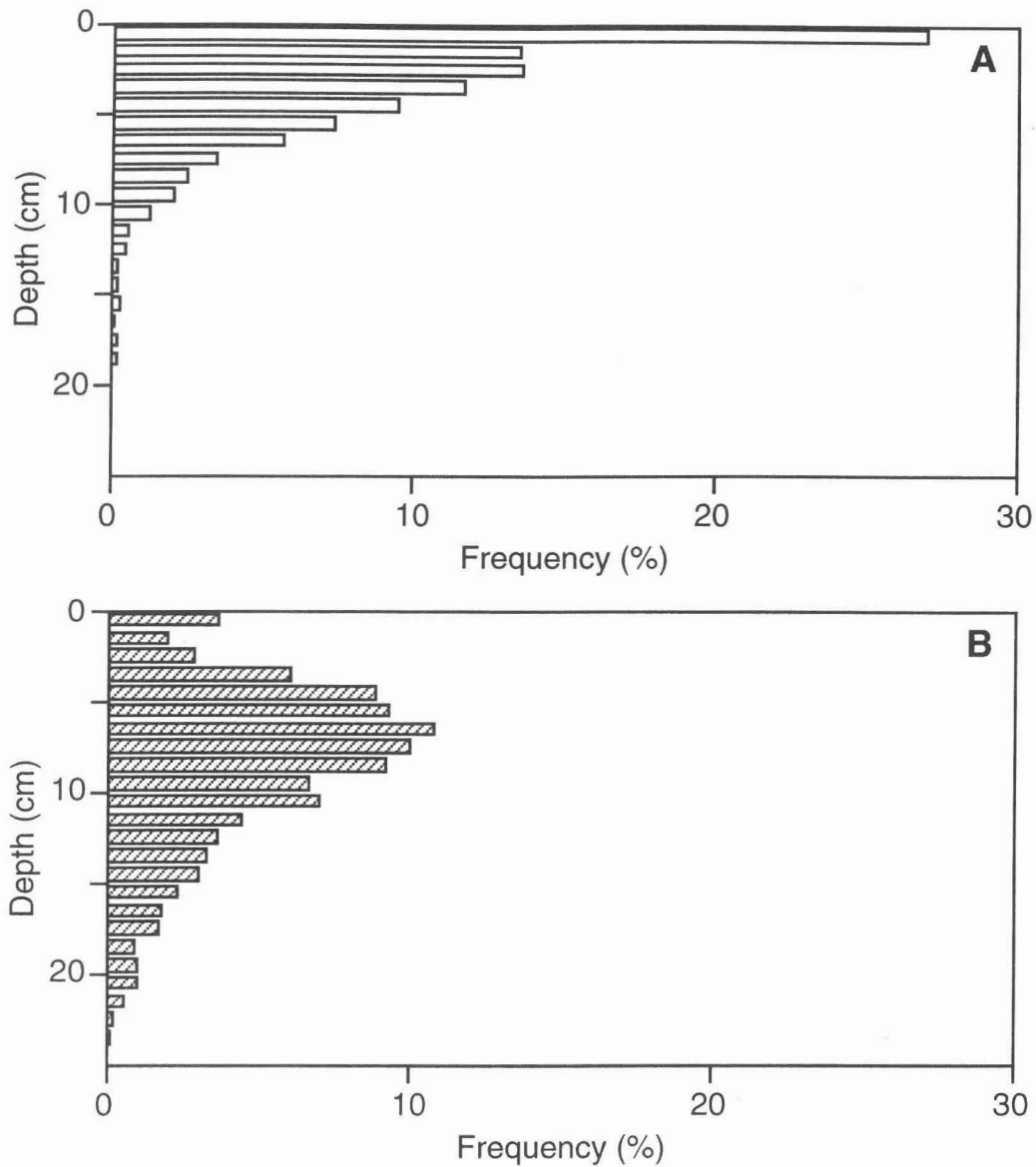


Fig. 5.16 Distribution with depth of the long-cell (A) and the short-cell (B) morphotypes given as frequency per 1 cm depth interval. The total number of observation was 3461 for long-cell and 1387 for short-cell trichomes.

The frequency of short-cell trichomes (Fig. 5.17) increased strongly towards the coast. A reason for this could be that the sulfate reduction rates on the shelf off Concepción generally increase towards the coast (FERDELMAN et al., 1997). Within the Bay of Concepción (Station 4), almost all trichomes were of the short-cell morphotype. However, *Thioploca* filaments occurred only

occasionally there, probably due to toxic sulfide concentrations above 1 mM (FERDELMAN et al., 1997, B. STROTMANN, in prep.). During a survey for *Thioploca* spp. in Namibian shelf sediments, only few *Thioploca* trichomes could be found and all were of the short-cell morphotype. This coincided with high sulfide concentrations, > 400 μm at the sediment surface (T. FERDELMAN pers. com.). Thus, it seems that a high proportion of the short-cell morphotype correlates with high sulfide concentrations.

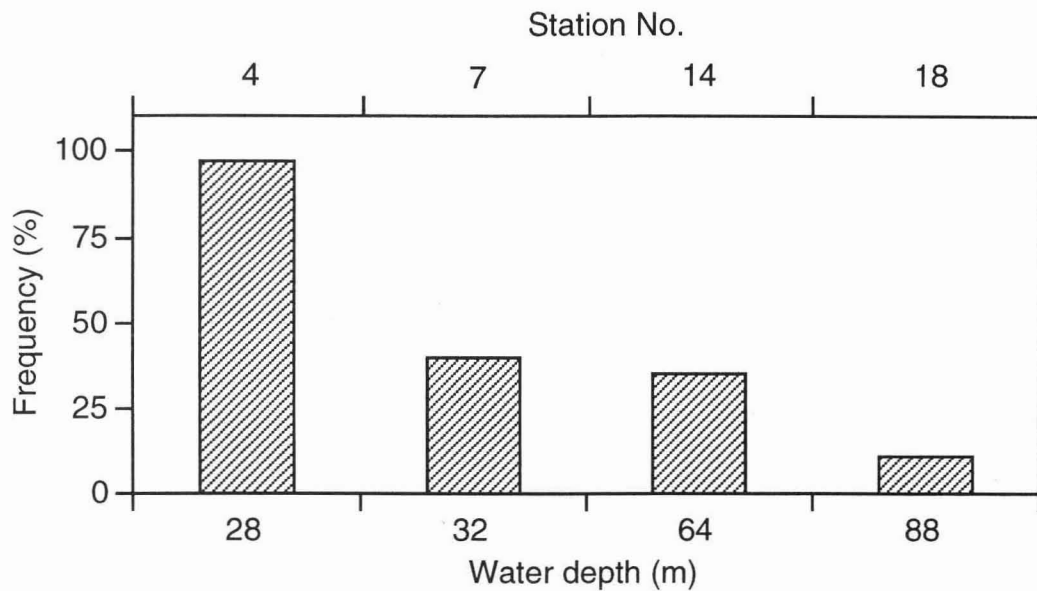


Fig. 5.17 Frequency of the short-cell morphotype of *Thioploca* at four stations. The total number of observation was 4848.

Comparison with *Beggiatoa* spp. The morphological appearance of the short cell morphotype of *Thioploca* is more similar to *Beggiatoa* spp. than the long-cell morphotype (NELSON, 1992; Strohl and Larkin, 1978). The ratio of cell-length : diameter becomes gradually smaller for larger filaments of the short-cell morphotype (Fig. 5.18), which is a tendency that can also be seen for *Beggiatoa* spp. (NELSON et al., 1989; LARKIN and HENK, 1996). The vertical distribution of short-cell filaments with a peak of frequency at 5 - 7 cm depth is in contrast to the accumulation of long-cell filaments at the sediment surface (Fig. 5.16). *Beggiatoa*s are usually reported to occur in a layer on top of the sediment, similar to the long-cell morphotype of *Thioploca*. However, a

Beggiatoa population living in greater sediment depths is less conspicuous than a population on the sediment surface and therefore can be overlooked (NELSON, 1992). An investigation of the depth distribution of *Beggiatoa* spp. in a Danish fjord e.g. showed a maximum number of *Beggiatoa* spp. below the sediment surface (2 - 3 cm) (JØRGENSEN, 1977a). Recent studies of the phylogenetic affinity of *Beggiatoa* spp. with *Thioploca* spp. reveal that the large vacuolated beggiatoas and thioplocas are a monophyletic group within the family of *Beggiatoaceae* (AHMAD et al., 1999; JØRGENSEN et al., submitted). The short-cell morphotype of *Thioploca* seems to be closer related to the vacuolated beggiatoas (Fig. 5.15) than the long-cell morphotype and also affiliates with the morphologically distinct *Thiomargarita* from Namibia. Obviously, the morphological and phylogenetical diversity in the group of nitrate-accumulating sulfur bacteria is higher than has been expected. Altogether, the sequence differences are quite small, so that further studies based on complete 16S rDNA sequences are needed to reveal the details of the phylogenetic relationships between the different morphological forms of the genera *Thioploca*, *Beggiatoa* and *Thiomargarita*.

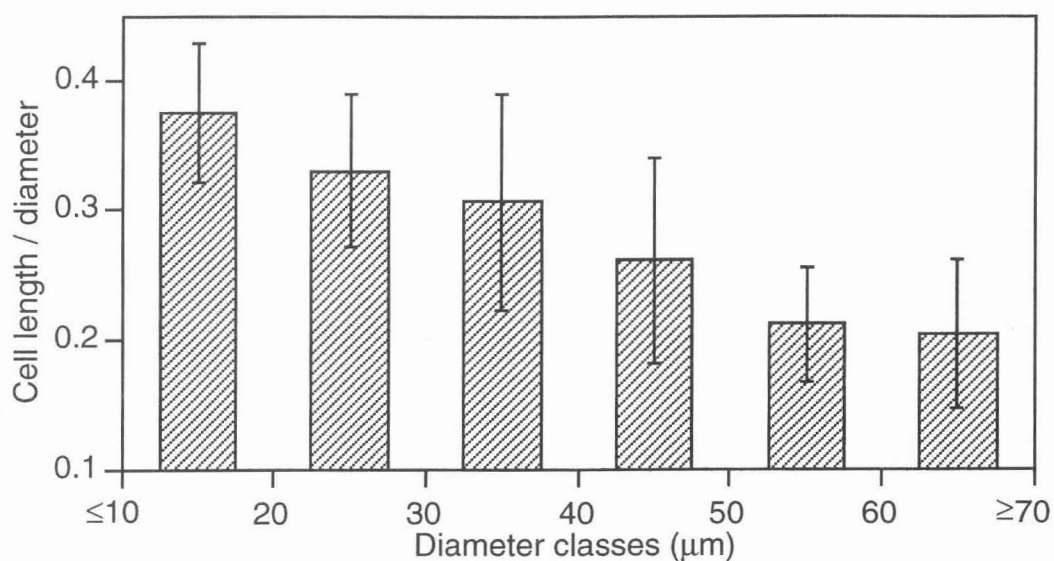


Fig. 5.18 Average ratios of cell length to trichome diameter in short-cell filaments of 6 size classes. Standard deviations are indicated.

5.2.6 Acknowledgments

We would like to thank the Captain of the RV Kay Kay, Miguel Monné, and the crew, Sergio Marileo and José Camaño, for their cooperation and helpfulness onboard, as well as the staff and colleagues at the University of Concepción and the Station for Marine Biology in Dichato, especially Miguel Torres, Mario Baltazar, Jaime Henríquez and Gonzalo Cid. Special thanks are owed to Paula Urrutia, Vanessa Madrid and Anyola Vega for uncountable favors. We also thank Andreas Teske and J. Gijs Kuenen for critical discussions of our results and Andrea Friedrich for helpful comments on the manuscript. The study was supported by the Max Planck Society, Germany, the University of Concepción, and the German Academic Exchange Service (DAAD).

5.3 Ecophysiological studies on partially purified mixed cultures of *Thioploca* species (summary)

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summary

The purpose of the study was to investigate the physiology of the marine *Thioploca* spp. with respect to A) the electron acceptor and the terminal product of its reduction, B) the electron donor and the terminal product of its oxidation, C) the rates at which oxidation and reduction occur, and D) the potential carbon sources of *Thioploca*. Experiments were performed during January and March 1997 at the marine station of the University of Concepción in Dichato, Chile. It was not possible to obtain pure cultures of *Thioploca* species. Therefore, investigations were carried out on whole sheaths with filaments which were taken fresh from natural sediment samples and washed several times in artificial sea water. The whole procedure was anaerobic and without removing the filaments from the water. Since *Thioploca* filaments could not be separated from the bacteria attached to their sheaths, controls with mechanically destroyed *Thioploca* trichomes were done. The filaments were incubated anaerobically in artificial seawater of 11 °C for several hours to days with addition of substrates. At specific time intervals samples were taken for analysis of ammonium, nitrite, sulfide, thiosulfate and sulfate. In addition, uptake experiments were carried out with ¹⁵N-labeled NO₃⁻, ¹⁴C-labeled NaHCO₃ and acetate, and filaments were incubated for microautoradiography. During the washing procedure and the incubation the filaments were kept under dinitrogen atmosphere, cooled and mechanical stress was avoided.

In the initial incubations nitrite accumulated in the medium, which was probably a stress reaction of the *Thioploca* filaments. With improved handling and a modified medium, nitrite concentrations were very low and ammonium accumulated instead. The average rate of ammonium production was around 1 nmol min⁻¹ mg protein⁻¹ and was independent of the sulfide concentrations of the medium. Incubation with ¹⁵N-labeled NO₃⁻ confirmed that ammonium was the major terminal product of the reduction of nitrate, although partial reduction to nitrogen could not be completely ruled out. Produced ammonium was only 50 % labeled indicating that part of the ammonium resulted from reduction of unlabeled nitrate stored in the central vacuole. Nevertheless, the specific label was higher than could be expected, if all labeled nitrate was taken up and diluted internally with unlabeled nitrate of the vacuole. Thus, presumably part of the incorporated, labeled nitrate was directly reduced before reaching the vacuole.

Sulfide oxidation rates were estimated by the rate of disappearance of sulfide in the medium and the accumulation of thiosulfate or sulfate. Sulfate accumulated in the medium with rates of 2-3 nmol min⁻¹ mg⁻¹ protein. Addition of sulfide led to a small accumulation of thiosulfate in the medium while the sulfate accumulation did not change significantly. Nevertheless, addition of thiosulfate instead of sulfide yielded only very low rates. A small accumulation of thiosulfate could also be observed in control incubations, suggesting that it was probably formed by bacteria living on the sheaths. Sulfide was incorporated at an average rate of 5 nmol min⁻¹ mg protein⁻¹. This rate could be increased to 10.7 nmol min⁻¹ mg protein⁻¹ after starvation. The average ratio between sulfide oxidation and ammonia production was 2.2 suggesting that part of the sulfide taken up from the medium was oxidized to elemental sulfur and the other part to sulfate. Both sulfate and ammonium production were not influenced by the sulfide concentration of the medium. These results are best explained if sulfide was first oxidized to elemental sulfur and that in a second independent step elemental sulfur was oxidized to sulfate.

Incubation with ¹⁴C-labeled NaHCO₃ and acetate revealed that both substrates were taken up at similar rates (0.4 nmol min⁻¹ mg protein⁻¹). Addition of sulfide did not increase the uptake rate. Incorporation of acetate and CO₂ by *Thioploca* filaments was confirmed by microautoradiography, showing that the importance of bacteria attached to the sheath was negligible. These results suggest that *Thioploca* spp. are facultative autotrophs capable of mixotrophic growth. ¹⁴C-labeled CO₂ was not formed during incubation with acetate, thus acetate was not used as energy, but only as carbon source.

In conclusion, it could be demonstrated that *Thioploca* spp. are lithotrophic bacteria gaining energy from the oxidation of sulfide to sulfate without significant potential to use thiosulfate. Oxidation of sulfide to sulfur and oxidation of internal sulfur to sulfate seem to be independent from each other. The nitrate stored in the central vacuole is used as electron acceptor and appears to be mainly reduced to ammonium, although alternative reduction to dinitrogen gas cannot completely be ruled out. Thus, the dense populations of *Thioploca* probably do not cause a loss of nitrogen from the sediments. *Thioploca* spp. are capable of fixing CO₂ but alternatively they may also use acetate as carbon source. Rates of sulfide oxidation by thioplocas correspond to 20 - 70 % of the sulfide produced in the natural environment, which

emphasizes the significance of thioplocas for the sulfur cycle in Chilean and Peruvian shelf sediments.

5.4 Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments

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5.4.1 Abstract

A previously unknown giant sulfur bacterium is abundant in sediments underlying the oxygen minimum zone of the Benguela Current upwelling system. The bacterium has a spherical cell that exceeds by up to 100-fold the biovolume of the largest known prokaryotes. Based on 16S rDNA sequence data, these bacteria are closely related to the marine filamentous sulfur bacteria, *Thioploca*, abundant in the upwelling area off Chile and Peru. Similar to *Thioploca*, the new bacteria oxidize sulfide with nitrate that is accumulated to ≤ 800 mM in a central vacuole.

5.4.2 Results and Discussion

Filamentous, nitrate-accumulating sulfur bacteria of the genus *Thioploca* form extensive populations of up to 120 g wet weight per m² along the coast of Chile and Peru (GALLARDO, 1977; FOSSING et al., 1995; SCHULZ et al., 1996). Similar to the South American continental shelf, the shelf off Namibia has strong upwelling with high plankton productivity and oxygen depleted bottom water (HART and CURRIE, 1960; CALVERT and PRICE, 1971; SHILLINGTON, 1998). In a search for *Thioploca* along the Namibian coast, we obtained sediment samples from water depths of ~100 m during a cruise in April 1997 aboard the R/V Petr Kottsov. *Thioploca* and its close relative *Beggiatoa* were present, but only in low numbers. Instead, we discovered large populations of a previously undescribed sulfur bacterium, that occurred at biomasses of up to 47 g m⁻². These giant bacteria grow as a string of pearls, which shine white due to refractive sulfur globules and are large enough to be visible to the naked eye (Fig. 5.19 A). We suggest the new genus and species name, *Thiomargarita namibiensis*, "Sulfur pearl of Namibia", for this organism.

Thiomargarita was found at stations between Palgrave Point and Lüderitz Bay. The highest biomasses were between Cape Cross and Conception Bay. The surface sediment in this area is a fluid, green diatom ooze (BRONGERSMA-SANDERS, 1983; BREMNER, 1983; SCHUETTE and SCHRAEDER, 1981). Oxygen concentrations were low, 0-3 μ M, in the overlying water at all stations, while nitrate was present at 5-28 μ M. Sulfate reduction rates measured by the ³⁵SO₄²⁻ tracer technique were high, 14-76 mmol m⁻² d⁻¹ in the upper 19 cm, and gave rise to high sulfide concentrations of 100-800 μ M in

the upper 3 cm of the sediment. Frequently, also the water directly overlying the sediment smelled of sulfide. Most of the bacteria were found in the top 3 cm of the sediment. The biomass of *Thiomargarita* declined exponentially with sediment depth down to 10 to 14 cm (Fig. 5.20 A).

The giant cells of *Thiomargarita* have many similarities to those of the gliding, filamentous relatives, *Thioploca* (FOSSING et al., 1995; SCHULZ et al., 1996; FERDELMANN et al., 1997; MAIER et al., 1990). *Thiomargarita* also occurred in an oxygen-poor environment with high sulfate reduction rates. Each cell possessed a large central vacuole (Fig. 5.19) in which nitrate was accumulated to a concentration of 0.1-0.8 M. Electron micrographs showed that the cytoplasm was restricted to a thin outer layer of 0.5-2 μm thickness (Fig. 5.19 D and E). The remaining 98% of the biovolume consisted of a liquid vacuole. The bacteria contained sulfur stored in the form of globules, which were situated in the thin outer layer of cytoplasm at a concentration per total biovolume equivalent to 0.4-1.7 M. The depth distribution of biomass in the sediment observed for *Thiomargarita* (Fig. 5.20 A) was similar to that of *Thioploca* off the Chilean coast (SCHULZ et al., 1996). In contrast to the multicellular *Thioploca* and *Beggiatoa*, the cells of *Thiomargarita* were not attached to each other but were evenly separated by a mucus sheath (Fig. 5.19). Motility was not observed. Most of the chains were linear and contained in average 12 cells, but sometimes they branched or coiled together in a ball. Long chains of e.g. 40-50 cells tended to break easily when manipulated.

Most cells had diameters of 100-300 μm (Fig. 5.20 B). Most cells in a chain were of a similar diameter (Fig. 5.20 C), but in some chains, a single cell occurred with a much larger diameter of up to 750 μm . These extremely large forms also occurred as single cells (Fig. 5.19 A). The average *Thiomargarita* with a diameter of 180 μm had a volume of $3 \times 10^6 \mu\text{m}^3$, the largest observed cells had a biovolume of $200 \times 10^6 \mu\text{m}^3$. In comparison, the largest known sulfur bacteria, *Beggiatoa* spp., found at hydrothermal vents in the Guaymas Basin, Gulf of California, can reach diameters of 160 μm (JANNASCH et al., 1989; NELSON et al., 1989). The height of their disc-shaped cells is ca 50 μm and their volume is $1 \times 10^6 \mu\text{m}^3$ per cell. The largest described bacteria, *Epulopiscum fishelsoni*, a symbiont of the surgeonfish (ANGERT et al., 1993), is typically 250 by 40 μm large, but individual cells can reach 600 by 80 μm . This corresponds to a volume of 0.3×10^6 - $3 \times 10^6 \mu\text{m}^3$ per cell.

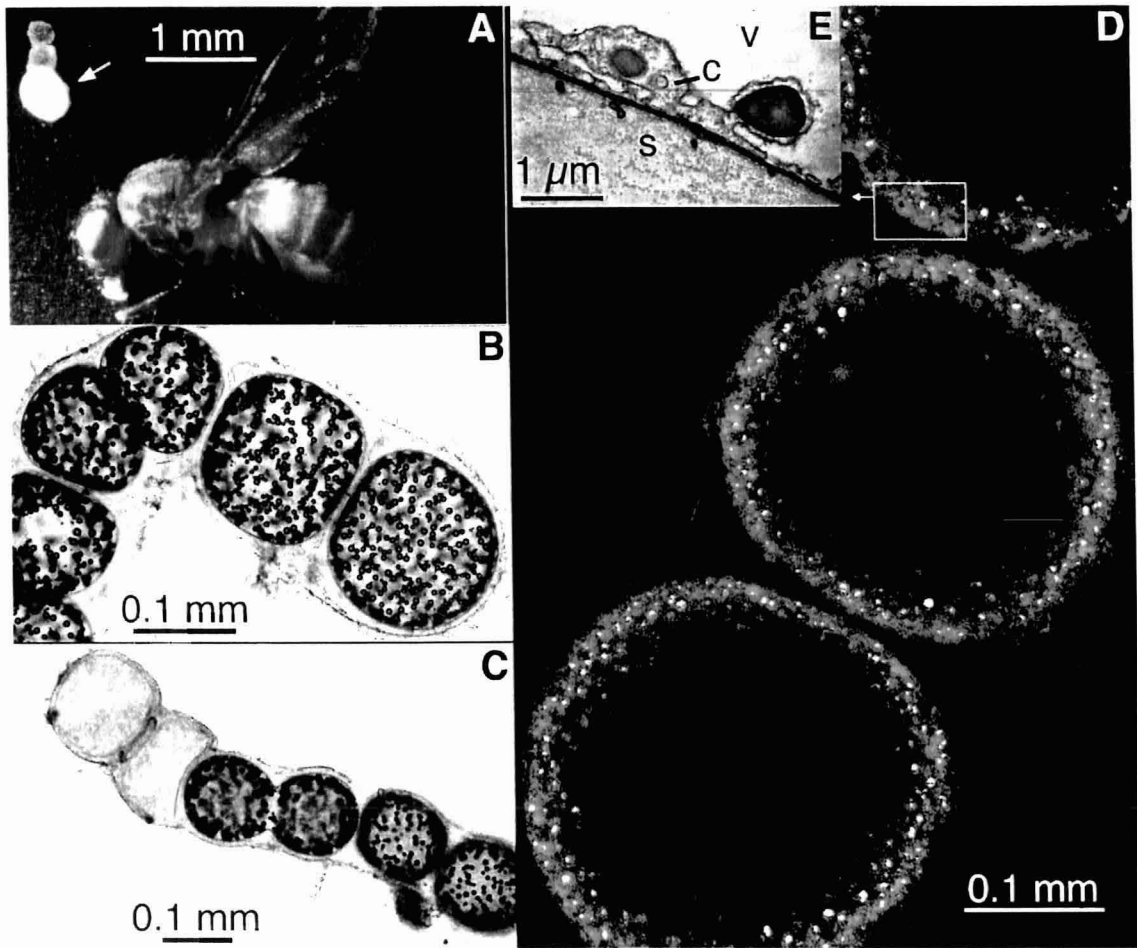


Fig. 5.19 *Thiomargarita namibiensis*. (A) The white arrow points to a single cell of *Thiomargarita*, 0.5 mm wide, which shines white because of internal sulfur inclusions. Above there is an empty part of the sheath, where the two neighboring cells have died. The cell was photographed next to a fruit fly (*Drosophila viriles*) of 3 mm length to give a sense of its size. (B) A typical chain of *Thiomargarita* as it appears in the light microscope. (C) At the left end of the chain there are two empty mucus sheaths, while in the middle a *Thiomargarita* cell is dividing. (D) Confocal laser scanning micrograph showing cytoplasm stained green with FITC and the scattered light of sulfur globules (white). Most of the cells appear hollow due to the large central vacuole. (E) Transmission electron micrograph of the cell wall showing the thin layer of cytoplasm (C), the vacuole (V), and the sheath (S).

The phylogenetic position of *Thiomargarita* was determined by fluorescent in situ hybridization and 16S rRNA sequencing. A hybridization analysis with competitive beta- and gamma-proteobacterial probes (MANZ et al., 1992) identified *Thiomargarita* as a gamma proteobacterium, a bacterial phylum which also harbors *Beggiatoa* and *Thioploca* (TESKE et al., 1995). We then tested *Thiomargarita* with the *Thioploca araucae* and *Thioploca chileae*-targeted probe 829 (TESKE et al., 1995) and found a positive hybridization. This probe was subsequently used as a specific primer to amplify positions 24-828 of the 16 S rRNA gene of *Thiomargarita* (To avoid contamination with other sulfur bacteria, the sheaths of *Thiomargarita* were dissolved using a common washing powder with enzymes and the cells were washed several times in DNA-free water until no other bacteria could be detected microscopically.). *Thiomargarita* was found to be the closest relative to the marine, vacuolated, nitrate-accumulating *Thioploca* species, *T. araucae* and *T. chileae*, thus separating them from the smaller freshwater species, which do not possess large vacuoles (MAIER and MURRAY, 1965) (Fig. 5.21). Apparently, the possession of a large vacuole in connection with intracellular nitrate accumulation is congruent with this phylogeny.

Our attempts to isolate *Thiomargarita* into pure culture have not been successful. The bacteria may survive and grow in the laboratory in samples of their natural sediment for at least a year. Nitrate and sulfide addition led to a doubling of the cell number within 1-2 weeks. Addition of organic substrates such as acetate or glucose had no immediately detectable effect on growth. Although *Thiomargarita* appear to thrive best under low oxygen or anoxic conditions, exposure to atmospheric oxygen levels were not toxic as has been observed for *Beggiatoa* (NELSON et al., 1986b) and *Thioploca* (MAIER and GALLARDO, 1984). *Thiomargarita* showed an unusual ability to survive without growing. Small samples of 15 cm³ fluffy surface sediment collected during an earlier research cruise, that were kept in 80 ml of air-saturated sea water and stored at 5°C without addition of nitrate or sulfide, contained intact cells after more than 2 years. The surviving cells were all rather small with diameters of 50-110 µm occurring singly or in pairs.

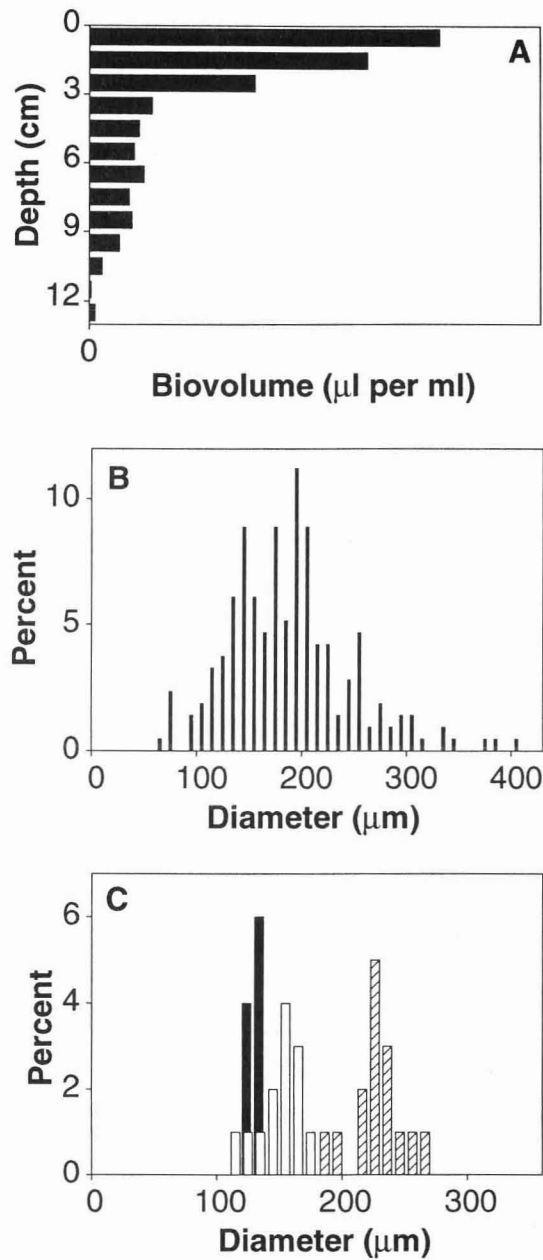


Fig. 5.20 Distribution of biomass and diameters. **(A)** Depth distribution of biovolume of *Thiomargarita* in $\mu\text{l per ml}$. Mean values of three measurements. **(B)** Frequency of diameters of 214 randomly chosen cells. **(C)** Cell diameter distributions in three different chains.

The thickness of the cytoplasm corresponds to the usual small width of bacteria, and its peripheral distribution counteracts a potential diffusion limitation within the cell (LARKIN and HENK, 1989). Since the thickness of cytoplasm is independent of cell size, the ratio of vacuole- to cytoplasm-volume increases with the diameter. By doubling the diameter, the volume of vacuole storage capacity relative to cytoplasm also doubles. The observed potential of *Thiomargarita* to survive nitrate starvation for long periods might, accordingly, be explained by the following calculation: the mean protein content of *Thiomargarita* was 4.5 mg cm⁻³ volume (including the vacuole), less than half of what has been measured for the large, vacuolated *Beggiatoa*, which also accumulate nitrate (MCHATTON et al., 1996). For a nitrate reduction rate of 1 nmol NO₃⁻ min⁻¹ mg⁻¹ protein as observed for *Thioploca* (OTTE et al., manuscript submitted), a *Thiomargarita* cell with a diameter of 180 μm and 0.3 M nitrate stored could survive for at least 40-50 days without taking up nitrate. As the intensity of the upwelling off the Namibian coast frequently changes (HART and CURRIE, 1960; CALVERT and PRICE, 1971; SHILLINGTON, 1998), *Thiomargarita* could survive until sulfide or nitrate appear in higher concentrations and can be stored again for later use.

In most marine sediments, the zones of nitrate and hydrogen sulfide do not overlap. *Thioploca* has developed a strategy to overcome the problem that their electron acceptor and energy source do not coexist. They live in sheaths that allow the filaments to glide up and down and thereby commute between nitrate uptake from the overlying sea water and sulfide uptake within the sulfate reduction zone of the sediment (FOSSING et al., 1995; SCHULZ et al., 1996). The high fluidity and instability of sediments at Walvis Bay (BRONGERSMA-SANDERS, 1983; BREMNER, 1983; SCHUETTE and SCHRADER, 1981), however, seem to prevent *Thioploca* from forming vertical sheaths and establishing dense populations. Instead balloon-shaped sulfur bacteria thrive here. The discovery of *Thiomargarita* expands the range of known adaptations of prokaryotic organisms to a life in sulfide gradients. Whereas motility is a fundamental prerequisite for the filamentous *Thioploca* and *Beggiatoa* (NELSON et al., 1986a; MØLLER et al., 1985; HUETTEL et al., 1996), *Thiomargarita* appear unable to move actively to an environment where its energy source and electron acceptor are optimally supplied. Instead, they may rely on passive transport by external processes such as periodic resuspension

of the loose sediment or on temporal variations in the chemical environment. In accordance with this, it is more resistant to high levels of oxygen and sulfide than are the filamentous relatives, which show a phobic chemotactic response to oxygen (MØLLER et al., 1985; HUETTEL et al., 1996). Both *Thiomargarita* and *Thioploca* face the same ecological challenge: to oxidize sulfide with nitrate, although their two substrates do not coexist. By their solution, to store both nitrate and sulfur, they may successfully compete with faster growing anaerobic sulfide oxidizers, such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*. With *Thioploca*, sulfide and nitrate are spatially separated, and *Thioploca* commute between these two sources. In contrast, *Thiomargarita* only obtain nitrate during occasional sediment resuspension events. Meanwhile they can effectively endure high sulfide concentrations until the next resuspension event occurs.

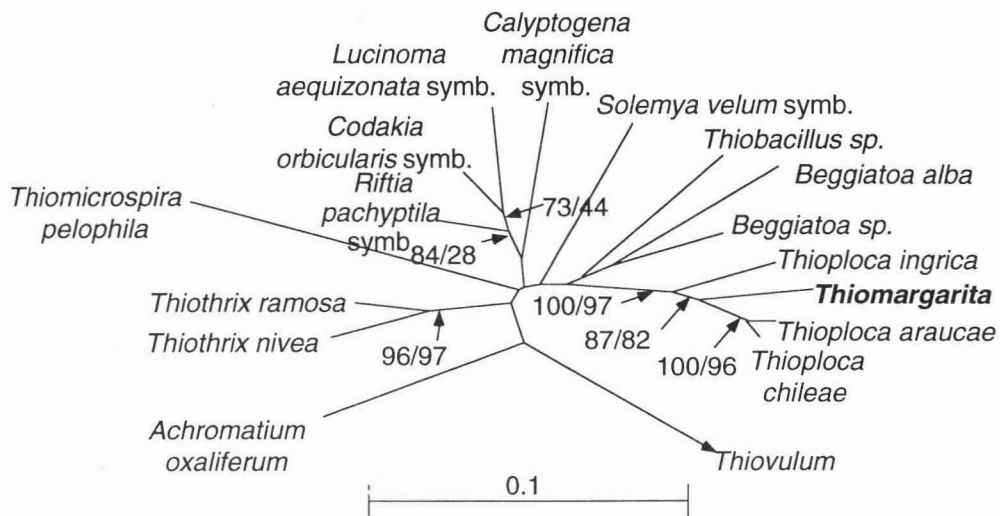


Fig. 5.21 Distance tree of *Thiomargarita namibiensis* and related sulfur-oxidizing bacteria of the gamma-proteobacterial subdivision. The distance tree is based on 16S rRNA position 358-802, which is the overlap of the partial 16S rDNA sequence of *Thioploca araucae*, *T. chileae* and *Thiomargarita*. The tree was rooted with *Thiovulum majus* of the epsilon-proteobacterial subdivision as outgroup. Bootstrap values (200 runs) are given for nodes which have at least 70 % support by distance (first) or parsimony bootstrap (second value). The scale bar corresponds to 0.1 Jukes-Cantor substitutions per nucleotide.

Sulfide production rates are high in coastal sediments around the world, wherever the sediment is rich in organic matter, particularly in upwelling regions (FERDELMANN et al., 1997). The bottom water in these areas is often depleted of oxygen because of intense heterotrophic respiration. As the second-most favorable electron acceptor, nitrate may be used for the oxidation of sulfide. This results in a close coupling of the sulfur and the nitrogen cycles through these specialized sulfur bacteria. *Thioploca* predominates along the Pacific coast of South America, whereas *Thiomargarita* is abundant along the Namibian coast. In both upwelling areas, sediments with extremely high organic content and sulfate reduction rates harbor dense and conspicuous populations of giant sulfur bacteria. However, even the well-known *Beggiatoa*, frequently encountered along the coast, have recently been shown in Baltic Sea sediments to accumulate nitrate (STROTMANN et al., in prep.). These new findings indicate, that a chemolithotrophic coupling of nitrate and sulfide through nitrate storing sulfur bacteria may be a widespread feature of coastal sediments.

5.4.3 Acknowledgments

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5.5 Nitrate storage by marine *Beggiatoa* spp. in Limfjorden, Denmark (summary)

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summary

As described in the previous chapters, it was found, that the impressively dense population of *Thioploca* spp. at the South American West coast and the very large *Beggiatoa* filaments living on hot vents use nitrate instead of oxygen for the oxidation of sulfide (FOSSING et al., 1995; McHatton et al., 1996). Therefore, the question arose, whether this alternative metabolism could also occur in the well known and frequently encountered smaller *Beggiatoa* filaments. A hint for this was, that during a study in Limfjorden, Denmark in 1974 / 1975 *Beggiatoa* filaments of 8 - 17 μm diameter were frequently observed in 2 cm sediment depth where no oxygen was available (JØRGENSEN, 1977a). This observation contradicted the common idea of *Beggiatoa* spp. living exclusively in the narrow overlapping zone of sulfide and oxygen (JØRGENSEN and REVSBECH, 1983; M. MØLLER et al., 1985; D. NELSON et al., 1986a). To address this question, in November 1997 we returned to one of the stations in Limfjorden, sampled in 1975 (Station 5), and took sediment and bottom water samples. For this study bottom water nitrate and oxygen concentrations were measured. In the sediment nitrate and oxygen profiles were measured using microelectrodes, and sulfate reduction rates, sulfide, iron and manganese concentrations were determined. The *Beggiatoa* community was described in terms of vertical distribution and diameter classes, and the internal nitrate concentration of several *Beggiatoa* filaments was measured.

During the time of investigation bottom water oxygen concentrations were around 290 μm and oxygen penetrated 2 mm into the sediment. Nitrate was present in 17 μm concentration, penetrating the upper 0.4 cm of the sediment. The areal sulfate reduction rates of 2.7 $\text{mmol m}^{-2} \text{d}^{-1}$ in the upper 10 cm were comparable to those measured in November 1974 (ca 3 $\text{mmol m}^{-2} \text{d}^{-1}$) and November 1975 (ca 4 $\text{mmol m}^{-2} \text{d}^{-1}$) (JØRGENSEN, 1977b). Highest sulfate reduction rates were found directly at the sediment surface (64 $\text{nmol cm}^{-3} \text{d}^{-1}$) decreasing exponentially with depth to ca 20 $\text{nmol cm}^{-3} \text{d}^{-1}$ at 10 cm. Hydrogen sulfide concentrations were around 1 mM in 10 cm depth decreasing continuously towards the sediment surface. In the upper 2.5 cm no free hydrogen sulfide could be detected. Total biomass of *Beggiatoa* spp. was 14 - 16 g m^{-2} which fits well to an average yearly biomass of ca. 15 g m^{-2} found at Station 5 in 1974 / 1975 (JØRGENSEN et al., 1977a). The *Beggiatoa* trichomes populated the upper 2.5 cm of the sediment, with very few filaments

found in 2.5- 3.3 cm depth. Two peaks of biomass occurred at 0.25 - 0.5 cm and 1.5 - 2 cm depth. Only 17 % of the total biovolume of *Beggiatoa* spp. was located directly at the sediment surface (0 - 2.5 cm). In the upper 2 cm of the sediment filaments of 12 μm diameter were most abundant. Filaments of this size class contained nitrate in 60 - 240 mM concentration (150 mM in average). The smaller *Beggiatoa* spp. (<5 μm), which had populated the sediment surface in 1974 / 75 (JØRGENSEN, 1977a) were found in low numbers in 0 - 1 cm sediment depth and very few in 1 -2 cm depth.

It could be shown, that also *Beggiatoa* filaments of moderate size (12 μm) accumulate nitrate in concentrations comparable to the marine *Thioploca* spp. (Fossing et al., 1995). In the upper 2.5 cm of the sediment, which were populated by *Beggiatoa* spp., no free hydrogen sulfide was detectable, although the sulfate reduction rates were high. This is also the case in Chilean sediments populated by *Thioploca* spp., although thioplocas penetrate much deeper into the sediment (FOSSING et al., 1995). From the biovolume and the average nitrate content of beggiatoas it can be calculated that the upper 2.5 cm of the sediment were 4-5 times enriched in nitrate (75 μm) compared to the bottom water (17 μm) due to the *Beggiatoa* population. If all the sulfide produced in the upper 3 cm of the sediment was oxidized by the beggiatoas, their nitrate pool would be turned over every 3 -4 days, which would equal a nitrate uptake rate of approximately 220 $\text{nmol cm}^{-2} \text{d}^{-1}$. This is in the same order of magnitude as the total flux of nitrate into the sediment of 123 $\text{nmol cm}^{-2} \text{d}^{-1}$, which was calculated from the maximal slope of a nitrate profile measured with a microelectrode. The upper peak of biomass at 0.25 - 0.5 cm occurred in a zone where oxygen was consumed but nitrate was still present, while the second peak at 1.5 - 2 cm was directly above the sediment depth where hydrogen sulfide could be detected. This might indicate, that like *Thioploca* spp. also these nitrate-accumulating *Beggiatoa* spp. shuttle between two sediment depths to take up either nitrate or sulfide, although the distances they would have to overcome are much smaller.

6. Conclusion and Outlook

Most of the "morphologically conspicuous sulfur bacteria" have been described within the last century and the beginning of this century during a time when microbiologists tried to approach the diversity of bacteria with morphological descriptions comparable to approaches in zoology and botany. As indicated by the name, the "morphologically conspicuous sulfur bacteria" are one of the few groups of bacteria mainly defined by morphology. Nevertheless, compared to most other bacteria they are rather large and have a lot of morphological features in addition to size and shape. For most bacteria, morphological descriptions are not very informative. Therefore, microscopic observations on bacteria are less recognized today than they used to be in the beginning of this century, especially when there is no pure culture isolated. In the case of the "morphologically conspicuous sulfur bacteria", however, there are still many new and interesting things to learn on the basis of observation, which is indicated by the discovery of a new genus and a new morphotype of sulfur bacteria in this study (chapter 5.2 and 5.4).

The newest taxonomic approach in microbiology is the comparison of 16S rDNA sequences. This tool has been applied on some genera of the "morphologically conspicuous sulfur bacteria". Obtaining sequences for non-cultivated species is more difficult than for pure culture strains. This has slowed down progress in obtaining secure phylogenetic trees of this group. Clearly, further studies are required to gain a more detailed impression of the phylogenetic affiliations among sulfur-accumulating bacteria. The data obtained until now indicate that the nitrate-storing sulfur bacteria are closely related to each other, regardless of prominent differences in morphology (chapter 5.2).

The ability to accumulate nitrate in a central vacuole occurs in the three genera *Thioploca*, *Beggiatoa*, and *Thiomargarita*, and sets these prokaryotes apart from the rest of the sulfur oxidizing bacteria. The use of nitrate instead of oxygen for the oxidation of sulfide has great ecological importance in coastal marine environments. High sulfate reduction rates which give rise to high sulfide concentrations in the sediment are typically found in eutrophic areas

where the sediment is rich in organic matter. For the degradation of organic matter sulfate is a less favorable but more abundant electron acceptor than oxygen or nitrate. Thus, high sulfate reduction rates occur in the sediment at depths where oxygen and nitrate are depleted. Therefore, most sulfide oxidizing bacteria can only use sulfide once it has diffused up to a zone where nitrate or oxygen are present. Only the nitrate-accumulating bacteria are capable of using sulfide as electron donor and nitrate as electron acceptor without depending on the co-occurrence of the two compounds. This enables them to use this source of energy, although compared to smaller sulfide oxidizers they have relatively low growth rates (chapter 5.1 and 5.3). Sulfide oxidation with nitrate is of great relevance in upwelling areas where dense populations of nitrate storing sulfur bacteria occur (chapter 5.1 and 5.4). Nevertheless, even in less productive coastal areas, the proportion of sulfide oxidized by nitrate-accumulating bacteria might be larger than previously assumed, which is indicated by the preliminary study of Limfjorden *Beggiatoa*s (chapter 5.5).

The "morphologically conspicuous sulfur bacteria" are not only characterized by a certain metabolism. Furthermore, it seems that each genus occupies a particular ecological niche often including an individual "trick" for obtaining their substrates. The microaerophilic *Beggiatoa* spp. and *Thiovulum* have an efficient chemotaxis to orient themselves in the sulfide / oxygen interface. The marine nitrate-storing *Thioploca* shuttle between the sediment surface and deeper parts of the sediment, thus keeping their electron acceptor and electron donor spatially separated. *Thiomargarita* is able to endure high concentrations of oxygen or sulfide and can survive for at least several months without nitrate or sulfide present. Thus, the special adaptation of *Thiomargarita* is to use two substrates that are not necessarily available at the same time.

Much valuable information on several of the "morphologically conspicuous sulfur bacteria" has been obtained by observation, field studies or with the use of purified enrichment cultures. Nevertheless, for some genera of this group there is still a lack of basic information on e. g. abundance, diversity or details of morphology. However, a goal of future studies on "morphologically conspicuous sulfur bacteria" has to be the isolation of these prokaryotes into pure culture in order to study their physiology and biochemistry in more detail.

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