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Community Structure of Filamentous, Sheath-Building Sulfur Bacteria, *Thioploca* spp., off the Coast of Chile

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The filamentous sulfur bacteria *Thioploca* spp. produce dense bacterial mats in the shelf area off the coast of Chile and Peru. The mat consists of common sheaths, shared by many filaments, that reach 5 to 10 cm down into the sediment. The structure of the *Thioploca* communities off the Bay of Concepción was investigated with respect to biomass, species distribution, and three-dimensional orientation of the sheaths. *Thioploca* sheaths and filaments were found across the whole shelf area within the oxygen minimum zone. The maximum wet weight of sheaths, 800 g m^{-2}, was found at a depth of 90 m. The bacterial filaments within the sheaths contributed about 10% of this weight. The highest density of filaments was found within the uppermost 1 cm of the mat. On the basis of diameter classes, it was possible to distinguish populations containing only *Thioploca* spp. from mixed populations containing *Beggiatoa* spp. Three distinct size classes of *Thioploca* spp. were found, two of which have been described previously as *Thioploca araucae* and *Thioploca chileae*. Many *Thioploca* filaments did not possess a visible sheath, and about 20% of the sheaths contained more than one *Thioploca* species. The three-dimensional sheath structure showed that *Thioploca* filaments can move from the surface and deep into the sediment.

Since the first description of the sulfur bacteria *Thioploca* spp. (16), these bacteria have been observed in various freshwater lakes and wells, although never in high amounts (13–15, 19, 20, 30).

In 1977, however, V. A. Gallardo, reported a previously unnoticed, extensive occurrence of *Thioploca* spp. (3) on the seabed. There have been many observations which have shown thick bacterial mats cover the shelf sediments along the coast of southern Peru and northern to central Chile for a distance of 3,000 km. These mats consist of interwoven bacterial sheaths containing up to 100 multicellular filaments called trichomes, and each filament is 10 to 40 μm thick (Fig. 1). The sheaths are usually 0.5 to 1 mm thick and 5 to 15 cm long. The combined wet weights of the filaments and their sheaths can amount to 1 kg m^{-2} (3).

Thioploca spp. are close relatives of *Beggiatoa* spp., as shown by their levels of 16S rRNA sequence similarity (28). The only visible differences between *Beggiatoa* and *Thioploca* trichomes on a microscopic scale are that the latter live in a thick, gelatinous sheath that is shared with other trichomes and frequently have tapered ends. Therefore, the presence or absence of a common sheath has been the principal criterion used to distinguish the two genera (17). *Beggiatoa* spp. are mainly restricted to a narrow zone where O_2 and H_2S overlap (12). However, this restriction apparently does not apply to *Thioploca* spp., which form mats that can be at least a few centimeters thick.

Only two marine *Thioploca* species, *Thioploca araucae* and *Thioploca chileae*, have been validly described (18a). A third species has been tentatively named "*Thioploca marina.*" Differentiation of the *Thioploca* species has been based entirely on trichome diameters. Nonetheless, this characteristic is consistent with the results of recent phylogenetic studies of the rRNA sequences of the two marine species, *T. araucae* and *T. chileae* (28). Although the marine *Thioploca* spp. have been found regularly on the shelf off Concepción, Chile (3, 4, 21, 24a) and along the coast of Peru (7, 22, 25), neither the spatial distribution of the *Thioploca* population nor the abundance of the different species has been investigated previously.

So far, it has not been possible to obtain pure cultures of *Thioploca* spp. The physiological properties of *Thioploca* spp. can therefore be determined only indirectly from observations of natural populations. *Thioploca* spp. are thought to be sulfide-oxidizing organisms because they accumulate elemental sulfur globules in their cells. High nitrate concentrations measured within *Thioploca* cells, as well as the absence of O_2 in the sediment, have led to the hypothesis that the marine *Thioploca* spp. found in the Bay of Concepción might use nitrate as an electron acceptor (24), as has been suggested previously for *Beggiatoa* spp. (27). It seems likely that *Thioploca* spp. could take up nitrate at the sediment surface and transport it down into the sediment to oxidize sulfide at some depth (2). For such a mode of living it is essential that the sheaths reach from the sediment-water interface to the deeper, sulfidic parts of the sediment.

In this study we investigated the structure of the *Thioploca* community, the relative abundance of thioplocas, and the coexistence of the different species. The purpose of this study was to gain a better understanding of the conditions that support the growth of *Thioploca* spp. and what possibilities and constraints the sheaths might provide for the movements of the trichomes.

MATERIALS AND METHODS

Samples were obtained in March 1994 along a transect across the shelf area near Concepción, Chile (Fig. 2), during a joint cruise on board the research vessel *Vidal Gormaz* which included participants from the Centro-Eula (University of Concepción, Concepción, Chile) and the Max Planck Institute for Marine Microbiology (Bremen, Germany). A preliminary investigation of the Bay of Concepción and the adjoining shelf area revealed the approximate distribution of the *Thioploca* community. On the basis of the general distribution pattern, six stations were selected for further investigation; two of these stations were in the Bay of Concepción (station 6 at 36°37′3″S, 73°00′5″W, and station 7 at 36°36′5″S, 73°00′6″W), and four were in the adjoining shelf area (stations 14, 20, 21, and 26 at 36°32′1″S, 73°03′0″W; 36°30′51″S, 73°08′79″W; 36°29′5″S, 73°11′7″W; and

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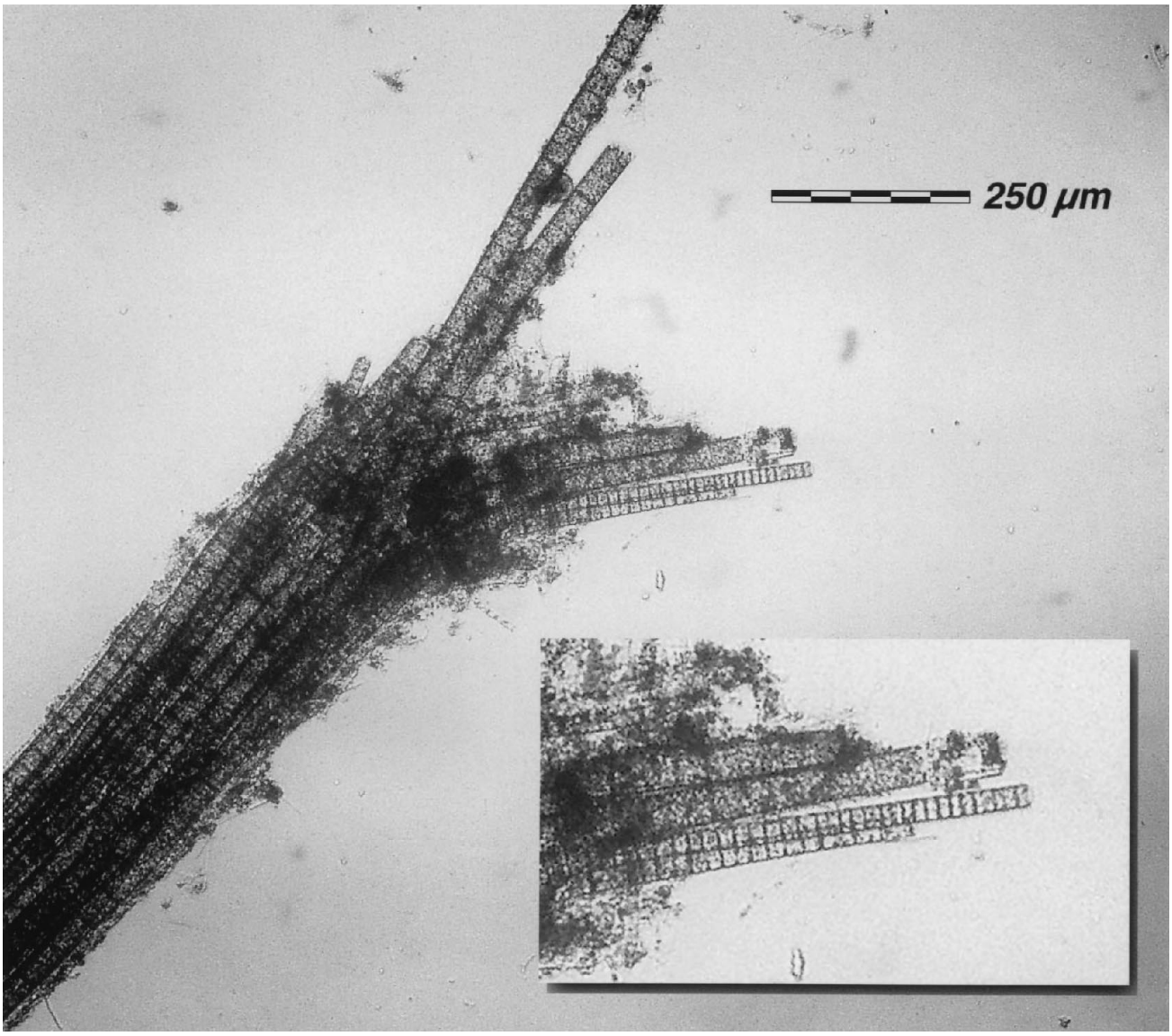

FIG. 1. Bundle of *Thioploca* trichomes in a common sheath with trichomes sticking out the end of the sheath. (Inset) Two large *T. araucae* trichomes and three smaller *T. chileae* trichomes. The black dots at the cell membranes are sulfur globules as they appear during phase-contrast microscopy.

36°25′9″S, 73°23′4″W, respectively). Stations 19 and 21 were in the middle of the *Thioploca* spp. area, while station 6 was at the inner boundary of the area inhabited by *Thioploca* spp. and station 26 was at the outer boundary of the area inhabited by *Thioploca* spp. During the investigation, the oxygen was totally depleted (concentration, <5 μM) in the water column over the area inhabited by *Thioploca* spp. (2). The bottom water temperatures were 11 to 11.5°C.

Biomass. To determine the weight of sheaths with trichomes, three samples were obtained with a multiple corer (inside diameter, 9.5 cm) or subcores were obtained with a box corer, and the samples were processed within 24 h. The cores were cut at 1- or 2-cm intervals starting at the sediment surface down to the depth where no sheaths could be found. *Thioploca* sheaths with trichomes were separated from the sediment by washing the sediment with freshwater through a 500-μm-pore-size sieve. The use of freshwater might have resulted in underestimation of the biomass because of osmotic stress on the filaments, but overestimation was avoided by adding crystallized salt. The sheaths were separated from the benthic fauna, the detrital material, and larger mineral grains with a binocular microscope. The samples were dried for 6 to 10 h at ca. 60°C and stored at −20°C until their dry weight could be determined. Before they were weighed, the samples were dried again for 2 h at 60°C. The ratio of wet weight to dry weight was determined with a single large sample (wet weight, 83 mg).

To measure the biomass of the trichomes alone, subcores obtained from the multiple corer or box corer with Plexiglas tubes (inside diameter, 3.6 cm; length, 20 cm) were stored at 5°C for up to 3 days. The sediment in each core was extruded from the tube and placed on a slightly tilted surface. The silt between the sheaths was then washed away carefully with seawater from a squirt bottle starting at the bottom of the core. After 1 cm of sediment had been washed away, the number of exposed sheaths was counted. In this way the number of sheaths throughout most of the core was recorded at 1-cm intervals initially and in smaller intervals (5, 3, and 2 mm) near the top. At each depth, four to eight randomly chosen sheaths were cut off and inspected with the microscope to assess how many trichomes of the different diameter classes they contained. To calculate the volume of the trichomes, the lengths of exposed sheaths, after 1 cm of sediment had been washed away, were recorded.

The number of sheaths per square centimeter multiplied by the average number of trichomes per sheath, the average diameter of trichomes (which was species dependent), and the average sheath length in the 1-cm interval gave the biovolume of trichomes per sediment volume at a given depth. Thus, the distribution of species was taken into account when the biovolume of trichomes was calculated. The biomass of trichomes was calculated from the biovolume by assuming that the *Thioploca* trichomes have a density of 1 g cm^{-3}.

Spatial orientation. To investigate the spatial orientation of the sheaths within the sediment, subsamples of multiple corer or box corer samples were obtained with copper tubes (inside diameter, 3.2 cm) and rapidly frozen in an isopentane (2-methylbutane) bath cooled with liquid nitrogen to −160°C. Cooling of large objects by immersion in cold isopenthane proceeds more rapidly than cooling by immersion directly in liquid nitrogen, as the nitrogen bubbles formed in the latter

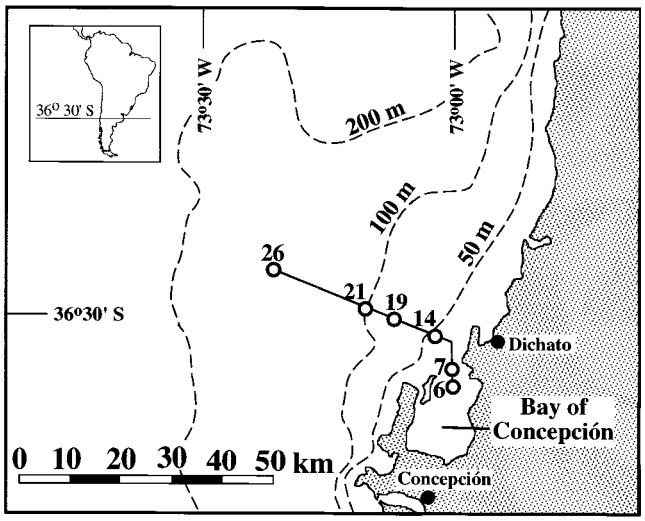

FIG. 2. Map of the area investigated, showing the Bay of Concepción (Chile) and the adjoining shelf area. The six sampling stations are marked with open circles. The dashed lines (isobaths) indicate the water depths across the continental shelf.

case insulate the sample and thus reduce heat conduction to the liquid phase. Before the tubes were frozen, they were closed with metal stoppers at the top, whereas the bottoms of the tubes were plugged loosely with Styrofoam stoppers. The firm, heat-conducting stopper at the top of each tube directed the expansion of ice toward the bottom of the tube to avoid distortions at the sediment surface. The frozen samples were stored for up to 6 months at −20°C.

To generate a three-dimensional picture of the sheaths within the sediment, the frozen cores were sliced perpendicular to the sediment surface in 100-μm increments with a cryomicrotome (−35°C; Microm model HM 505 E). The cores were embedded in a rectangular form with strongly diluted OCT embedding solution (Miles), and pencil leads in the corners served as reference points. After each slice had been cut away, the exposed surface of the remaining core was photographed with a Nikon model 801 camera and Kodak Gold ASA 200 film. Before each picture was taken, the surface was polished with a soft cotton cloth and coated with oil to enhance the contrast between the dark sediment and white *Thioploca* trichomes. The core was illuminated with polarized light, and a polarizing filter perpendicular to the illumination filter was mounted on the camera. This filter combination removed much of the reflected light, which made the light-scattering sulfur globules within the trichomes appear as white objects against a relatively uniform dark background. Since the presence of sulfur globules was important for recognizing sheaths, only those sheaths that were inhabited by *Thioploca* spp. were included in the analysis.

A total of 101 sequential pictures were taken to produce a three-dimensional view of the *Thioploca* sheaths in a 1-cm-thick sediment column. Each picture was scanned into a Macintosh computer by using an Apple, Color One Scanner and a model 6100/60 AV Apple computer and processed with the program NIH Image (Wayne Rasband, National Institutes of Health), a public domain computer program freely available by anonymous FTP from zippy.nimh.nih.gov. The grey tones of the individual pictures were inverted (i.e., white to black), and the pictures were aligned manually by using the reference points. The background intensity of each picture was reduced by subtracting a smoothed copy of the picture from the original. The resulting pictures showed the *Thioploca* filaments as black objects on a relatively uniform grayish background. A three-dimensional projection was produced with NIH Image from a stack of all 101 pictures by making all of the white regions of each image appear transparent. A dark object that continued through several pictures was a sheath filled with trichomes.

The average angles of individual sheaths with respect to the defined surface plane were calculated from the coordinates of points within the path of a sheath. Each angle was calculated from the coordinates of two points separated vertically by 500 μm. The mean and standard deviation were calculated from all of the angles measured in a 1-mm interval.

RESULTS

Biomass. *Thioploca* spp. were found at all stations within the shelf area, starting at the mouth of the Bay of Concepción at a water depth of 34 m and reaching out almost to the edge of the continental slope at a water depth of 122 m (Fig. 3). Near the shelf edge, a reef composed of massive phosphate concretions prevented sampling. At the stations sampled, the total wet weight of *Thioploca* trichomes plus sheath material ranged from 100 to 800 g m^{-2}. The highest values were found in the middle of the shelf area at a water depth of 87 m. The biomasses of filaments alone, not including the surrounding sheath material, were estimated from the biovolumes at stations 7, 19, and 21 and were between 50 and 120 g m^{-2}. These values seemed to follow the pattern of the total wet weights of trichomes with sheaths. A direct comparison in one selected core confirmed that the biomass of the living trichomes was about 10% of the total wet weight of sheath material and filaments, which agrees with the results shown in Fig. 3. The sediments inside the Bay of Concepción were characterized by a well-developed *Beggiatoa* spp. population on the sediment surface which co-occurred with the *Thioploca* population at the mouth of the bay (stations 6 and 7).

The vertical distributions of *Thioploca* trichome biomass calculated from the biovolumes in the sediments were similar at all of the stations investigated. Typically, the biomasses were greatest in the top 0 to 1 cm and decreased gradually below a depth of 1 cm. Many filaments reached down to a depth of 4 to 8 cm (Fig. 4), and occasionally single sheaths containing filaments were found much deeper; the maximum depth observed was 15 cm below the sediment surface. Empty sheaths were observed even deeper in the sediment (depth, >20 cm).

The vertical distribution of sheath material retained after sieving was generally similar to the biovolume distribution except in the top 0 to 1 cm, where sieving retained significantly less material (data not shown). The reason for this discrepancy is probably that large fractions of the thinner sheaths and sheathless trichomes observed near the surface were not retained by sieving. Thus, it is our impression that the biovolume distribution shown in Fig. 4 is a more accurate estimate of vertical filament abundance than are the results from sieving.

Distribution of species. The results of the diameter measurements obtained for randomly selected sheathed *Thioploca* trichomes at stations 7 and 19 are shown in Fig. 5A and B, respectively. All of the trichomes measured belonged to one of three diameter classes, all of which showed a typical Gaussian size distribution. The same three size classes that were found for trichomes within sheaths were also found for sheathless

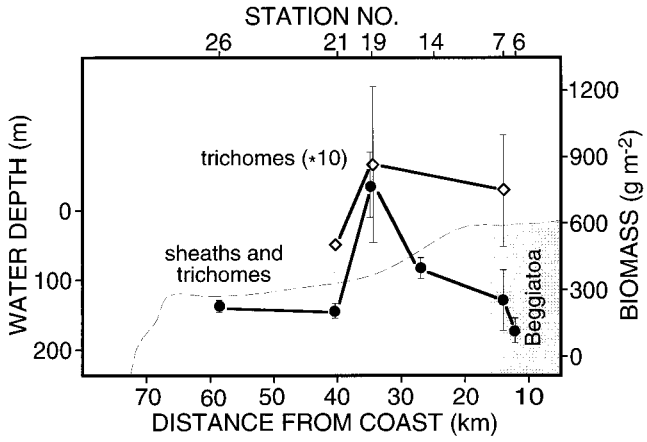

FIG. 3. Biomasses of *Thioploca* trichomes and sheath material with trichomes, expressed as grams of fresh weight per square meter. The biomass of trichomes was multiplied by 10 to fit on the same axis. The dashed line shows the water depths across the continental shelf. The area with *Beggiatoa* spp. in the Bay of Concepción is indicated by dark shading. The *Thioploca* population co-occurred with *Beggiatoa* spp. at the entrance of the bay and was present on the shelf area almost out to the shelf break.

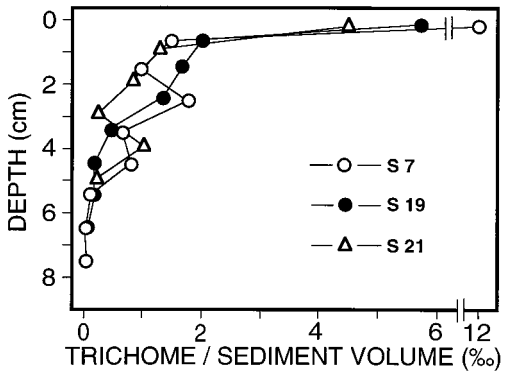

FIG. 4. Distribution of biovolume of *Thioploca* trichomes with depth at stations 7 (S7), 19 (S19), and 21 (S21), expressed as volume of trichomes per volume of sediment. The value at the sediment surface at station 7 was twice the value found at the other two stations. The highest biovolume of trichomes was found in the uppermost 1 cm of the sediment. The biovolume decreased rapidly with depth down to a depth of 4 to 8 cm.

filaments at station 19 (Fig. 5D). However, the sheathless filaments that were <25 μm in diameter at station 7 (Fig. 5C) did not exhibit a size distribution similar to the size distribution of the sheathed *Thioploca* spp. at that station (Fig. 5A), which indicated that other species (e.g., *Beggiatoa* spp.) were present. The two larger *Thioploca* spp. size groups (diameters, 28 to 42 and 12 to 22 μm) corresponded to the two previously described species, *T. araucae* and *T. chileae*, respectively. The smaller filaments (diameters, 1 to 8 μm) were probably "*T. marina*" (18). Occasionally, we observed very large mostly sheathless trichomes with diameters between 50 and 60 μm.

In approximately 20% of the sheaths examined we found more than one *Thioploca* species. In 85% of these sheaths with mixed populations *T. araucae* was numerically dominant. The sheaths with mixed species occurred in the whole area investigated, but their distribution was very patchy. The *T. araucae* population was, altogether, larger in both biomass and number than the *T. chileae* population (Fig. 6). The percentage of *T. chileae* filaments in the sediment samples increased toward the continental slope; the percentages of these filaments were about 7% at station 7, 42% at station 19, and 62% at station 21. A comparison of the diameters of *T. araucae* trichomes living in neighboring sheaths showed that there was more variability in trichome diameter between different sheaths than within the same sheath (Fig. 7), suggesting that the filaments within each sheath had a monoclonal origin.

Spatial orientation. The three-dimensional pictures revealed a layer of thinner and more horizontally oriented sheaths near the sediment surface, while the sheaths underneath this layer extended downward in a more vertical orientation (Fig. 8). The average angles of the sheaths at different depths reflected this general pattern (Fig. 9), although many sheaths changed direction at some depth so that their ends bent back up toward the surface. In the three-dimensional reconstructions (Fig. 8) the horizontal layer at the top appeared to be slightly thicker at station 21 in the center of the *Thioploca* spp. area (8 to 12 mm) than at station 7 at the mouth of the Bay of Concepción (6 to 8 mm). Still, the distribution of average angles (Fig. 9) revealed that below a depth of 6 mm the sheaths turned toward a more vertical orientation at both stations. At station 21 we observed a second horizontal layer underneath the surface (Fig. 8, arrow). This layer was slightly tilted with respect to the surface, and therefore it did not show up very well in the average

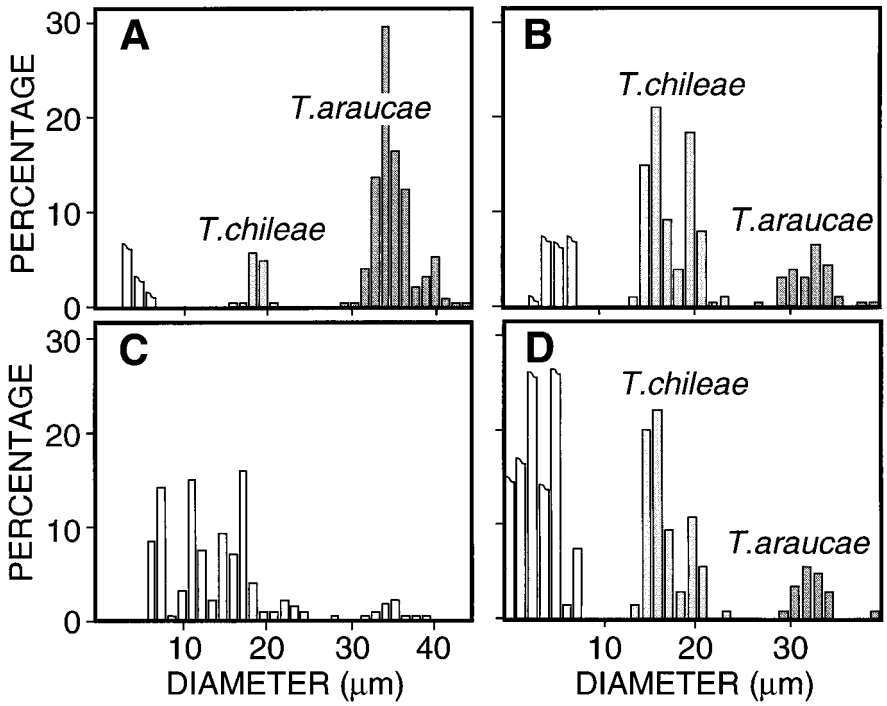

FIG. 5. Distributions of diameters of randomly chosen *Thioploca* trichomes within sheaths at station 7 (A) and station 19 (B) and of trichomes which were not within visible sheaths at station 7 (C) and station 19 (D). The diameter distributions are expressed as percentages of the total population. The bars for the smallest-size groups are open at the top because the values were only estimated and therefore not included in the total percentages. All of the trichomes measured were from the uppermost 1 cm of sediment. At station 7, some of the trichomes outside sheaths (C) were *Beggiatoa* spp. trichomes whose size distribution is different from the size distribution of *Thioploca* trichomes within sheaths (A). At station 19, the trichomes outside sheaths (D) had the same size distribution as the trichomes within sheaths (B).

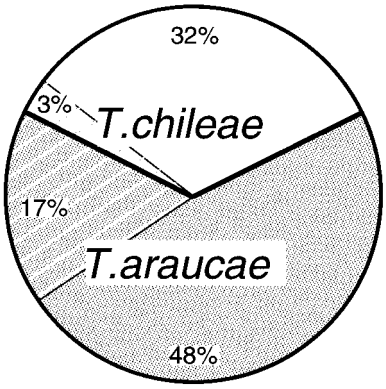

FIG. 6. Species composition in randomly selected sheaths. The white area shows sheaths with only *T. chileae* trichomes, and the shaded area shows sheaths with only *T. araucae* trichomes. The striped areas indicate sheaths with mixed populations that were numerically dominated by trichomes of *T. chileae* (white with gray stripes) or *T. araucae* (gray with white stripes). A total of 20% of the sheaths contained both species. In these sheaths *T. araucae* was the dominant organism numerically six times more often than *T. chileae* was.

distribution of angles. Branching of sheaths was observed on rare occasions.

DISCUSSION

Distribution of biomass. The wet weights of *Thioploca* filaments with sheaths found during our study in March 1994 (up to 800 g m^{-2}) were within the range of weights which have been reported previously for random samples taken in the same area (up to 1 kg m^{-2}) (3). Within our investigation area almost all of the 40-km-wide shelf was covered with *Thioploca* spp. The thickest mats were found at water depths between 50 and 100 m (Fig. 3). The *Thioploca* community was apparently restricted to the oxygen minimum zone, where the overlying water was anoxic (O_2 concentration, <5 μM) (6) at the time of our study. This distribution agrees with the hypothesis that the marine *Thioploca* spp. use nitrate rather than oxygen as an electron acceptor (2).

The measurements of trichome biovolume revealed that the bacterial biomass probably constitutes only 10 to 15% of the weight of the sheath material. The highest biovolume of trichomes (120 g m^{-2}) greatly exceeded the value reported for *Beggiatoa* spp. populations (20 g m^{-2}) (10). However, in marine *Thioploca* spp., about 80% of the volume is taken up by a central vacuole (21). Therefore, only 20% of the biovolume is living cytoplasm. When this fact is taken into account, the metabolically active biovolume of *Thioploca* spp. off the Chilean coast is only slightly higher than the reported biovolume of *Beggiatoa* spp. in Limfjorden, Denmark. The sulfate reduction rates that were determined during this study in most of the shelf area off Concepción (30 to 56 mmol of SO_4 reduced m^{-2} day^{-1}) (29) were two- to threefold higher than the rates determined during the summer in Limfjorden (15 to 18 mmol of SO_4 reduced m^{-2} day^{-1}) (8, 11). With comparable sulfate reduction rates, the amounts of sulfide that are available for growth of sulfide-oxidizing bacteria should also be similar. This suggests that the amounts of metabolically active biomass of these filamentous sulfur bacteria could ultimately be restricted by the amount of sulfide produced by sulfate-reducing bacteria.

Although the total biovolumes differed considerably at the different stations, the depth distributions of the biovolume of *Thioploca* trichomes within the sediment were comparable at all of the stations investigated (Fig. 4). The investigation took place in late summer, at the end of the growth season for *Thioploca* spp. (5). This might be why very well-developed mats were found in a large area.

Differences between *Beggiatoa* spp. and *Thioploca* spp. The density of *Thioploca* spp. decreased close to the coast, near the entrance to the Bay of Concepción (stations 6 and 7). In this area we also observed large populations of *Beggiatoa* spp. that seemed to become more dominant the closer we approached the shore. The sediment in this region was black and rich in sulfide (the sulfide concentration was up to 1,200 μM at station 6), while the sediments out on the shelf in the main distribution area of *Thioploca* spp. were brownish and contained very low levels of sulfide (the concentrations were generally less than 7 μM) (1) despite extremely high sulfate reduction rates. The sulfide concentrations in the Bay of Concepción were even higher (1,400 μM at station 4) (1), which may have been because *Thioploca* spp. were not present, but the high sulfide concentrations may also have been one of the reasons for this absence. Oxygen is found more frequently in the shallow bottom waters of the bay than on the shelf, whereas the nitrate concentrations generally decrease in the inner bay (29). The difference in the availability of the two major electron acceptors might have been responsible for the observed shift in the community structure from a community that was dominated by *Thioploca* spp. on the shelf to a community that was dominated by *Beggiatoa* spp. in the bay.

The distributions of *Thioploca* spp. and *Beggiatoa* spp. (Fig. 3) suggest that these organisms differ not only in morphology but also in physiology. The distributions of *Thioploca* spp. and *Beggiatoa* spp. overlapped at the entrance of the Bay of Concepción. Thus, it was possible to compare the vertical distributions of the two genera under the same general environmental conditions. The *Thioploca* community at this location had the typical structure, with sheaths reaching 4 to 8 cm down into the sediment, while the *Beggiatoa* community covered the *Thioploca* mat as a thin surface layer (Fig. 8, station 7). Since the photographic technique which was used was not able to resolve the dense population of thin, free-living *Beggiatoa* filaments, filaments appear in Fig. 8A as a greyish shade at the surface.

Because of our indications that there are sheathless *Thioploca* filaments (Fig. 5), it does not seem appropriate to distinguish *Beggiatoa* spp. and *Thioploca* spp. simply on the basis of the presence or absence of a sheath. Still, the majority of the *Thioploca* filaments were found within a sheath. The *Thioploca* trichomes without sheaths might have moved out of their sheaths, as has been reported previously (4).

Coexistence of species. We found that many sheaths contained trichomes of more than one *Thioploca* species (Fig. 6). Sheaths containing filaments with very different diameters

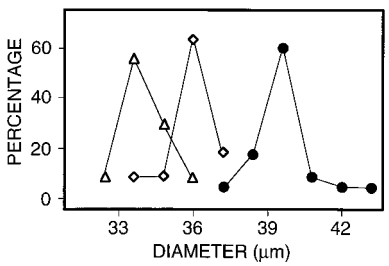

FIG. 7. Diameters of *T. araucae* trichomes from three neighboring sheaths in the uppermost 1 cm of a sample from station 7. Each sheath is represented by a different symbol. The mean trichome diameters in the sheaths differed, which suggests that individual sheaths are frequently populated by clones of a single trichome.

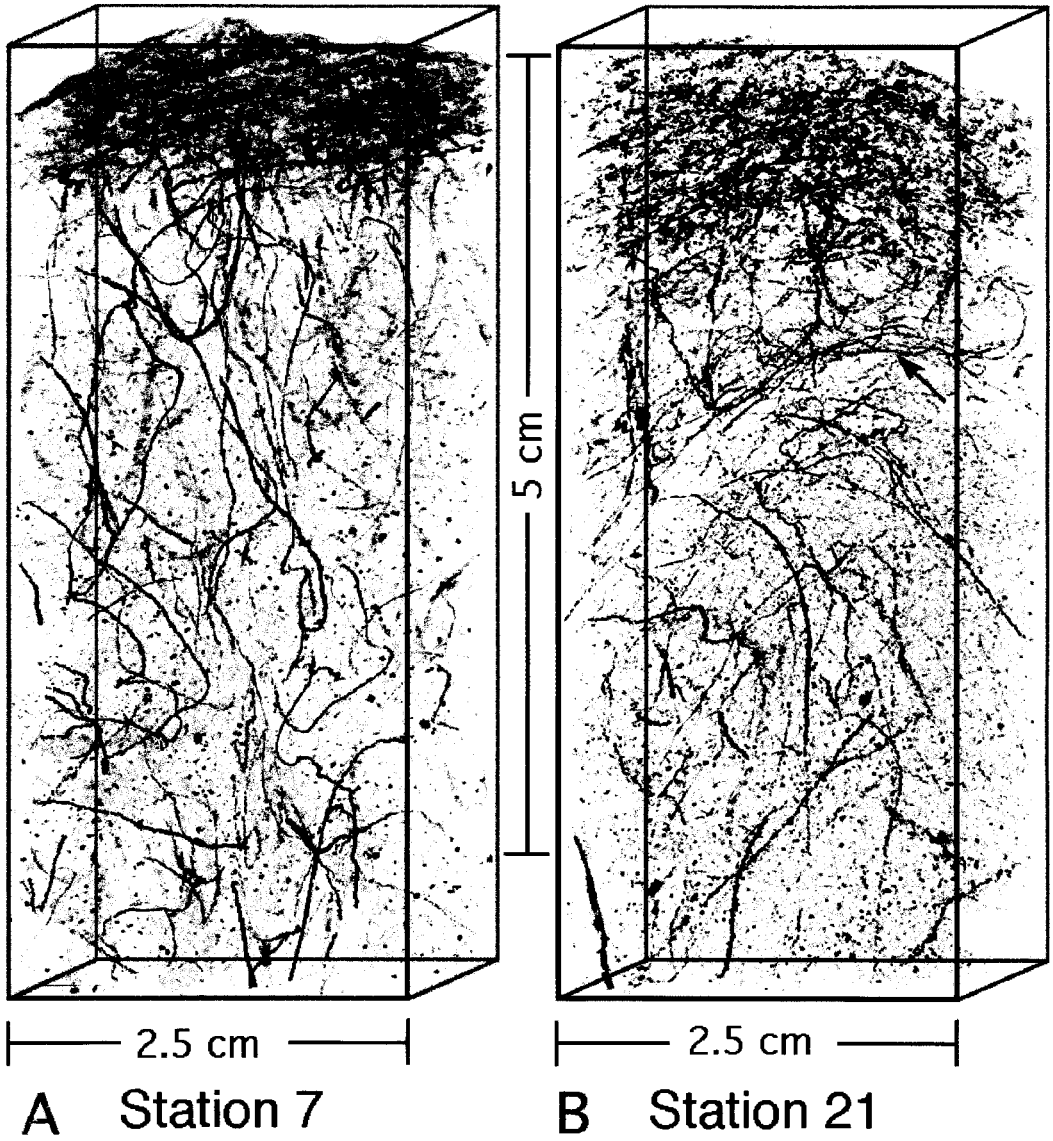

FIG. 8. Three-dimensional reconstruction of *Thioploca* sheaths (black lines) in blocks of sediment (5 by 2.5 by 1 cm) from station 7 (A) and station 21 (B). At the sediment surface there was a layer of relatively horizontally oriented sheaths. Underneath this the sheaths were oriented more or less vertically. The arrow in panel B indicates a second layer of more horizontally oriented filaments beneath the horizontal surface layer. The lower ends of the sheaths frequently bend back up toward the surface. At station 7 there was a mat of *Beggiatoa* filaments on the sediment surface, which appears gray.

have also been found in freshwater populations of *Thioploca* spp. (15), but these organisms have been interpreted as members of a new species. The presence of sheaths containing mixed populations and the occurrence of sheathless trichomes (Fig. 5) suggest that trichomes leave their sheaths occasionally and may glide into other sheaths. Since *T. chileae* trichomes are much smaller and more flexible than *T. araucae* trichomes, it is more likely that they coincidentally glide into the relatively larger *T. araucae* sheaths than vice versa. This argument is supported by the observation that the larger species, *T. araucae*, was the dominant organism in the sheaths containing mixed populations six times more frequently than *T. chileae* was (Fig. 6). The free *Thioploca* trichomes may enter a foreign sheath by gliding on other trichomes as these withdraw into their sheaths. It should be noted that the filaments are several centimeters long.

Although there is evidence that trichomes leave their original sheaths, a comparison of *T. araucae* trichome sizes in neighboring sheaths revealed that the mean diameters were slightly different (Fig. 7). Therefore, we assume that sheaths are originally populated by single clones. Considering that the occurrence of sheaths containing more than one species was extremely patchy, it could be that a change in local chemical conditions induced trichomes to glide up very far and stretch into the overlying water, which resulted in partial mixing of trichomes in individual sheaths when they glided back down. Filaments reaching out into the water were observed frequently during experimental investigations of intact sediment cores (9). In one case in which cores from the multiple corer were kept closed and standing on the seafloor for more than 24 h many filaments left the sediment altogether.

Spatial distribution. The reconstruction of the spatial orientation of *Thioploca* sheaths within the sediment revealed two structurally different parts of the *Thioploca* mat, a top layer

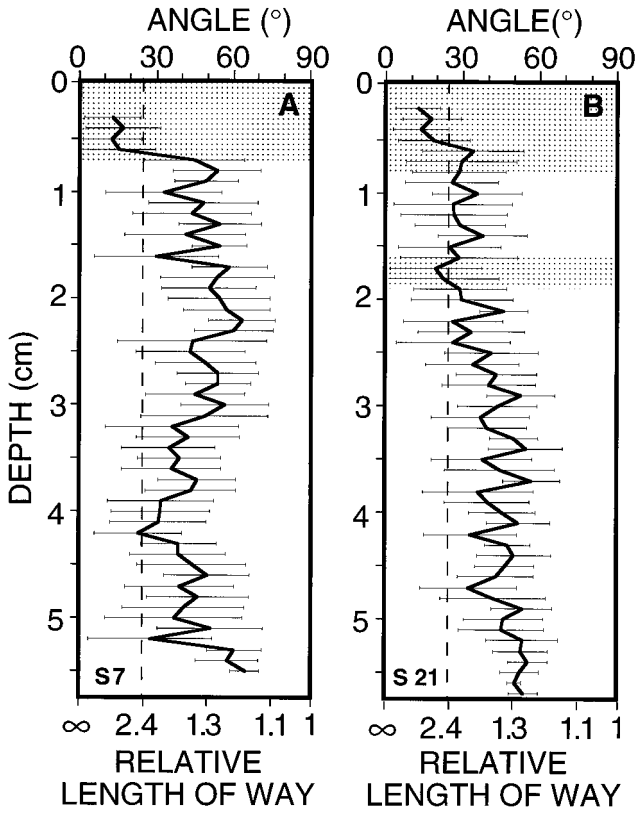

FIG. 9. Depth distribution of mean angles of *Thioploca* sheaths with respect to the surface plane at station 7 (A) and station 19 (B). The axis at the bottom indicates the additional distance that a trichome has to travel to move through a vertical depth interval as a result of the nonvertical orientation of its sheath. The relative length of way is thus a distance factor. The angles were calculated from the coordinates of the sheaths shown in Fig. 8. The stippled areas show which parts seemed to contain more horizontally oriented layers of sheaths. Beneath the uppermost 6 mm almost all of the sheaths have angles of more than 25° relative to the surface. With these angles, the distances traveled by trichomes would be less than 2.4-fold longer than a straight vertical path.

with thinner, more horizontally oriented sheaths and a zone underneath with thicker sheaths that were more vertically oriented (Fig. 8 and 9). This general structure was also observed when sheaths were carefully washed out of the sediment. The sheaths were frequently so long that they ended outside the reconstructed sediment block. The three-dimensional reconstruction made it possible to follow the paths of single sheaths in the sediment. This, together with observations made during the washing out of the sheaths, revealed that most of the sheaths did offer access to deeper parts of the sediment. Therefore, metabolism which involves taking up nitrate at the sediment surface and transporting it into deeper parts of the sediment to oxidize sulfide is indeed possible for trichomes that move inside the sheaths. The sheaths may thereby provide a tunnel system which enables *Thioploca* filaments to commute vertically between their electron acceptor, nitrate, and their electron donor, sulfide. Such a directional movement over many centimeters within the sediment is probably more efficient with the directional guidance of the surrounding sheaths than if single filaments were to move over the same distance. Accordingly, we did not find free *Thioploca* filaments without sheaths below a depth of a few centimeters in the sediment.

The average orientation of sheaths as a function of depth (Fig. 9) shows that in the uppermost 5 to 6 mm of sediment the sheaths had angles of 10 to 20° relative to the surface plane, while at depths greater than 6 mm the angles were mainly 30 to 50° and usually greater than 25°. Even when the angle of a sheath relative to the surface is as low as 25°, the distance which a trichome has to move to get down into the sediment is only 2.4-fold longer than the optimal vertical path (90°). The average angles for sheaths at depths greater than 6 mm at stations 7 and 21 were 43 ± 22 and 35 ± 21° (± standard deviation), respectively. This means that the resulting distances for trichomes moving down inside the sheaths were only 1.5- and 1.7-fold longer than a vertical path. Therefore, we concluded that the sheath orientation at depths greater than 6 mm provides efficient vertical connections between different sediment horizons, although the average angles are lower than the optimal angle, 90°. In the uppermost 6 mm, the average angles of the sheaths were 14 ± 13 and 19 ± 15° at stations 7 and 21, respectively; these angles increased the path length to move a given vertical distance 4.1- and 3.1-fold, respectively. We suggest that the function of the mostly horizontal surface layer is to maintain a larger part of the long filaments within a sediment layer containing nitrate.

At station 21 we found a second dense layer of more horizontally oriented sheaths below the surface layer (Fig. 8B). A possible explanation for this is that a former sediment surface was buried. Although the function of a more horizontally oriented top layer appears to be understandable, it remains unclear how such a layer develops, as the formation of sheaths has not been observed over long periods of time yet.

The structure of the *Thioploca* mat investigated differs from previous descriptions of U-shaped *Thioploca* sheaths observed in Lake Kinchezero (Russia) by Perfil'ev (26). Perfil'ev argued that the trichomes glide down until they reach a reduced layer, where they take up sulfide. In this layer the sheaths are oriented horizontally. The sulfur is oxidized to provide energy in the upper oxidized zone of the sediment, where the sheaths are oriented more vertically. The occurrence of two very different sheath structures demonstrates that the spatial orientation of the sheaths depends on the local environmental conditions. It is likely that the freshwater *Thioploca* spp. do not accumulate nitrate, as they lack a central vacuole (19). The different structures of *Thioploca* mats in Chile and Lake Kinchezero may, therefore, also be due to differences in physiology.

The three-dimensional reconstructions of the sediment cores reveal details of the mat structure. The lower ends of the sheaths often bent upward (Fig. 8), which is consistent with observations made during the biovolume determinations. As sheaths are presumably built by trichomes moving according to chemical gradients, the bends may be interpreted as a chemotactic response of trichomes that glide back into their preferred area. These bends occurred at different depths in individual sheaths. Apparently, the chemical concentrations that are important for the trichomes did not change at the same sediment depth when the sheaths were formed. This could be due to heterogeneity of the sediment, to differences in the number of trichomes in different sheaths, or to temporal variations. Branching of sheaths seldom occurs. This suggests that during the development of the sheaths in the sediment the trichomes tend to stay close to each other, although they apparently separate when they stretch into the overlying water at the upper end of the sheath (9). The average thickness of the sheaths seems to be a feature that varies between locations. At the entrance of the Bay of Concepción, where the sulfide concentrations were relatively high and the *Thioploca* mat grew underneath a *Beggiatoa* spp. population, the sheaths were thicker than in the shelf area (Fig. 8), although the total biomass was lower (Fig. 3). This observation has been mentioned previously (23). The

sheaths may get thicker in response to higher sulfide concentrations.

It is not known how new sheaths that reach 10 cm or more down into the sediment are formed. Free *Thioploca* filaments that occur outside sheaths were not found during this study below the uppermost few centimeters. The three-dimensional image in Fig. 8 shows only those sheaths that were inhabited at the time of fixation, but many empty sheaths also occurred in the sediment down to depths of 10 to 15 cm.

Implications for the ecosystem. The *Thioploca* community builds up high biomasses in a vast area (3, 7, 18, 20, 22, 23, 25). It is not clear whether the mats investigated in this study occur at a similar density along the entire coasts of Chile and Peru, but at least off the Bay of Concepción they covered most of the continental shelf, which is particularly broad in this area. The nitrogen and sulfur cycles of these sediments must be strongly influenced by the *Thioploca* mats (2). The network of interwoven horizontal sheaths that is held in place by the root-like vertical sheaths stabilizes the sediment very efficiently. Considering the fine grain size of the sediment, the surface layer of sample cores was surprisingly hard to disturb during handling of sediment cores. In this upwelling area, with its high sedimentation rates and a steep continental slope, strong stabilization of the sediment should have a great effect on the amount of carbon that is retained in the sediment.

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