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THE ECOLOGY OF MARINE MICROBENTHOS

IV. STRUCTURE AND FUNCTION OF THE BENTHIC ECOSYSTEM, ITS CHEMICAL AND PHYSICAL FACTORS AND THE MICROFAUNA COMMUNITIES WITH SPECIAL REFERENCE TO THE CILIATED PROTOZOA

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CONTENTS

ABSTRACT

The paper describes the ecosystem constituted by marine sediments and their microflora and fauna but with special emphasis on the ecology of ciliated protozoa. This description is based on quantitative studies of the vertical and horizontal distribution of the fauna, the physico-chemical factors (O_2, H_2, S, Eh, pH, grain size, organic matter, salinity) and the microflora, and on model experiments with artificial and natural sediments.

Factors controlling the oxidation-reduction properties of sediments, the O_2-uptake of reduced sediments and the respiration and photosynthesis of undisturbed sediments were studied.

Among other things it is demonstrated that the microfaunal communities can be correlated with the oxidation-reduction properties of sediments and with their mechanical composition and that the endproducts of anaerobic decomposition (notably H_2S) are of large trophic significance to the sediment ecosystem through the activity of chemo- and photoautotrophic bacteria. The energetic role of the microfauna in sediments is estimated; mostly, the respiration of the microfauna exceeds that of the macrofauna. In fine and medium sands the ciliates constitute the most important group among the microfauna.

SUMMARY

The present paper describes various physico-chemical and biological aspects of the ecosystem constituted by marine sediments and their microflora and fauna but with special reference to the ecology of ciliated protozoa.

The significance of the mechanical properties of the sediment is discussed (total surface area, porosity, permeability, capillarity, diffusion rate of dissolved compounds and the size of individual interstitia) and the extent to which these properties can be predicted from sieving data. The permeability (governing the oxygenation of the sediments) and the size of the interstitia (limiting the size of animals which can live in the sediment) are of greatest significance. Comparisons between different localities in the Øresund show that an interstitial fauna does not occur in well sorted sands with a median grain size below 90-100 μ. In finer sediments practically only the burrowing nematodes are present. In fine sands

(125-250 μ) ciliates constitute the dominating animal group, often exceeding the metazoan microfauna in biomass. The metazoans (except nematodes) become numerous in sands coarser than 200-250 μ. In median and coarse sands metazoans (except nematodes which here are rare) dominate. This distribution is attributed to the small size and the high degree of morphological adaptation found in ciliates.

Only 2-4 % clay and silt totally excludes the presence of interstitial animals in a sediment.

The detritus layers covering clays and muds may contain a rich microfauna but here the ciliates play a modest role.

Temperature and salinity generally cannot explain field distributions of ciliates though a few relatively stenohaline ciliates (*Remanella, Kentrophorus, Geleia*) exist. In general even dilute brackish waters contain typical marine ciliate faunas.

The vertical zonation of chemical factors and microbial processes have been studied and are discussed. Below a certain depth anaerobic conditions prevail in most sediments resulting in bacterial production of reducing compounds of low molecular weight (H_2S, NH_3, CH_4, H_2). These compounds diffuse upwards and are oxidized in the presence of oxygen by chemoautotrophic bacteria or abiologically or under anaerobic conditions by the activity of photoreducing organisms.

Measurements of oxidation-reduction potentials in sediments have been made and their significance and chemical interpretation are discussed. A characteristic redox-profile and a well defined redox discontinuity layer is nearly always present in sediments reflecting the vertical zonation of chemical factors and microbial processes. Negative potentials are controlled by a sulphur – sulphide system. Systems poising higher potentials are not known in detail.

The quantitative vertical distribution of O_2 and H_2S and the pH-profile were studied and correlated with the redox-profile.

The mutual effect of microorganisms and the redox conditions of the environment was studied and discussed. Heterotrophic organisms reduce and autotrophic organisms oxidize their environment.

The oxygen uptake of reduced sediments was studied; it can to a large extent be explained by abiological oxidation of H_2S.

Factors influencing the redox properties of sediments were investigated. Anaerobic decomposition of organic matter is always the primary cause of reducing conditions. In exposed localities irregular import of organic matter (mainly driftweed) and water turbulence are the most important factors. In lentic localities where reducing conditions prevail close to the sediment surface, light is, through the activity of photoautotrophic organisms, the main factor controlling the depth of the redox discontinuity layer. Diurnal vertical migrations of the redox discontinuity layer due to light was demonstrated in model experiments and in the field; it was shown to involve vertical migrations of sulphurbacteria.

The vertical and horizontal distribution of various protophytes was studied. Representatives of some groups (diatoms, flagellates) occur far below the photic zone of the sediments and it is proposed that these may be able to live heterotrophically.

The significance of bacterial activity for the utilization of dead organic matter by the microfauna is stressed. It is shown that the end products of anaerobic decomposition are of large trophic significance for the fauna of sediments since the energy of the reduced low molecular compounds is utilized for the synthesis of particulate organic matter by chemoautotrophic bacteria. These processes take place at the redox discontinuity layer explaining the high concentrations of microfauna in this layer which is often situated several cm below the sediment surface. When reducing conditions prevail close to the surface also photoautotrophic bacteria are of trophic significance.

Among the various types of chemo- and photoautotrophic bacteria those belonging to the sulphur cycle seem to be of greatest significance.

The sediment ecosystem is compared with that found in stagnant water basins with an anaerobic hypolimnion as recently described by Sorokin.

Measurements of respiration and photosynthesis of undisturbed shallow water sediments were made. Respiration always exceeded photosynthesis somewhat, even under conditions which seemed optimal for photosynthesis, thus indicating a preponderance of heterotrophic processes in sediments even in shallow water.

The composition, zonation and food relationships of various microfaunal communities were studied with special reference to the ciliates. Three communities were studied in most detail: the "sublittoral sand microbiocenosis" (i.e. well sorted and detritus poor subtidal sands), the "estuarine sand microbiocenosis" (i.e. sands, rich in organic detritus found in lentic, shallow bays and lagoons) and the sulphuretum (localities quite dominated by the bacteria of the sulphur cycle, found in connection with large accumulations of drift-weed in lentic places).

The fauna of sublittoral sands shows a characteristic vertical zonation. Three groups of species can be recognized: those living in the oxidized surface layers, those living in the vicinity of the redox discontinuity layer and those living in the reduced, anaerobic and sulphide containing layers. The latter two groups are mainly represented by ciliates and to some extent nematodes, most other metazoans being confined to the oxidized surface layers. With the exception of localities where growth of protophytes in the surface layers is extremely rich, greatest numbers of ciliates occur in the vicinity of the redox discontinuity layer several cm below the surface. Sands oxidized throughout (coarser sands and sands mixed by the surf) do not show this characteristic zonation.

In "estuarine" sands the redox discontinuity layer is situated closer to or at the sediment surface and consequently the different fauna zones are compressed towards the surface. Many species are characteristic for these sediments due to the presence of certain bacteria and protophytes (photosynthetic bacteria, blue-green algae) never or rarely found in cleaner sands.

In sulphureta reducing properties prevail at all levels of the sediment and often also in the overlying water, and the vertical zonation is therefore compressed to a few cm or mm. The development of a sulphuretum (chemistry, microflora and fauna) through a year is described.

The importance of various food items for the ciliates was studied and compared in different localities and at different seasons. Carnivorous ciliates mostly preying on other ciliates play a relatively small role (about 10 % of the ciliate fauna) as do histophagous ciliates. Most important are herbivorous forms (eating diatoms, phytoflagellates, blue-green and other algae) which often dominate in summer in shallow water localities, and bacterivorous ciliates which dominate in deeper water localities and also during autumn-spring in shallow water localities. In estuarine sediments and in sulphureta ciliates eating chemoautotrophic and photosynthetic bacteria play a large role, thus forms eating sulphurbacteria may constitute 25-50 % of the total ciliate fauna.

Some other microfauna communities were also studied though in less detail: the splash zone of beaches, gravels, carpets of *Vaucheria* and detritus layers covering clays and muds.

The correlation between the vertical distribution of the various species and the redox-profile is explained by oxygen requirements, the tolerance to various reduced, toxic compounds (notably H_2S) and specialization to feed on various microorganisms which require certain redox conditions. Evidence is put forward that the animals orientate themselves in gradiants of H_2S and O_2. The tolerance to H_2S of various species based on field distributions and experiments is discussed. Some species living in the sulphide zone of sediments were cultured anaerobically for several months in water containing 100-200 mg H_2S/l (Eh: – 197 to – 210 mV). No metazoans have a tolerance to H_2S comparable to that found in some ciliate species.

The taxonomic composition of the ciliate faunas of different communities is discussed and it is shown that some groups dominate in certain communities. Some morphological adaptations of benthic ciliates and the formation of cysts are discussed. Encystation is induced by starvation. Cysts are viable for long periods but do not survive desiccation.

Various laboratory models of marine sediments were studied, some of which had totally oxidized conditions and some in which anaerobic decomposition, chemosynthesis and photoreduction could be studied. The food chain H_2S – sulphurbacteria – ciliates was demonstrated as was the trophic significance of the redox discontinuity layer. The effect of light was studied; it accelerates oxidation of reduced systems due to the activity of photoautotrophic organisms. Growth of certain protophytes (e.g. diatoms, blue-greens) is stimulated by the development of reducing conditions.

The succession of microflora and fauna based on organic material in artificial and natural sediments was followed. In such successions the microbial processes: aerobic decomposition – anaerobic decomposition – chemosynthesis – photore-

duction and photosynthesis dominate in the mentioned sequence which again results in characteristic microfaunal successions in which ciliates dominate.

The initial growth of ciliate populations is often exponential but rarely attains the potential rate of the species in question. This is explained by limited food supply and shows that one cannot infer from pure cultures to population growth in the field.

Values of biomass and the number of individuals of the ciliates and various representatives of the metazoan microfauna are given. In some localities these values were compared with the quantitative importance of the macrofauna.

In a sublittoral fine sand (10 m depth, Øresund) the proportion macrofauna: metazoan microfauna: ciliates was found to be 1:40:1500 (number of individuals) and 190:1.5:1 (biomass). The relative metabolic rates were estimated and the proportion 3.7:1:1.4 was found. In an estuarine sediment (Nivå Bay) the proportions were 1:10:150 (individuals), 170:10:1 (biomass) and 4:2:1 (metabolic rate).

The general energetics of marine sediments are discussed. It is concluded that bacterial metabolism in all cases exceeds that of the fauna. It is further concluded that attempts to construct a quantitative picture of the trophic relationships between all representatives of the benthic community is still premature due to insufficient knowledge on the feeding biology of several animal groups and due to the extreme complexity of the food webs.

Finally the very sparse evidence of ciliates as food for other representatives of the benthic fauna is reviewed. It is believed that the role of ciliates as prey has been underestimated since ciliates leave no recognizable remains in the digestive tracts of predators.

1. INTRODUCTION

The present paper is the last in a series (Fenchel, 1967, 1968a, b) describing the ecology of the microfauna in marine sediments. The first paper dealt with the quantitative importance of different microfaunal groups and the latter two dealt with the food and the reproductive potential of ciliates, respectively.

The purpose of the present paper is to give a general description of various aspects (physico-chemical as well as biological) of the ecosystem constituted by the sediment and its microflora and fauna but with special emphasis on one of its elements: the ciliated protozoa.

There are several reasons for this restriction and for choosing the ciliates as main object for the present study. The field of ecology comprises a number of concepts and theories entitling it to be considered as a special branch of science. However, starting his work the ecologist finds himself restricted to use methods belonging to a large number of other disciplines which he may not all master to a sufficient degree, e.g. taxonomy, physiology, chemistry, statistics, hydrography, bacteriology, etc. During the present investigation, taxonomic knowledge of the large number of animal and plant groups encountered in the community studied was one of the limiting factors and consequently it was necessary to restrict detailed analysis to one taxonomic group. Ciliates (of which there are several hundred species in marine sediments) were chosen due to my previous taxonomic knowledge. Other reasons were also decisive. Ciliates are, as shown in Fenchel (1967), of large quantitative importance in certain marine sediments, a fact which has hitherto been overlooked. Also their ecology is poorly known in spite of a rather recent and very comprehensive taxonomical literature.

Some information on other representatives of the microfauna will also be found in the present paper.

Most ecological studies on ciliates and communities of microorganisms deal with freshwater biotopes (e.g. Bick, 1958, 1964; Lackey, 1938; Noland, 1925; Stout, 1956; Wetzel, 1929, and others). Very important is the work of Picken (1937) who used the community concept for describing assemblages of protozoa and demonstrated the significance of food interrelationships and the mechanical structure of the substratum for the maintenance of the communities. These ideas were also developed by Fauré-Fremiet (1950 a, d, 1951a, b, d) in his studies on the protozoan associations found in colonies of *Beggiatoa* in freshwater and those found in marine sands. Also the work of Webb (1956) on the ecology of the ciliates in a salt marsh is an important contribution. Bock (1952-53, 1960) described the qualitative composition of the ciliate fauna in various sediments from the Bay of Kiel. Persoone (1968) has made studies on the infusorian fauna on submerged slides and includes considerations of food chains. Borror (1968) gives some discussion on the significance of the mechanical structure of algae and of sand for the ciliates inhabiting these biotopes. Most other works on marine ciliates have mainly taxonomical and faunistic scope and will be referred to in later chapters. However, the taxonomic monographs of Borror (1963a) and Dragesco (1960) should be mentioned here as they contain some ecological information.

Lackey (1961) noted that the distribution of many ciliates apparently depends on the presence of sulphurbacteria. This author as well as Bamforth (1961) call upon investigations on the microdistribution of protozoa.

The usual way to sample protozoa has been to fill large jars and other containers with water or sediment and search for the animals in the laboratory. This sampling method, however, excludes studies on the microhabitats of protozoa since these microhabitats are much smaller than the samples and consequently several protozoan communities are mixed together.

One of the aims of the present study has been to study the microhabitats of benthic protozoa and their extension in the field.

Nearly all natural ecosystems from the beech wood and the water masses of the open sea to cushions of moss show a vertical zonation of physical and chemical factors and of the flora and fauna. A vertical zonation of factors and fauna could therefore also be expected in marine sediments. Such studies have until now been carried out in beaches (e.g. Jansson, 1968a; Renaud-Debyser, 1963, and others) where factors such as water contents, salinity and temperature are of importance. In subtidal sediments, where other factors could be expected to be of significance, no studies on the vertical zonation of the microfauna have yet been made except the work of Fenchel & Jansson (1966) which describes the vertical zonation and microfauna in a sediment in the Baltic Sea. This material will be redescribed in the present paper.

Besides describing the vertical zonation of factors and fauna in sediments it has been the aim of the present paper to show that an understanding of the function of the benthic ecosystem presupposes knowledge on this zonation.

Much emphasis has been laid on quantitative work which was facilitated by following the recently described extraction method for interstitial fauna (Uhlig, 1964, 1966). Unfortunately, however, while the methods of sampling and enumeration used in the present study give a reliable picture of the quantitative importance of various groups and a picture of the delimitation of the micro communities they do not lend themselves to statistical analysis. The reasons for this will be discussed in Chapter 2.

The concepts micro-, meio-and macrofauna are not very clearly defined in the literature. The school of workers studying the interstitial fauna of sands have, following the works of Remane (e.g. 1933), called the interstitial animals for microfauna in contrast to the macrofauna which burrows in the sediment. This definition has, in contrast to that discussed below, a biological meaning but naturally the upper size limit of the microfauna will be different in different sediments.

Many marine biologists, however, following the work of Mare (1942) who worked on muddy bottoms, divide the fauna quite arbitrarily into microfauna (bacteria, protophytes and protozoans excluding the foraminifers), the meiofauna (foraminifers and small metazoans, e.g. nematodes, turbellarians, and also juvenile macro invertebrates) and the macrofauna. Usually the metazoans passing through a sieve with a certain mesh size are considered as meiofauna. As mentioned above these definitions have no biological meaning. Furthermore sieving is a poor way to sort animals according to size since for example worm shaped animals may pass a sieve in which an equally large animal of another shape is retained.

In the present paper the term microfauna is quite arbitrarily defined as animals weighing less than 10^{-4} g and the term meiofauna is used to some extent to designate the metazoan microfauna, i.e. nematodes, gastrotrichs, turbellarians, harpacticoids, ostracodes, rotifers, tardigrades, and archiannelids.

In recent years many papers have treated the food chains and the energetics of the sediment ecosystem (e.g. Carey, 1967; Darnell, 1967; Kanwisher, 1962; Renaud-Debyser & Salvat, 1963; Teal & Kanwisher, 1961; Wieser & Kanwisher, 1961). The role of protozoa in the benthic ecosystem has, however, been totally neglected in the literature and will therefore be another topic of the present paper.

My gratitude is due to Mr. Holger Knudsen for his careful photographic work and for skillfully constructing many of the more or less useful devices which I designed during my work. My thanks are also extended to Jan Fabricius B.Sc. for taking samples from the Hornbæk Bay by SCUBA diving. It is a pleasure to express my thanks to the artist Kaj Olsen for drawing Figs 66 and 75, which required not only artistic skill but also hours of microscopical studies.

My sincere gratitude is due to Dr. Bengt-Owe Jansson (University of Stockholm) and the Askö team of zoologists for hospitality in their laboratory at the Baltic Sea during my five summer visits and for valuable discussions. Also, I am very grateful to Dr. Erik Rasmussen for the opportunity to stay and to work in his Isefjord Laboratory and to Dr. Gotram Uhlig (Helgoland) for discussions on quantitative extraction of microfauna. My thanks are also extended to Dr. Anne Thane-Fenchel for identifying the rotifers of my samples and to Dr. Willy Nicolaisen for information on the macrofauna of Hornbæk Bay.

My particular gratitude is due to Miss Harriet H. Hansen who has been of invaluable help in all respects during the work. Only due to her help could the laborious and tedious quantitative work be carried out within a reasonable period of time.

2. MATERIALS AND METHODS

The localities investigated

All the investigated localities are situated within three main areas: The northern part of the Øresund, the Isefjord area, both at the Danish island Sjælland (Fig. 1), and on the small Swedish island Askö situated south of Stockholm in the Baltic Sea.

In the Øresund quantitative samples were taken in a line directed NE-SW off Hornbæk. Samples were taken at 5, 8, 11, 15, 20, and 22 m depth in the period February-March 1968 (Fig. 2).

The sediments at 5 m depth consist of an about 15-20 cm thick layer of coarse sand (median grain size: 800 μ) resting on fine clayey sand. Scattered on the bottom large stones with algae are found. The coarse sand is poor in organic contents and oxidized throughout.

Seawards from this locality the seabottom slopes gently down to depths of about 27 m, the sediments becoming finer and with increasing contents of silt with increasing depth. At 8 m the median grain size is 140 μ and the content of clay and silt 0.7 %; at 11 m: 160 μ and 0.5 %; at 15 m: 135 μ and 3.4 %; at 20 m: 120 μ and 5.5 %, and at 22 m: 95 μ and 9.6 %. In these localities a reduction layer is always developed but generally situated relatively far below the sediment surface.

The macrofauna assemblage on the localities below 15 m may be characterized as a *Venus gallina* community (see Thorson, 1957) the dominating animals being lamellibranchs as *Venus gallina, Tellina fabula, Cultellus pellucidus,* and *Corbula gibba;* polychaetes as *Pectinaria koreni, Scoloplos armiger, Nephtys longisetosa, Spio martinensis,* a.o.; and the echinoderms *Echinocardium cordatum* and *Ophiura albida.* At depths greater than 19 m animals such as *Amphiura filiformis, Nucula* spp and *Abra nitida* are common in the finer sediments.

Another series of quantitative samples were taken off Ålsgårde (north of Hellebæk) in a line perpendicular to the coast at 5, 10, 15, 20, and 25 m depth in May-June 1967. Here the finest sediments are found closest to the coast (Fig. 2). At 5 m the median grain size is 150 μ and the contents of silt and clay 0.2 %. Seawards for a long distance depth varies between 9 and 12 m and the sediment is fine well sorted sand with a median grain size of around 175 μ containing about 0.3 % silt and clay. Here, as well as in shallower water, a well developed layer of reduced sediments is found 5-10 cm below the surface.

At approximately 12 m, depth increases seawards to about 20 m within a short distance. On this slope clean medium sand (median grain size: 400 μ, 0.02 % silt and clay) which is completely oxidized to at least 20 cm depth is found. From 20 m and deeper the sediment is still relatively coarse, but the amount of silt and clay increases with increasing depth (about 0.2 % at 20 m and about 4 % at 25 m).

At depths until 15 m the assemblage of macroinvertebrates must be characterized as a *Macoma balthica* community as found on sandy bottoms (Thorson, 1957). Common benthic invertebrates are: lamellibranchs: *Tellina tenuis, Cardium* spp, *Mya arenaria, Corbula gibba, Mysella bidentata;* polychaetes: *Ophelia bicornis, Pygospio elegans, Scoloplos armiger, Eumenia crassa, Magelone* sp., *Pectinaria belgica, Spio* sp.; crustaceans: *Bathyporeia* spp, *Diastylis* sp., *Haustorius arenarius.*

In addition to the 5 quantitative samples a large number of qualitative samples were studied at all seasons of the year in the period from winter 1966 to summer 1968.

At Julebæk between Helsingør and Hellebæk the shore is sheltered by a number of sandbars lying parallel to the shore (Fig. 4). Between these sandbars and the shore a quantitative sample was taken (May 1967) and studies on the vertical distribution of diatoms, H_2S, O_2 and the redox conditions were made. Also numerous observations on the microfauna were carried out on this locality. The sediment consists of fine (190 μ) well sorted sand (Fig. 3). The average water depth is about 0.5 m. Due to the sheltered position and to the large amounts of drift-weed mixed with the sand during winter storms the sediment is reduced and contains relatively high amounts of H_2S few cm below the surface. Growth of diatoms and dinoflagellates is extremely rich colouring the surface yellowish. Sporadically small areas where white sulphurbacteria dominate the flora of the sediment surface are found.

The macrofauna is dominated by the lamellibranch *Macoma balthica,* the polychaetes *Arenicola marina, Pygospio elegans, Nereis diversicolor,* and *Capitella capitata,* and the haustoriid amphipods *Bathyporeia sarsi* and *B. pilosa.* The physical factors and the metazoan microfauna of the Julebæk beach above the water line was described by Fenchel et al. (1967).

The beach off the Marine Biological Laboraotry in Helsingør (Fig. 4) was studied in detail. Quantitative (31) and qualitative studies as well as studies on the redox conditions of the sediments were made regularly in the period October 1966-

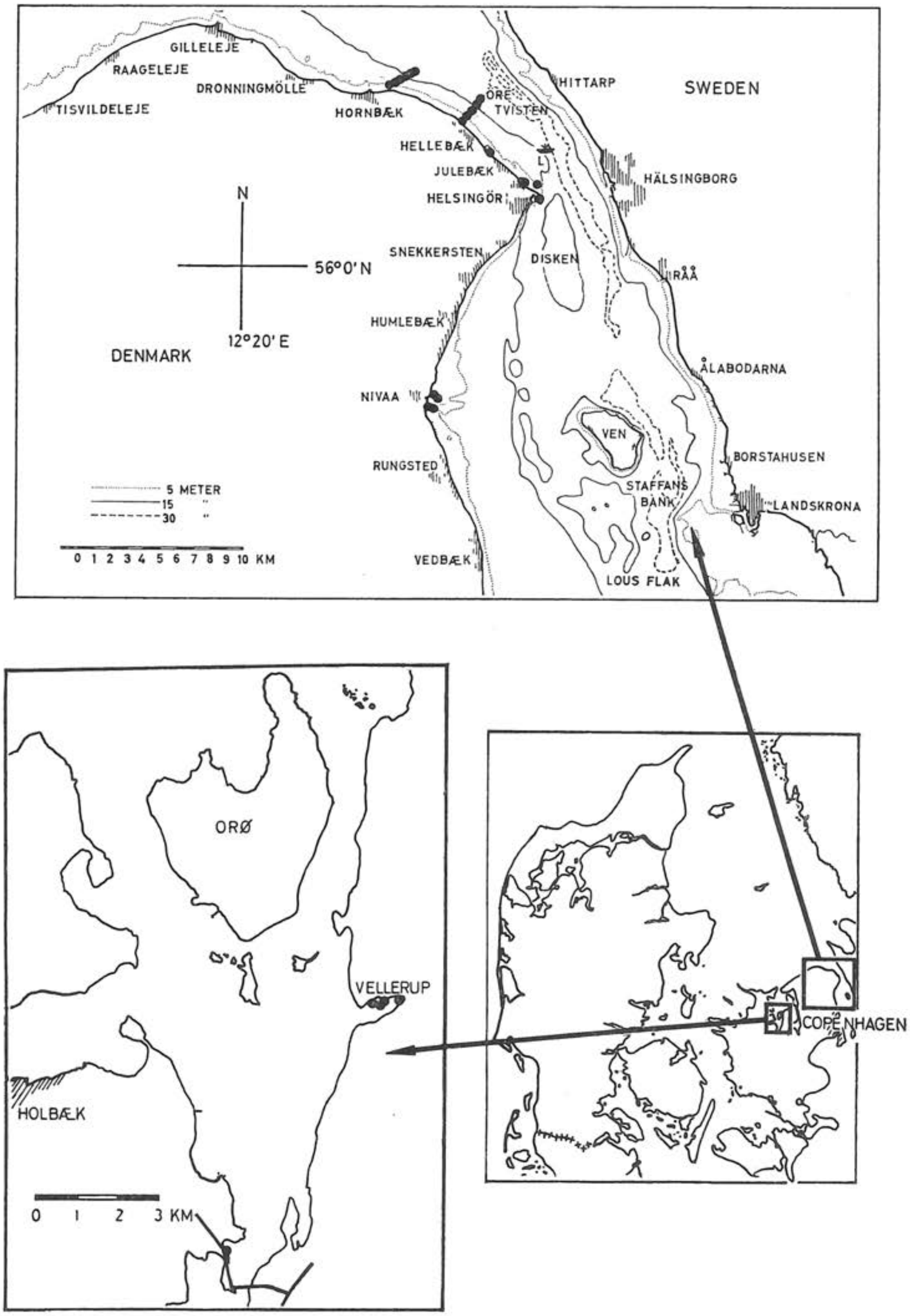

FIG. 1. Danish localities sampled quantitatively.

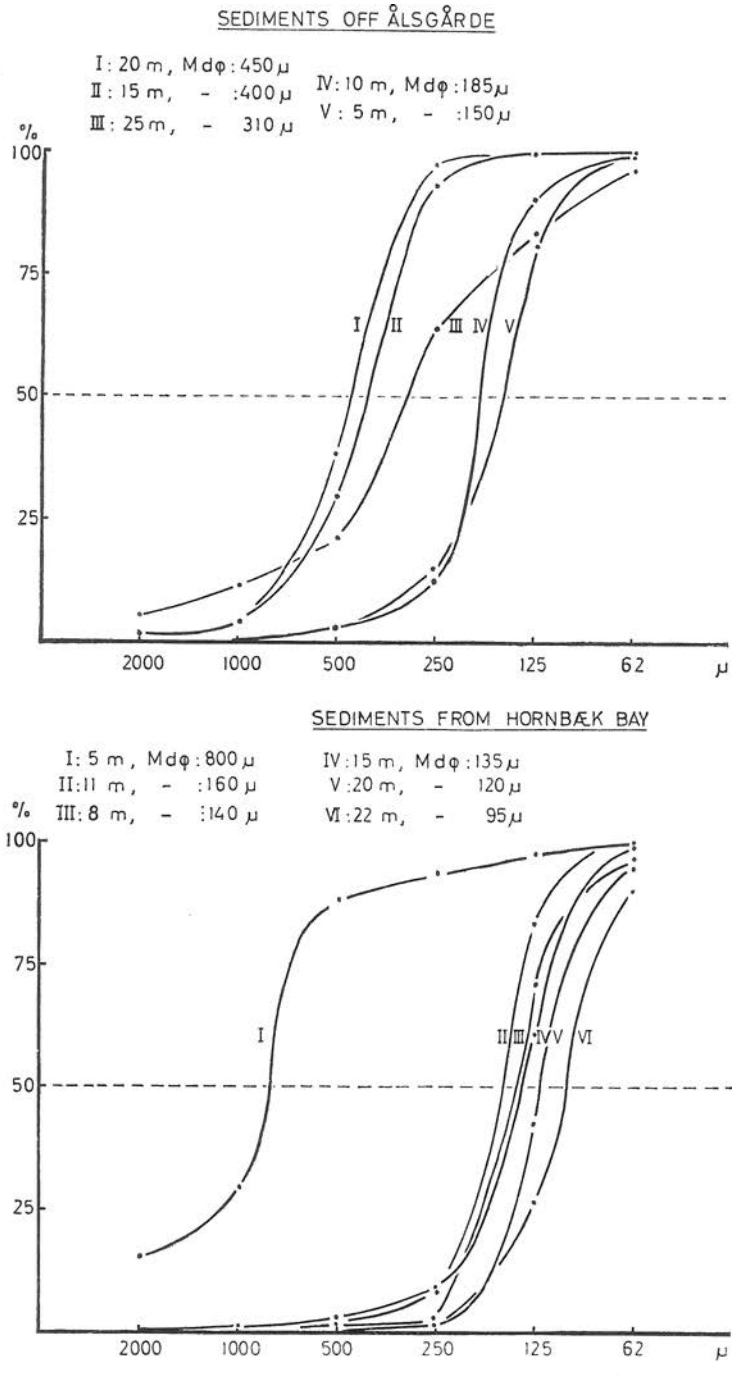

FIG. 2. Grain size distributions of sediment samples from Ålsgårde and the Hornbæk Bay.

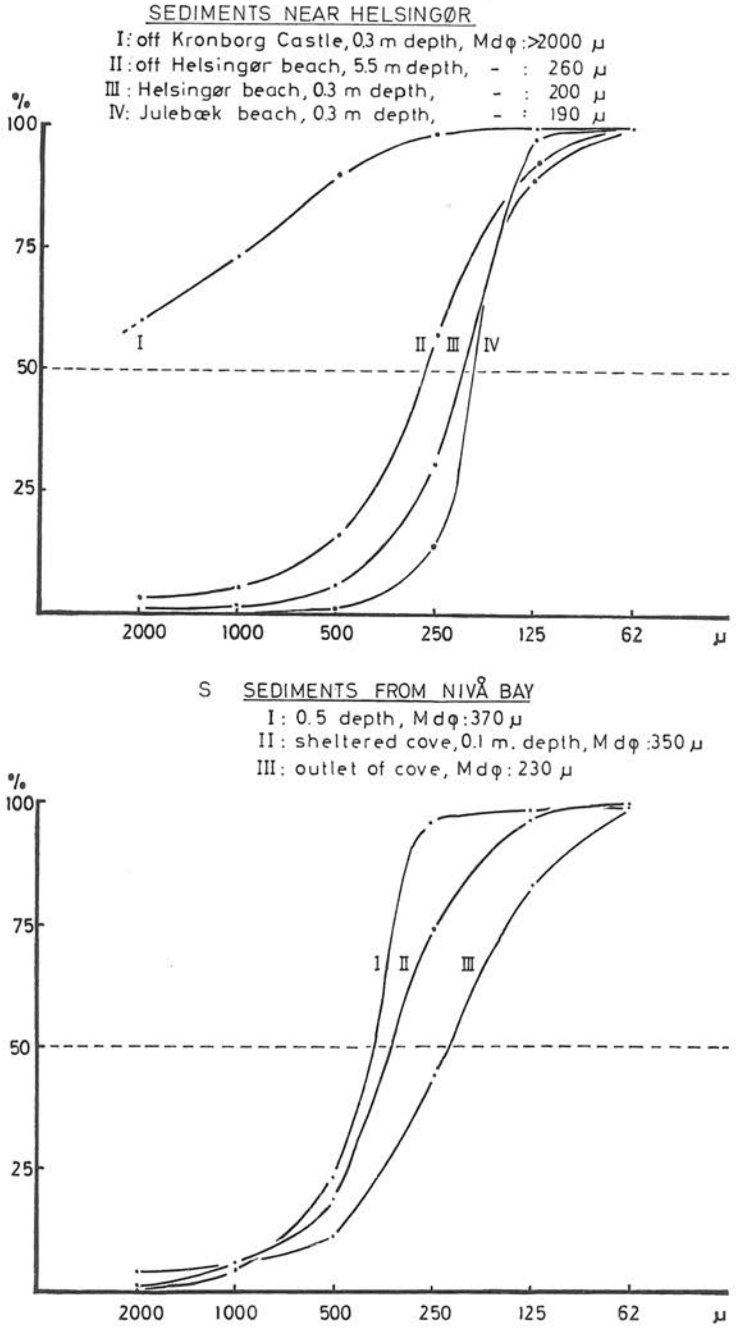

FIG. 3. Grain size distributions of sediments from Helsingør Beach, Kronborg, the Julebæk Beach, and the Nivå Bay.

August 1967 and studies on the vertical distribution of the microflora and of H_2S in spring-summer 1968.

The depth of the fixed sampling station is 0.2-0.5 m according to the water level. The median grain size of the sediment is about 200 μ (Fig. 3). The beach is relatively exposed to surf with winds from NE and the sediments are regularly mixed during winter storms. At the same time large amounts of drift-weed are mixed into the sand resulting in anaerobic and reducing conditions during calm periods. The redox conditions of the sediments therefore vary from completely oxidized to strongly reducing.

The poor macrofauna consists mainly of haustoriid amphipods and oligochaetes.

Only few metres seawards at about 1 m depth, where conditions are more stable, a typical sandbottom *Macoma balthica* community is found (*Cardium edule, Mya arenaria, Arenicola marina,* etc.).

At the sampling station the diatom flora is relatively poor and mainly confined to the deeper layers of the sediment probably due to the wave exposure of the surface.

In January 1968 a quantitative sample was taken at 5.5 m depth off the Helsingør Beach (Fig. 3; median grain size: 260 μ); qualitative samples were often studied from this locality.

Two quantitative samples were taken off the south side of the Kronborg Castle (Fig. 3). Here the sediment is extremely coarse (median grain size: >2000 μ) due to the strong water currents at this locality.

Nivå Bay is a sheltered shallow bay situated about 15 km south of Helsingør. About 250 m from the shore the sheltered area is bordered by sandbars consisting of clean hard sand. Inside the sandbars the sediment consists of detritus-rich medium sand, in some places with a relatively high content of silt. Landwards the bay is bordered by a dense growth of *Phragmites*. The bottom vegetation of the bay consists mainly of *Zostera* and *Ruppia*, but also *Ulva* and on the sporadic stones *Fucus, Enteromorpha* and *Cladophora* are growing. The sediments contain rather much organic detritus mainly derived from dead *Zostera*. Below a surface zone of various thickness consisting of oxidized sediments conditions are reducing and the interstitial water contains high amounts of H_2S. A rich microflora dominated by diatoms, dinoflagellates, cyanophyceans and sulphurbacteria lives in the sediments.

The macro- and meiofauna as well as some physical factors of the Nivå Bay have recently been described by Muus (1967). The macrofauna assemblage characteristic of Danish estuaries and lagoons was designated a "*Cardium lamarcki* – *Hydrobia ventrosa*" community, a variety of the *Macoma balthica* community. Dominating animals are the lamellibranchs *Mya arenaria, Macoma balthica* and *Cardium lamarcki,* the crustaceans *Corophium, Sphaeroma, Jaera,* gammarids, and *Crangon,* the polychaetes *Nereis diversicolor, Arenicola marina* and *Pygospio*

FIG. 4. Above: The Helsingør Beach; below: The Julebæk Beach.

elegans, the gastropods *Hydrobia* and *Littorina*, and oligochaetes which are very numerous.

Quantitative and qualitative samples were taken in the period 1966-68 about 50 metres off the shore. Other samples were taken close to the shore where accumulating drift-weed produces large amounts of H_2S and the surface of the sediments is quite dominated by purple sulphurbacteria (a "sulphuretum", see Fig. 71).

FIG. 5. Above: Cove in the northern part of the Nivå Bay; below: The southernmost locality in the Nivå Bay.

FIG. 6. Above: Vellerup Vig; below: The Munkholm Bridge locality.

About 1 km north of this locality is a small artificial cove (originally made for obtaining clay for a brick field but now in open connection with the sea; Fig. 5). Quantitative samples were taken from the sand inside the cove in the period October 1966-August 1967 and qualitative samples were taken in the period from 1966 to 1968. In this locality the sediments (Fig. 3) are very rich in H_2S and the sediment surface is dominated by white sulphurbacteria (*Beggiatoa, Thiovolum*, etc., see Fig. 63) except in June-August when purple sulphurbacteria dominate. Diatoms, flagellates and cyanophyceans are numerous throughout the year. A few quantitative samples were taken in the outlet of the cove and qualitative studies were made in the carpets of *Vaucheria* frequently found along the shore of the cove.

The salinity conditions of the Øresund are complex due to the outflow of brackish water from the Baltic Sea and the inflow of oceanic water from the North Sea. A salinity discontinuity layer is therefore usually developed. At the surface the salinity varies from 8‰ to 24‰, the average being around 14‰. At 5 m depth the same range of salinity is found but the average is around 16‰. At 10 m the salinity varies from 11‰to 32‰, the average being around 21‰. At 15 m salinities from 15 to 33‰ are found with an average of 26‰, and at 20 m the salinity varies from 21 to 34‰ and the average is 32‰.

In the Isefjord area several localities were sampled in a small cove – Vellerup Vig (Fig. 6). Two quantitative samples were taken on fine detritus-rich sand (median grain size: 125 μ) deposited on glacial clay. On this locality a dense growth of *Zostera* was found. Another sample was taken on coarser sand (median grain size: 400 μ) also containing much detritus and with patchy growth of *Zostera*. The associated benthic macrofauna assemblage is typical for Danish estuaries (*Macoma balthica, Mya arenaria, Hydrobia, Corophium, Nereis* spp, etc.).

One sample (salinity 2.8‰) was taken near an outlet of a freshwater stream in a coarse sediment.

Other samples were taken in accumulated drift-weed along the shore. Qualitative observations were made on a larger number of samples collected at various places in the cove.

Quantitative and qualitative samples were taken at the Munkholm Bridge about 10 km south of Vellerup Vig in sulphureta (dominated by purple sulphurbacteria) along the shore (Fig. 6).

The salinity of the Isefjord fluctuates around 19‰. All samples were taken in April 1967 and in May-June 1968.

A few samples of purple sulphurbacteria were taken in marshes along the shore of the Roskildefjord at Frederikssund in May, 1968. These samples were mainly used for experiments.

The last area to be mentioned is the island of Askö. The majority of the quantitative (13) as well as of the qualitative samplings and measurements were carried out in the so-called "Harbour", a small cove off the laboratory sheltered on all sides by rocks (Fig. 7). The locality was described by Fenchel & Jansson (1966).

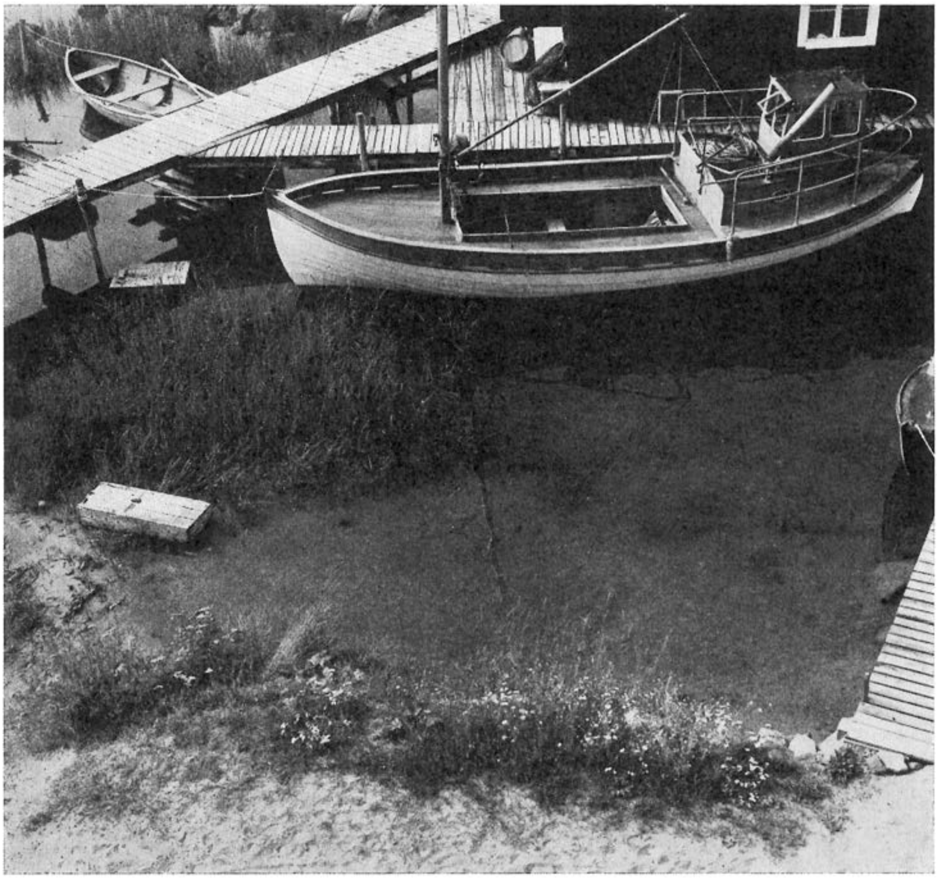

FIG. 7. The main sampling locality in the Askö Harbour.

The sediment is coarse sand but the median grain size varies from 230 to 820 μ in the different samples. There is a patchy growth of *Scirpus* and the sulphide concentrations are high in the sediments in most places. Samples were also taken on places sheltered by *Phragmites*. Here the sediment is glacial clay covered by a thick layer of detritus. Common benthic macroinvertebrates living in the harbour are *Mya arenaria, Cardium lamarcki, Macoma balthica, Corophium, Jaera, Lymnaea* spp, *Theodoxus fluviatilis*, large numbers of oligochaetes and chironomid larvae, and other insects.

In addition a few samples were studied from the other side of the island taken in coarse exposed sand in the splash zone.

The salinity of the Baltic Sea surrounding Askö is constantly around 6‰. All samples were taken in July-August 1966 and June 1967.

In none of the areas studied are lunar tides of any significance.

Practically all material used for laboratory experiments described in the present paper derives from the above mentioned localities.

Sampling and extraction of the fauna

Qualitative samples of bottom sediments were collected by a spoon, a pipette or by the pvc-tubes described below. Sediment samples from deeper water were taken with a bottom grab or a dredge. Samples were studied by shaking a spoonful of sediment with sea-water in a petri dish and collecting the animals under the dissecting microscope or by extracting them with the Uhlig method (see below). Undisturbed samples of the sediment surface were collected and taken to the laboratory in pvc-tubes and studied under the dissecting microscope in reflected light.

The quantitative sampling methods were described in Fenchel (1967) and will only be briefly redescribed here.

All quantitative samples were taken with pvc-tubes with a sharpened cutting edge. The internal diameter of the tubes is 2.1 cm corresponding to an area of 3.5 cm^2 (samples from Askö, summer 1966 were taken with tubes with a diameter of 1.3 cm, however). When sampling in shallow water the tube was pushed into the sediment by hand and corked in the upper end. The tube was withdrawn and corked in the lower end. In the laboratory the upper cork was replaced by another cork with a bore fitted with a rubbertube closed by a clamp.

By removing the lower cork and by opening and closing the clamp, the sediment core was allowed to slide downwards and was cut into segments of 1, 2 or 3 cm length and these were allowed to fall into cylindrical containers with the same diameter as that of the sampling tube. This way of splitting the sediment cores was found superior to the conventional way, i.e. pushing the core upwards, since it preserves the original zonation better.

In samples with non-capillary sediments (clay, clayey sand, mud) the surface layer of detritus was sucked of the sample in the tubes by a pipette.

The sediment segments were used for measuring the vertical distribution of factors such as pH, Eh (in many cases, however, Eh was measured in complete cores), H_2S, grain size, and microflora, and for the extraction of the microfauna. In the latter case subsamples corresponding to 1 cm^2 were mostly taken.

Sampling in deeper water off Ålsgårde was carried out with a Moore and Neill corer modified to support the sediment core from below when ascending through the water (see Fenchel, 1967, for description). The samples from Hornbæk Bay were taken by a SCUBA-equipped diver.

A few samples for enumerating the macrofauna were taken with a 1/10 m^2 Smith-McIntyre bottom grab (deep water) or by a pvc-tube with an area of 85 cm^2 in shallow water.

The sampling method employed for the microfauna is in some respects not fully satisfactory. The pvc-tubes with an internal diameter of 2.1 cm cannot be pushed down more than about 20 cm into most sands without spoiling the zonation due to friction along the sides. This is regrettable since in some localities microfauna may be present below this depth. On the other hand is was not possible to cut sediment cores horizontally with any precision if these had a larger diameter.

It was often desirable to extract and enumerate the microfauna from several cores taken on the same locality and at the same time for statistical reasons but it was rarely possible to treat more than one sample per day due to the large number of animals (more than 4000 ciliates per cm^2, i.e. in one sample, has been found). There is no way of preserving samples and it was not possible to make subsamples smaller than 1 cm^2 in sand.

Extraction of the microfauna was made by the sea-water ice method of Uhlig (1964, 1966). Sand samples are placed in pvc-tubes closed below by nylon gauze with a mesh size which is sufficiently small to prevent the sediment to pass (120 μ was used for fine sands and 265 μ was used for coarser sands). The extraction tube is suspended over an evaporating dish containing sea-water and the sediment sample in the tube is covered with ice made of sea-water with approximately the salinity of the sample. Ice is refilled regularly and the evaporating dishes containing the extracted animals are exchanged every now and then. Extracted animals are in good condition and can be used for initiating cultures.

As a standard the sediment samples (usually 1, 2 or 3 cm^3) were extracted for 1 hour with two refillings of ice.

Discussions on the efficiency and detailed descriptions of the method is found in Uhlig (1964, 1966), Thane-Fenchel (1968) and Fenchel (1967). In the last mentioned paper the efficiency for ciliates was tried by extracting sand samples with a known number of specimens and it was found that efficiency varies between 70 and 90 %.

The method seems to be most efficient in extracting ciliated animals (ciliates, flagellates, turbellarians (for which Uhlig (1964) found an efficiency of 75 %) gastrotrichs and archiannelids) while other groups (nematodes, rotifers, harpacticoids and tardigrades) are less well extracted. Fenchel (1967) found an efficiency of approximately 60 % for nematodes. Thane-Fenchel (1968) found that prolonged extraction and the use of extraction tubes with a larger diameter (3.5 cm as opposed to 2.1 cm used during the present investigation) increases the efficiency for rotifers and nematodes. Uhlig (1964, 1966, and personal communication) also uses the large extraction tubes but in connection with larger volumes of sand (25 cm^3).

It must be concluded that the numbers of rotifers, tardigrades and nematodes given in the present paper may be systematically 10-20 % too low in comparison to the enumerations of the other microfauna.

The animals were counted by removing them one by one with a pipette under the dissection microscope. All samples were extracted and counted on the day of collecting; nearly all samples were taken between 8 and 10 a.m. Core segments were kept at 5-10°C prior to extraction (at room temperature generation time of small ciliates may be down to 2 hours).

Samples of detritus and masses of sulphurbacteria were not extracted but sorted directly in petri dishes under the dissecting microscope.

A few additional samples were taken on some of the localities in order to enumerate the metazoan microfauna more exactly than possible by the small samples used for ciliates. Samples were taken with the normal sampling tubes (3.5 cm^2), usually 4 or 5 samples per locality, or one sample was taken corresponding to an area of 10 cm^2. The samples were extracted according to Jansson (1968a) by washing each sample with about 5 times its own volume of 10 % alcohol in sea-water 7 times and filtering the fluid, after the sand has settled, through a nylon gauze with 80 μ meshes. The animals are transferred to dishes with sea-water for enumeration under the dissection microscope.

Besides these samples for special purpose a total of 83 quantitative samples have been collected and counted. They contained in total about 90,000 ciliates in addition to representatives of other animal groups.*

One group of animals, the foraminifera, were not enumerated since this would require a special technique. These animals were probably present though in relatively small numbers in most localities studied. For description and quantitative data on the foraminifera in the Hornbæk Bay area the reader is referred to Hansen (1965).

The vertical distribution of diatoms in the sediment was studied in two ways. In the Helsingør Beach, where the diatom flora is relatively poor, the number of living diatoms per 50 sand grains were counted under the microscope on sand samples from different depths in the sediment. In other localities sand from different depths (usually each cm) was shaken with a constant volume of water and a constant volume of this suspension was placed on a microscopic slide, covered by an 18 × 18 mm coverslip and the average number of living diatoms per field of vision (430 ×, phasecontrast) was used as a measure of relative abundance.

Identification of species

Elton (1962) in his classical textbook on ecology rightly stresses the importance of correct identifications of all species in ecological work. Unfortunately fully satisfactory identifications are for several reasons often difficult to obtain when working with microfauna. The number of species encountered is high and I have mainly concentrated on the ciliates.

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* Photocopies of 79 tables showing the countings and measurements of most of these samples can be obtained from Marine Biological Laboratory, Strandpromenaden, DK-3000 Helsingør, Denmark. Price 7 $ including postage.

In spite of many recent significant contributions to the taxonomy of marine ciliates (Agamaliev, 1967; Borror, 1963a, b, 1965a, b; Dragesco, 1960, 1963a, b, 1965a, b, 1968a; Fauré-Fremiet, 1950a, 1951b, 1962, 1963a, b; Raikov, 1962; Tuffrau, 1954, 1960) many groups still lack sufficient taxonomic treatment. This is unfortunately also the case with some quantitatively important genera like *Tracheloraphis, Loxophyllum, Strombidium, Mesodinium,* and several genera within the Oxytrichidae.

A number of previous works, viz Kahl (1928, 1931), Kirby (1934), Sauerbrey (1928), and Villeneuve-Brachon (1940), are still important for identification and the large monographic work of Kahl (1930-35) is indispensable for identifying ciliates.

The greatest obstacle for making exact and complete identifications of quantitative samples lies in the fact that all methods available for preservation of ciliates and many other microfauna groups are far too time-consuming to be of any help. Thus all animals in the samples had to be counted and identified simultaneously on the day of collecting and time did not allow more than a few observations under the compound microscope. (For this purpose the "Roto-compressor" described by Heunert & Uhlig, 1966, in combination with phase-contrast is of great help).

Thus the majority of the animals were identified under the dissecting microscope (magnifications: 25-50×, transmitted light). This is possible for most species, but a number of forms, especially the small species of *Uronema, Cyclidium,* many oxytrichids, *Euplotes, Aspidisca,* could usually not be identified in this way.

In non-quantitative samples, in the succession experiments, etc. a larger number of species were identified.

In spite of these difficulties the majority of the ciliates in the quantitative samples were identified to species.

Several hitherto undescribed ciliates were found during the investigation. These are mentioned here by their generic name only.

Investigations of food relationships

The food of marine benthic ciliates was the topic of a previous paper (Fenchel, 1968a). Here information about the food of 260 species was brought together.

The findings for the most important forms are given in Table 1. The construction of food chains in the present investigation is based on that paper. Information about the food of other groups derives mainly from the literature referred to in the text.

TABLE 1. The food of 84 species or genera of ciliates playing an important role in marine sediments. XXX, food item exclusively or dominantly eaten; XX, important food item; X, food item of secondary importance.

	Diatoms	Phytoflagellates	Unicellular cyanophyceans	Filamentous cyanophyceans	Filamentous sulphurbacteria	White sulphurbacteria	Purple sulphurbacteria	Other bacteria	Small zooflagellates	Carrion	Mechanically damaged living animals	Ciliates	Small metazoans
Coleps spp.	.	XX	.	.	.	.	.	.	.	XX	XX	X	.
Helicoprorodon gigas	.	.	.	.	.	.	.	.	.	.	.	XXX	.
Chaena spp.	.	.	.	.	.	.	.	.	.	.	.	XXX	.
Lacrymaria marina	X?	X	.	.	.	.	.	.	.	.	.	XXX	.
Lacrymaria spp.	.	X	.	.	.	.	.	.	.	.	.	XXX	.
Plagiopogon loricatus	.	XX	.	.	.	.	.	.	.	XX	.	.	.
Prorodon discolor	XX	XX	.	.	.	.	.	.	.	XX	XX	X	X
P. morgani	.	XXX	.	.	.	.	.	.	.	.	.	.	.
Pseudoprorodon arenicola	.	XXX	.	.	.	.	.	.	.	.	.	X	.
Tracheloraphis kahli	XX	XX	.	.	.	.	.	.	.	.	.	.	.
Tracheloraphis spp.	XX	XX	.	.	.	.	.	.	.	.	.	XX	.
Homalozoon caudatum	.	XXX	.	.	.	.	.	.	.	.	.	.	.
Paraspathidium fuscum	.	XXX	.	.	.	.	.	.	.	XX	XX	X	.
Mesodinium pupula	.	XX?	.	.	.	.	.	.	.	.	.	.	.
Litonotus spp.	.	.	.	.	.	.	.	.	.	.	.	XXX	.
Loxophyllum spp.	.	.	.	.	.	.	.	.	.	.	.	XXX	.
Ciliofaurea mirabilis	.	XXX	.	.	.	.	.	.	.	.	.	.	.
Remanella margaritifera	XX	XX	.	.	.	.	.	X?	X	.	.	X	.
R. rugosa	XX	XX	.	.	.	.	.	.	.	.	.	X	.
R. brunnea	XX	XX	.	.	.	.	.	.	X	.	.	X	.
R. gigas	XXX	.	.	.	.	.	.	.	.	.	.	.	.
Kentrophorus spp.	X?	.	.	.	.	.	.	.	.	.	.	.	.
Geleia fossata	XX	XX	.	.	.	.	.	.	.	.	.	XX	.
G. decolor	X	XX	.	.	.	.	.	.	.	.	.	XX	.
G. orbis	.	.	.	.	.	.	.	.	.	.	.	XXX	.
G. nigriceps	XX	XX	.	.	.	.	.	.	.	.	.	.	.
Chilodontopsis elongata	XXX	.	.	.	.	.	.	.	.	.	.	.	.
Chlamydodon triquetrus	XXX	.	.	.	.	.	.	.	.	.	.	.	.
C. mnemosyne	X?	.	.	XXX	.	.	.	.	.	.	.	.	.
C. obliqus	.	.	.	XXX?	.	.	.	.	.	.	.	.	.
Lynchella gradata	XXX	.	.	.	.	.	.	.	.	.	.	.	.
Trochiloides recta	X	.	.	.	XXX	XX	.	.	.	.	.	.	.
Discotricha papillifera	XXX	.	.	.	.	.	.	.	.	.	.	.	.
Plagiopyla frontata	XX	X	X	.	.	XX	XX	X	.	.	.	.	.
Sonderia schizostoma	X	.	.	XX	XXX	X	.	.	.	.	.	.	.
S. vorax	XX	X	.	X	X	X	XX	.	.	.	.	.	.
S. sinuata	XX	.	.	XX	X	X	XX	.	.	.	.	X	.
S. cyclostoma	.	.	.	.	.	XX	XX	.	.	.	.	.	.
Conchostoma longissimum	.	XXX	.	.	.	.	.	.	.	.	.	.	.
Uronema spp.	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Cohnilembus spp.	.	.	.	.	.	.	.	XXX	.	.	.	.	.

	Diatoms	Phytoflagellates	Unicellular cyanophyceans	Filamentous cyanophyceans	Filamentous sulphurbacteria	White sulphurbacteria	Purple sulpurbacteria	Other bacteria	Small zooflagellates	Carrion	Mechanically damaged living animals	Ciliates	Small metazoans
Helicostoma notatum	.	.	.	.	.	.	.	.	.	.	XXX	.	.
Ophryoglena spp.	.	.	.	.	.	.	.	.	.	.	XXX	.	.
Cardiostomella vermiforme	X	.	.	.	.	.	XX	XX	.	.	.	.	.
Paramecium calkinsi	.	XX	XX	.	.	XX	XX	.	.	.	.	.	.
Frontonia marina	XXX	X	X	X	.	.	.	.	.	.	.	X	X
F. microstoma	XXX	.	.	.	.	.	.	.	.	.	.	.	.
F. arenaria	XXX	X	X	.	.	.	.	.	.	.	.	X	.
F. algivora	X	.	.	XXX	.	.	.	.	.	.	.	.	.
Pleuronema marinum	XX	XX	X	.	.	.	XX	XX	.	.	.	.	.
P. coronatum	X	X	.	.	.	XX	XX	XX	.	.	.	.	.
Cyclidium citrullus	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Cristigera media	.	.	.	.	.	XX	XX	.	.	.	.	.	.
Blepharisma clarissimum	XXX	X	X	.	.	.	X	.	.	.	.	.	.
B. salinarum	X	.	.	.	.	XX	XXX	.	.	.	.	.	.
Gruberia sp.	XX	.	X	.	.	XX	XX	.	.	.	.	.	.
Parablepharisma pellitum	X	X	X	.	.	.	XXX	X	.	.	.	.	.
P. chlamydophorum	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Spirostomum teres	XX	XX	.	.	.	.	.	XX	.	.	.	.	.
Condylostoma remanei	XX	XX	.	.	.	.	.	.	X	.	.	XX	X?
C. patulum	XX	XX	X	.	.	.	X	.	X	.	.	XX	X?
Peritromus faurei	X	X	X	.	.	XX	XX	.	.	.	.	.	.
Metopus contortus	X	.	.	.	.	XX	XX	XX	.	.	.	.	.
M. vestitus	.	.	.	.	.	XX	XX	X	.	.	.	.	.
Caenomorpha levandri	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Saprodinium halophila	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Myelostoma bipartitum	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Strombidium sauerbreyae	XXX	.	.	.	.	.	.	.	.	.	.	.	.
S. latum	XXX	.	X	.	.	.	.	.	.	.	.	.	.
Holosticha arenicola	.	.	.	.	.	.	.	XX	XX	.	.	.	.
H. kessleri	XXX	.	.	.	.	.	X	.	.	.	.	.	.
Keronopsis rubra	XX	XX	X	.	.	.	.	.	.	.	.	.	.
Trachelostyla pediculiformis	.	.	.	.	.	.	.	XXX	.	.	.	.	.
Urostrongylum caudatum	XXX	.	.	.	.	.	.	.	.	.	.	.	.
Euplotes harpa	XX	.	.	.	.	.	XX	X	.	.	.	.	.
E. vannus	.	.	.	.	.	.	.	XX	XX	.	.	.	.
E. moebiusi	.	.	.	.	.	.	.	XX	XX	.	.	.	.
E. elegans	X	.	.	.	.	XX	XX	.	.	.	.	.	.
Discocephalus ehrenbergi	XX	XX	.	.	.	.	.	.	.	.	.	.	.
Swedmarkia arenicola	.	XXX	.	.	.	.	.	.	.	.	.	.	.
Diophrys scutum	XXX	X	X	X	.	X	X	.	.	.	.	X	.
D. appendiculata	XX	.	X	.	.	.	.	X	.	.	.	.	.
Uronychia transfuga	XX	XX	.	.	.	.	.	.	XX	.	.	XX	.
Aspidisca spp.	.	.	.	.	.	.	.	XXX	.	.	.	.	.

Estimating the weight of the microfauna

The weights of ciliates were estimated as described in Fenchel (1967). Single ciliates were pressed in the Roto-Compressor (Heunert & Uhlig, 1966) until they had plane parallel sides. The thickness was measured by the scale of the fine adjustment of the microscope, the outline traced by a camera lucida and the volume was calculated. The specific gravity of ciliates was considered to be 1. Variable numbers of specimens of different species were measured in this way and the results given as a graph of the relation between length and volume from which the weight of different species could be estimated. Weight data of micro metazoa were taken from Renaud-Debyser & Salvat (1963) and are average values for whole groups such as nematodes, turbellarians, etc.

Mechanical analysis of the sediment

This was in the main carried out according to Morgans (1956). Fresh sediment samples were washed in distilled water (with care not to lose the silt and clay fractions), oven-dried at 110°C and sieved through a number of sieves according to the Wentworth Scale (mesh sizes: 2000 μ, 1000 μ, 500 μ, 250 μ, 125 μ, 62 μ). The material passing the 62 μ sieve was considered as the silt and clay fraction and was not treated further. The fractions were weighed and the results plotted cumulatively (Figs. 2-3). The median grain size (Mdφ) was read directly on the graph. As a measure of sorting the "Phi quartile deviation" (QDφ) was used. The grain sizes were converted to the "Phi scale" ($-\log_2$ of the grain size) and $QD\varphi = \frac{Q3\varphi - Q1\varphi}{2}$, where Q3φ and Q1φ are the values for Phi of the third and the first quartile, respectively. A small QDφ thus signifies a high degree of sorting.

Other measurements of physical and chemical factors

Salinity was measured as specific conductivity with a Philips conductivity cell (Pr 9513) in connection with a Wheatstone bridge provided with an a.c. source and earphones (Normameter 185 RW, Norma, Vienna) and the values of conductivity were converted to salinity by tables (National Inst. Oceanogr. G. B. and Unesco, 1966).

For pH measurements the following pH-meters were used: Beckman N pH-meter and Radiometer 4 and 25 models fitted with Beckman 41263 or Radiometer K401 glass electrodes and Beckman 41240 or Radiometer K4025 calomel electrodes.

For measurements of redox potentials the above mentioned pH-meters were used as potentiometers fitted with the same calomel electrodes as reference electrodes. As indicator electrodes platinum electrodes were used. The reference electrodes were connected with sediment samples through a KCl-agar bridge.

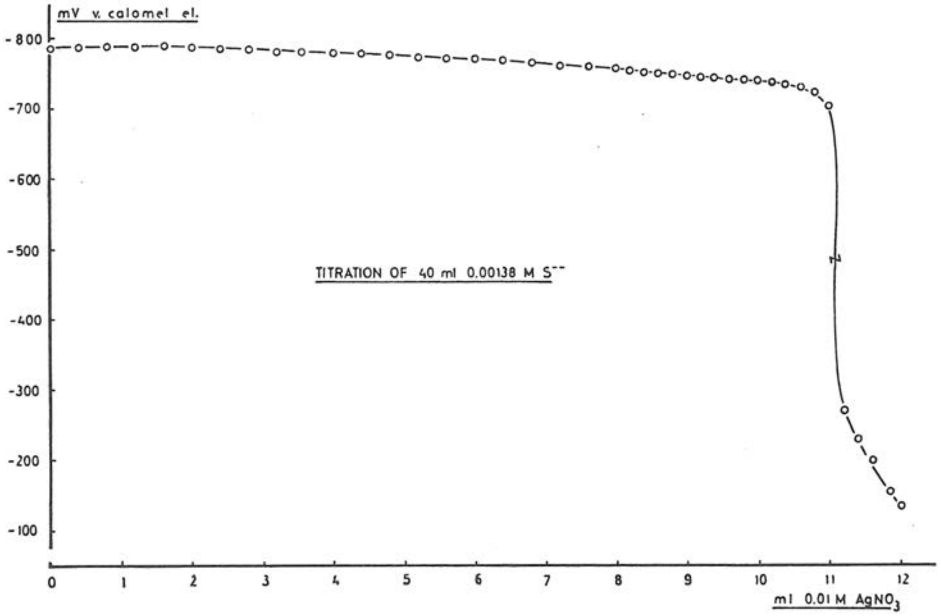

FIG. 8. An example of a titration curve from a potentiometric H_2S titration. The endpoint of the titration is indicated.

Some redox measurements were made with a Beckman 39273 Pt electrode; for the majority of measurements the Pt electrodes used were made by the author by sealing 0.7 mm Pt wire into 4 mm thick pyrex tubing. These electrodes could more easily be inserted into sediment cores. Pt electrodes were cleaned with scouring powder and distilled water between the measurements. – A detailed discussion on the theory, significance and methods of measurements of redox potentials in sediments is given in Chapter 5.

Total H_2S was measured by potentiometric titration using the Radiometer 25 pH-meter as potentiometer, silver wire as indicator electrode and a calomel as reference. The sample was added to 20 ml N KOH in a beaker and titrated with 0.01 M $AgNO_3$. A typical titration curve is shown on Fig. 8. Once the endpoint is determined it is not necessary to plot a titration curve but only to read the burette when the endpoint is reached.

When measurements were made in sediments, cores as used for quantitative fauna samples were taken to the laboratory and core segments of desired length were dropped into the KOH solution and titrated at once while constantly stirring the suspension. This procedure gives minimal aeration of the sample. When the porosity of the sediment and the sample volume is known, the H_2S contents of the interstitial water is easily calculated.

Water samples were taken with a pipette, added to the KOH solution and titrated.

KOH is added to the sample prior to titration in order to transform the larger part of the H_2S and HS^- into S^{--}; this makes the solution more stable and also improves titration. (Only S^{--} is titrated by Ag^+ so that it is important that the relative concentration of S^{--} is high during the titration. Since the acid/base balance is displaced during the titration the sum of H_2S, HS^- and S^{--} is actually titrated).

The sum of H_2S, HS^- and S^{--} expressed as g H_2S/l is $17.04 \times \frac{mb}{a}$ when m is the molarity of the Ag^+ solution, b the volume of the titrant, and a the volume of the sample.

Organic matter in sediments was determined by oven drying at 110°C, weighing, ignition at 550°C in a muffle oven and repeated weighing and was expressed as weight percentage of the total dried sample.

Oxygen contents were measured with galvanic oxygen electrodes built by the author according to the descriptions given in Mancy & Westgarth (1962) and Duxbury (1963) using Ag and Zn as electrode materials, N KOH as electrolyte and polyethylen as membrane.

The current produced by these electrodes, which is directly proportional to the oxygen tension, was measured with a μ-ammeter.

The galvanic oxygen electrodes, which work well when measuring in free water in the field and under experimental conditions (O_2 uptake of sediments, respiration and photosynthesis of the undisturbed sediments, etc.), could unfortunately not be used in the sediments due to the relatively large size of the electrode and the rise of membrane rupture.

For obtaining a crude picture of the vertical distribution of oxygen in the sediments the polarographic method described by Lemon & Erickson (1954) was used. By applying a voltage of about 0.8 V between a reference electrode (Ag-AgCl) used as anode and a naked cylindrical platinum electrode (constructed as the above mentioned redox electrodes) used as cathode, a current is created which is proportional to the number of oxygen molecules reduced per sec at the cathode (Odén, 1962). The value obtained may be expressed as g $O_2 \cdot cm^{-2} \cdot sec^{-1}$. It is a measure of the availability of oxygen which is dependent on oxygen tension, diffusion rate of dissolved oxygen (again dependent on mechanical structure of the sediment and temperature), and on turbulence. The measured value was designated "oxygen diffusion rate" by Lemon & Erickson, but it seems to be more dependent on turbulence than on the diffusion rate of oxygen and the term "oxygen availability" is therefore used here. The electrode is extremely sensitive to poisoning, especially in sediments containing sulphide, but poisoning is to some extent counteracted by grinding of the platinum electrode against sand grains in sediments.

The method has proved valuable in terrestrial sediments and in sandy beaches (Jansson, 1966a, b, 1968a, b; Fenchel et al., 1967) for investigating the oxygen climate in beaches and coastal groundwater.

In completely water logged soils with little movement of the interstitial water the measurements will in the main give a picture of the distribution of oxygen and a number of readings were made on the vertical distribution of the "oxygen availability" in Julebæk Beach, Nivå Bay, and in Askö Harbour.

As mentioned above the electrode is very sensitive to turbulence and when oxygen tension and temperature is kept constant it may be calibrated as a flow meter. In this way water flow in sands was studied.

Methods employed in experiments with artificial sediments, O_2-uptake of sediments, etc. are described together with the results in Chapters 5, 7 and 9.

3. MECHANICAL PROPERTIES OF SEDIMENTS AND THEIR SIGNIFICANCE FOR THE FAUNA

The grain sizes of sediments constitute a factor which is easy to quantify and it has therefore attracted much attention by workers on microbenthos (e.g. Jansson, 1967; Gray, 1967; Renaud-Debyser, 1963; Webb, 1958; Wieser, 1959 and others referred to below). Regrettably, some authors have considered correlation between the granulometry of sediment samples and the distribution of certain animals almost synonymous with the ecology of microbenthos.

The grain size factor is extremely complex and it is therefore often difficult to interprete the correlations found. It is probable that this factor in some cases influences the animals indirectly or that the distribution of various animals and of grain sizes is governed by some common factor which has not been studied.

Usually the following measures are considered: the median grain size (easily read on cumulative curves), the grade of sorting ($QD\varphi$ or a similar measure) and often the percentage of fine particles for example those smaller than 62 μ (silt and clay) or those smaller than 250 μ in order to emphasize the significance of small particles for the properties of the sediment.

These easily available data on sediment samples are then taken as measure of several significant properties of the sediments: The total surface area, porosity, permeability, capillarity, diffusion rate of dissolved substances in interstitial water and the size of the individual interstitia. Geometrical packing and the shape of the sand grains are rarely measured even though they influence the above mentioned properties.

ZoBell (1943) demonstrated that the bacterial growth in sea-water samples is dependent on the internal surface of the container and that growth could be increased by adding glass beads or sand to the samples. These observations were explained by the fact that many bacteria are sessile and, more important, that dissolved organic compounds are concentrated by adsorbtion to surfaces. The amounts of organic substances in sea-water are very small but adsorbed to surfaces the concentration becomes sufficiently large for utilization by bacteria.

Wood (1965) has shown that microorganisms tend to adsorb to fine sediment particles and also that sulphide containing sediments oxidize more slowly when high amounts of silt are present. Also a positive correlation between contents of organic compounds (and of organic nitrogen) and the contents of fine sediment particles has been demonstrated (Dietrich et al., 1965; Newell, 1965). This may partly be due to adsorbtion phenomena and partly to the fact that organic detritus is more liable to be sedimented where conditions for sedimentation are better, and also to the fact that fine sediments have a lower permeability which means slower oxidation of organic matter. There is certainly often no correlation between contents of organic material and grain size in many shallow water sediments where large amounts of drift-weed become embedded in the sediments during storms.

Since the internal surface area is of significance for the fauna it would be of interest to be able to calculate its size. If the sediment consisted of equally large spheres the surface area would be $k \cdot \frac{1}{r}$ where k is a constant dependent on the geometrical packing and r is the radius of the spheres, since the surface area of each grain is $k_1 \cdot r^2$ and the number of spheres per unit volume is $k_2 \cdot \frac{1}{r^3}$. Prenant (1960) constructed a formula according to which the number of grains in each sieve fraction may be calculated. If the grains are considered to be spheres consisting of quartz (Prenant also calculated constants for other materials and shapes) then $\log n = -0.14225 - \frac{5 \log d_1 + \log d_2}{2}$ where n is the number of grains retained by a sieve and d_1 and d_2 are the mesh sizes in cm in between which the sand is retained ($d_1 > d_2$). If the average radius of the grains retained by d_2 is considered to be $\sqrt{\frac{d_1 d_2}{4}}$ the surface of each fraction can be calculated and by addition the total internal surface of the sand sample is found.

The internal surfaces of 10 cm³ from different localities in the northern part of the Øresund (all well sorted sands) were calculated (Fig. 9). The data show that the internal surface of natural sands are equal to $k\frac{1}{\mathrm{Md}\varphi}$ and is thus easily estimated from sieving data. Deviations are due to different degrees of sorting in the different samples.

If we consider the biologically active part of the sediment to extend to a depth of 20 cm, 1 cm² bottom consisting of a sand with a median grain size of for example 300 μ will actually have a surface area of about 1/10 m².

The porosity of sediments, i.e. the total pore volume, has been studied by several authors (viz Frazer, 1935; Webb, 1958). These authors found the porosity to be dependent on a number of factors in a complex way and porosity cannot be predicted precisely from sieving data, but it can of course easily be measured

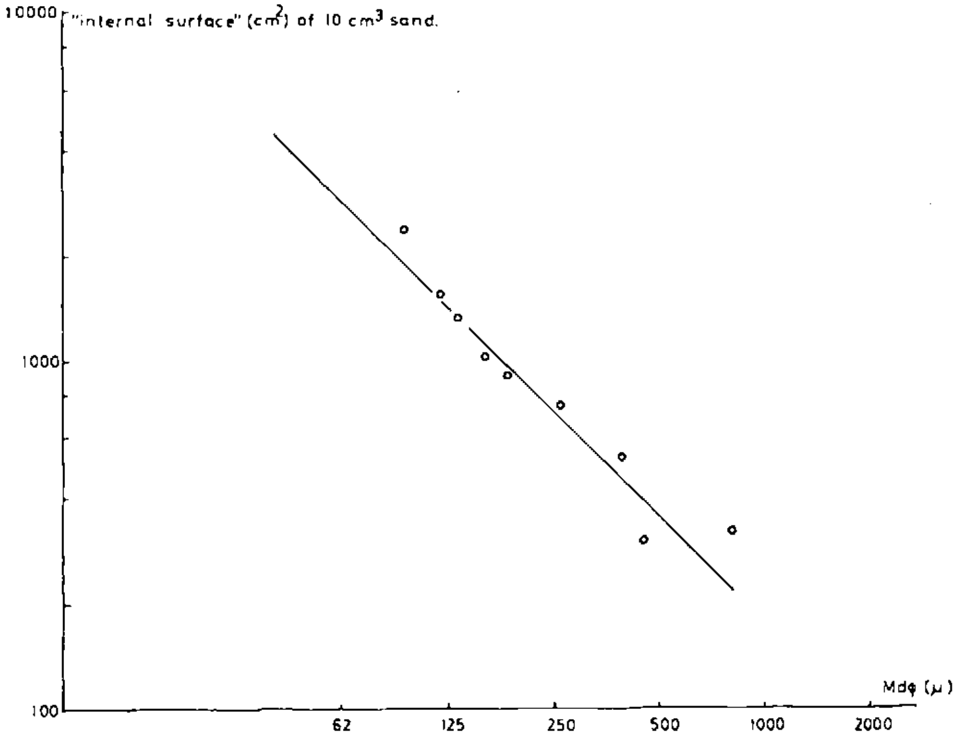

FIG. 9. The "internal surface" of 10 cm³ sand from 9 localities plotted against the median grain size.

directly. Sorting plays a large role as does the shape of the sand grains. Small grains below a certain "critical size" tend to decrease porosity by filling the spaces between larger grains, but grains above the critical size tend to increase porosity by pressing the larger grains apart thus increasing the size of the interstitia. Porosity will not be discussed further here. In all sand samples studied during the present investigation porosity varied between 25 and 40 % (volume), the highest values were found in well sorted sands.

By permeability is understood the speed by which water passes through a cylinder containing the sediment sample. Permeability was studied in detail by Frazer (1935) and by Webb (1958). Permeability is, in contrast to porosity, greatly influenced by the absolute sizes of the sand grains. If all grains are spheres of equal size, permeability is proportional to the square of the diameter. Addition of small particles to sediments decreases permeability strongly. The presence of 2.5 % silt decreases permeability 4 times (Webb, 1958). This author also demonstrated that addition of particles smaller than 200 μ decreases permeability strongly.

The permeability of sediments is very important for the microfauna since it determines the behaviour of the interstitial water and thus the availability of oxygen. Webb (1958) demonstrated a correlation between the depth of the "black layer" (i.e. the sulphide containing layers) and the permeability of the sediments in Lagos Lagoon. Brafield (1964) found a correlation between a high percentage of fine grains and low contents of oxygen in the interstitial water of beaches.

Such relations were also often evident during the present investigation. Samples taken off Ålsgårde at 15, 20 and 25 m depth all consisted of medium sand (Mdφ: 310-480 μ). At 15 m the contents of clay and silt were about 0.02 %, at 20 m 0.2 % and at 25 m 4 %. At 15 m the sediment was oxidized throughout, at 20 m Eh decreased significantly at about 5 cm depth and at 25 m the sediments were reducing already at 0.5 cm depth. The oxidized condition prevailing deep in the sediment is an important feature of the coarse, well sorted "*Amphioxus* sand" with its characteristic micro- and macrofauna. This sediment type is not developed characteristically in the Øresund and has therefore not been studied during the present investigation. Clays and muds are often reducing a few mm below the surface while in sand there is usually at least a layer of 0.5 cm oxidized surface sand unless large amounts of organic material are present in connection with lentic conditions. These correlations are undoubtedly due to the permeability of the sediments but the relations can often be overshadowed by other factors like amounts of organic material, water movements above the sediment (see Chapter 5).

Since water movements within the sediments are of great significance for the transport of dissolved substances it is of interest to know how deep in the sediments water movements are formed by water currents above the sediments. This was studied in the case of a laminar water flow above the sediment. Sand samples were placed in a layer of about 10 cm in an oblong aquarium constructed so that the water flows in laminar currents through it. Tap water was used for the water flow. A naked oxygen electrode (a 0.5 cm long horizontally orientated 0.7 mm Pt-wire) was calibrated as a flow meter by using stained water and a stop watch (see Chapter 2, p. 29). Temperature and oxygen contents of the tap water was considered constant during each experiment. The water flow above the sediment was adjusted to 8 cm/sec and the water current was measured at various depths in the sediment and just above the sediment surface. Some of the experiments are shown in Fig. 10. Here sands retained by a 500 μ, a 250 μ and a 125 μ sieve and a natural sand sample is shown. In all cases the water flow was reduced to about 50 % already at the surface and no water movements could be measured at depths below 0.5, 0.3, 0.2, and 0.4 cm, respectively. Even though water currents could be measured at somewhat greater depths in coarser sands it must be concluded that in all types of sediments laminar water flow above the sediment will only influence conditions a few mm below the surface.

In exposed beaches and in shallow water when waves are present the sediment will be exposed to turbulent water currents which will undoubtedly influence conditions considerably deeper in the sediments according to permeability.

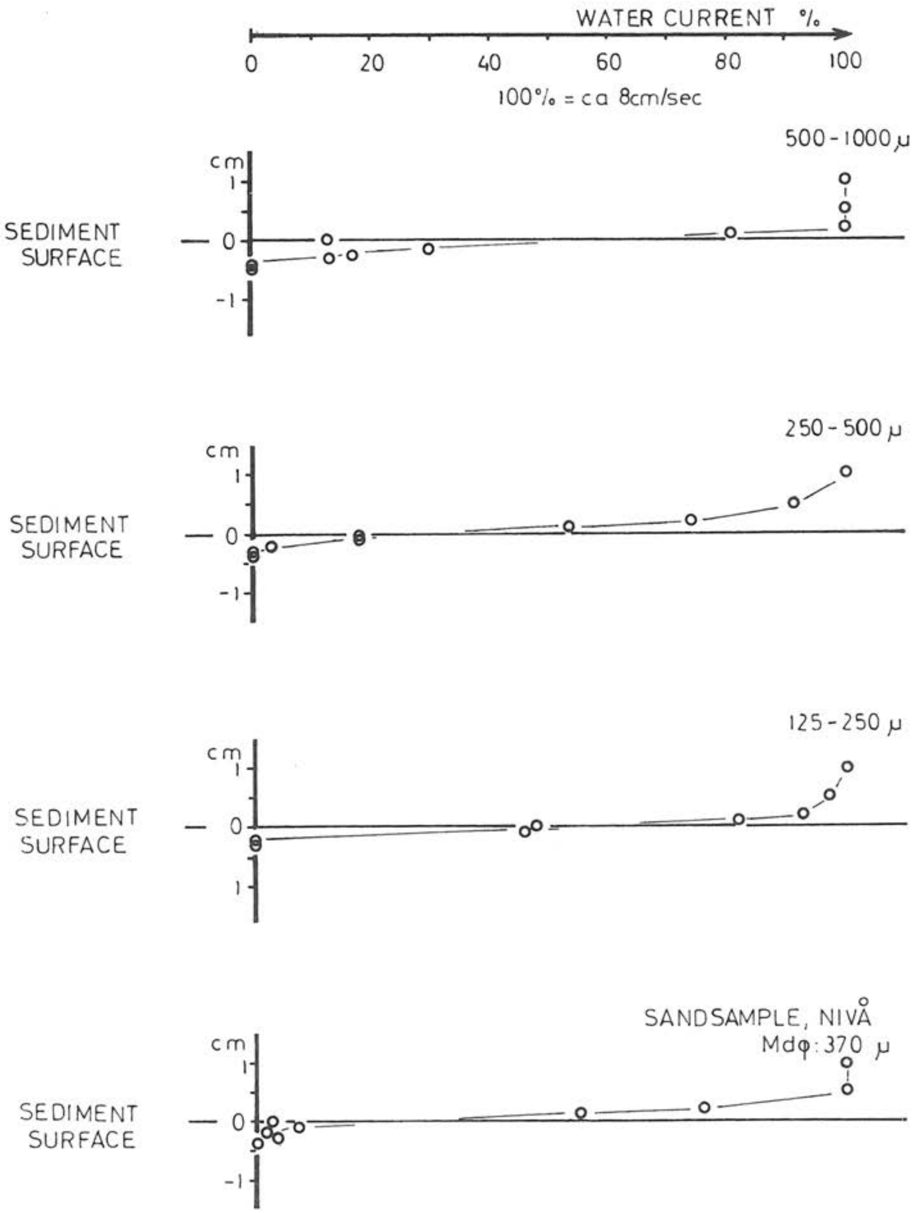

FIG. 10. The water movements in sands exposed to a laminar water flow.

The influence of waves on the oxygen climate of the interstitial water of beaches was studied by Jansson (1968b).

The diffusion rate of dissolved substances in various kinds of sediments has not, to my knowledge, been studied though this must be of great significance as the interstitial water at a certain depth is stagnant in most sediments. It seems probable that the diffusion rate is smaller in finer sediments and in sediments with a low porosity.

Capillarity is very important for the ecology of beaches since this property, together with permeability and porosity, determines the water content of sediments not permanently covered by water. The significance of this was demonstrated by Jansson (1967). However, in the permanently flooded sediments studied by me capillarity is not important. Capillarity is mainly a function of grain size.

The size of individual interstitia is the property of sediments which has attracted most attention by students of the interstitial fauna. Already Remane (1933) demonstrated several morphological features characteristic of interstitial animals (oblong shape, small size, etc.) which are explained as adaptations to life in small interstitia. Since then much work has been concerned with adaptation to life in interstitia of various sizes (see Swedmark, 1964; Renaud-Debyser, 1963, and papers cited below for references). Also the ability to move in different kinds of sediments is probably related to the size of the interstitia (Wieser, 1959) and experiments demonstrating preference of certain interstitial animals for certain sediments (Gray, 1967; Jansson, 1966a, 1967) probably support this view.

The size of the interstitia cannot be predicted precisely from sieving analysis alone since also shape of the sand grains and their geometrical packing is of significance. The property is related to porosity, but is also dependent on absolute grain sizes so that generally larger median grain sizes and higher degrees of sorting signify larger volumes of the interstitia.

Wieser (1959) correlated the distribution of a number of micrometazoan species with median grain size of sediments from several localities in Puget Sound. He found that most interstitial animals which move by "sliding" do not occur in sands with a $Md\varphi < 200\ \mu$ and no sliders were found in sands finer than 100 μ while "burrowers" (mainly nematodes) occur in finer sands. However, the author believes that some nematodes are able to move by sliding in fine sediments too, due to their shape.

The results of this sort of investigation must be interpreted with caution since only one factor is studied without taking notice of chemical and biological conditions. This also applies to the present investigation of the distribution of the microfauna according to grain size (Figs 11-13). However, in order to reduce the influence of other factors only a number of localities from the northern part of the Øresund were compared excluding samples from the fertile Julebæk Beach and Nivå Bay with their strong development of reducing conditions and the presence of microfauna living on rather than in the sediments. There remain 13 localities

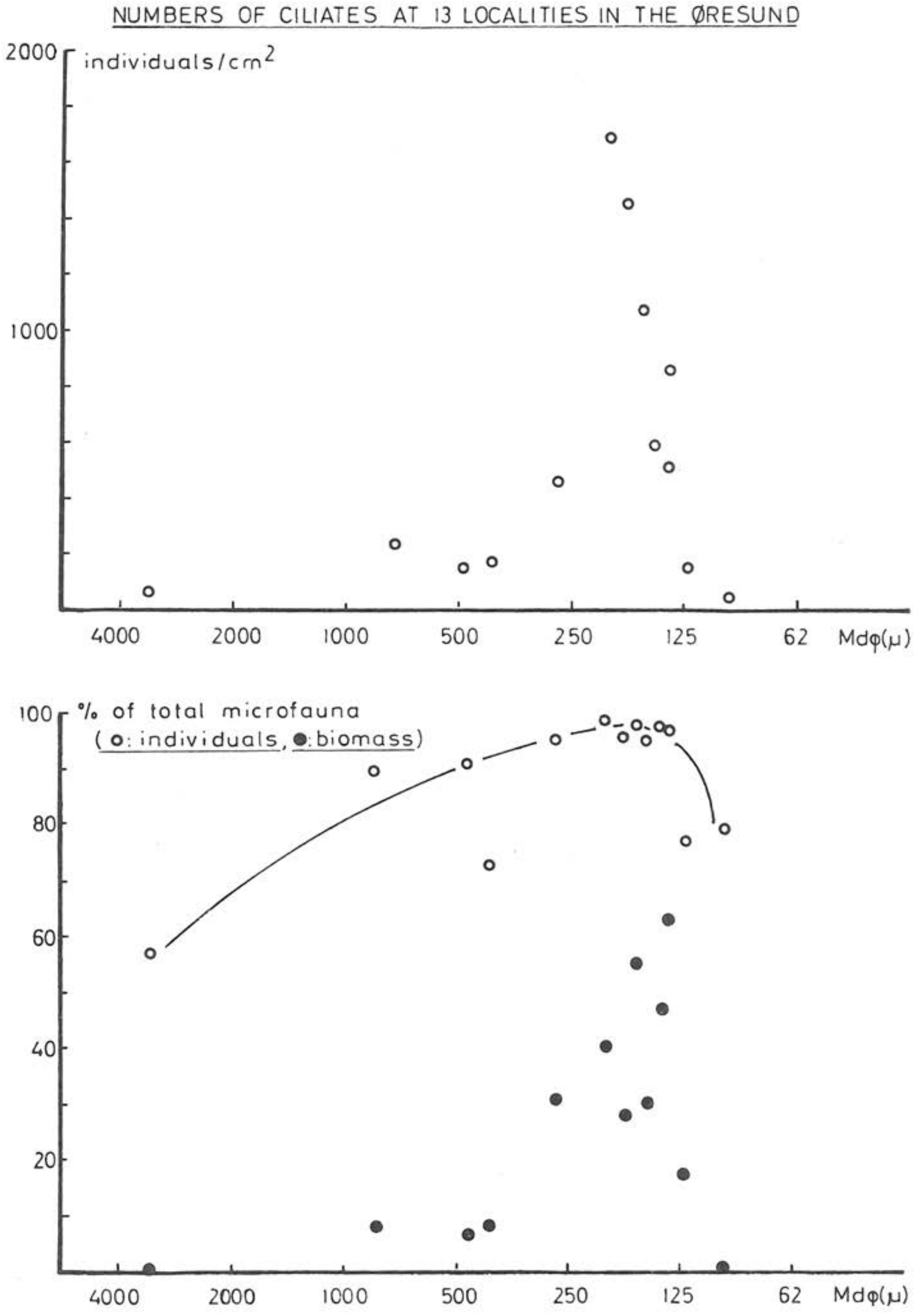

FIG. 11. The occurrences of ciliates in sands of different median grain sizes in 13 localities in the Øresund.

from the Øresund from 0.5 to 22 m depth with relatively clean, well sorted sand ranging in median grain sizes from 95 to ca. 3000 μ. In spite of the attempt to choose localities rather identical in other respects this was not quite possible as some of the sediments – mainly the coarser ones – were oxidized throughout while others showed a distinct redox discontinuity layer at some depth. The points on the graphs represent either single samples of 1 cm² or the average calculated from more samples from the same locality.

Fig. 11 shows that the ciliates have a maximum occurrence in fine sands (150-250 μ). In finer sands their numbers decrease drastically and also in coarser sands they occur in smaller numbers. The same is seen when their relative occurrence is considered; in fine sand they constitute more than 95 % of the animals present and 30-60 % of the biomass of the microfauna. Their maximum of relative importance is, however, somewhat displaced towards finer sands.

The explanation for this distribution lies mainly in the fact that many ciliate species are better adapted to life in small interstitia than are metazoans. Most ciliates are smaller than the great majority of the micrometazoans. It is often stated (see for example Swedmark, 1964) that in the interstitial environment the ciliates belong to the largest animals present. This, however, is not true. Many sand ciliates are extremely long and flattened or slender and actually ciliates have a volume 10 to 1000 times smaller than that of most interstitial metazoans (see Chapter 10). Only few metazoan groups (rotifers, chaetonotoid gastrotrichs) comprise forms smaller than 10^{-6} cm³ while the majority of the ciliates are smaller than 10^{-6} cm³. A few species: *Helicoprorodon gigas, Pseudoprorodon arenicola* and few others, do attain body volumes a little above 10^{-6} cm³ and are thus comparable with small turbellarians, harpacticoids, etc. Very small ciliates (*Cyclidium, Uronema,* etc.) are only 10-30 μ long with body volumes of $1\text{-}5 \times 10^{-10}$ cm³, sizes much below those of metazoans.

Although many interstitial metazoans show remarkable morphological adaptations there seems to be a limit to their morphological plasticity while interstitial ciliates have evolved quite grotesque body shapes like the few microns thick *Remanella, Kentrophorus* and *Loxophyllum* or the extremely long *Tracheloraphis* (see Figs 40-41). Only nematodes are comparable to the ciliates in evolving thread like body shapes, but nematodes are very stiff and rigid compared with ciliates. Thus it is understandable that ciliates are well adapted to and play a dominant role in fine sands.

The fact that many ciliate species (and also nematodes) can endure or will even demand reducing and anaerobic conditions probably also explains their dominance in fine sands.

The ciliates cannot be suspected to burrow in the substrate; they are all ciliary sliders or are free-swimming in the interstitia. Thus Fig. 11 shows that for well sorted sands without large amounts of silt and clay the lower limit for interstitial animals is found at a grain size of 90-100 μ. Organisms living in finer sediments

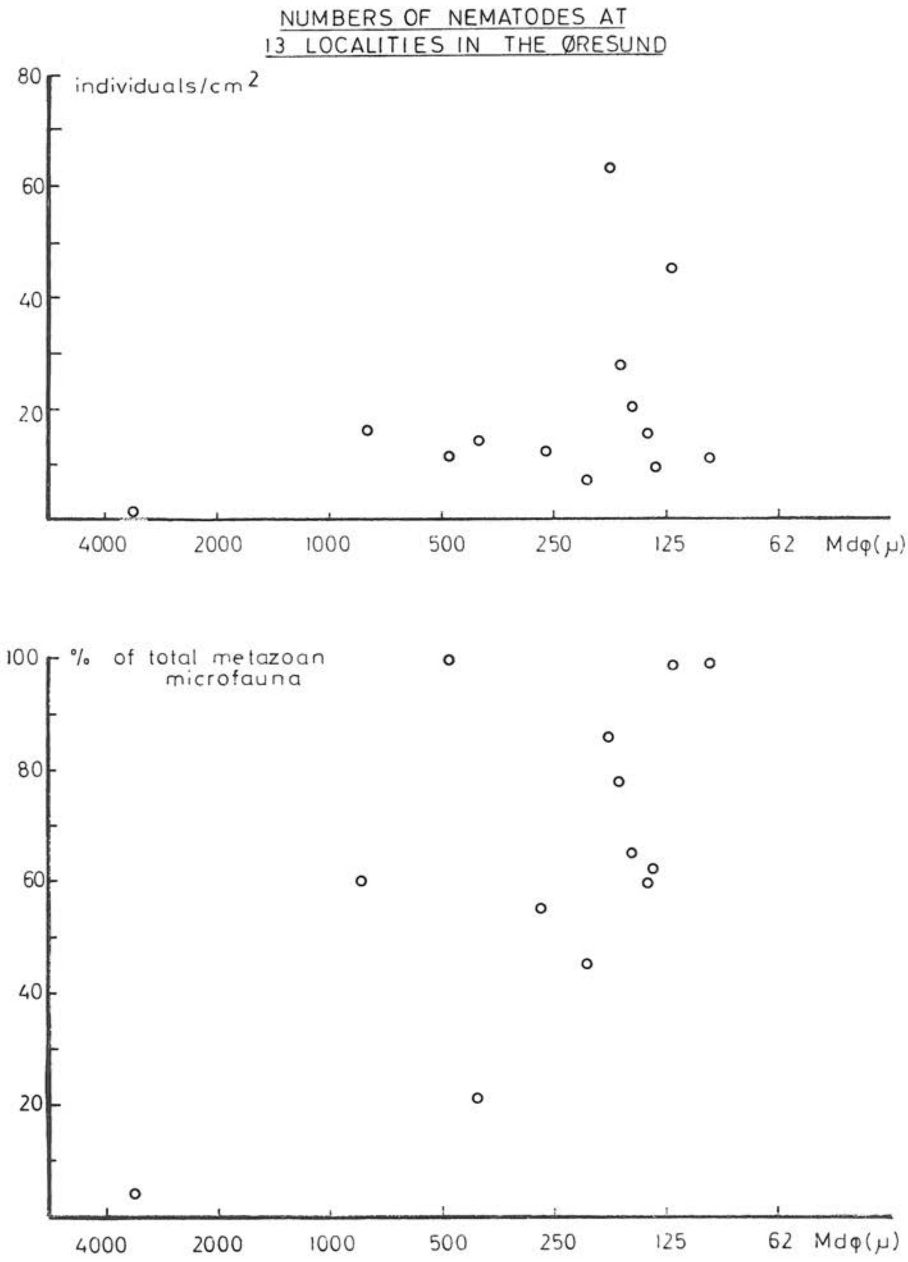

FIG. 12. The occurrences of nematodes in sands of different median grain sizes in 13 localities in the Øresund.

must either be smaller (very small flagellates, bacteria) or be sufficiently robust to burrow in the sediment (nematodes). Naturally if coarser sediments contain enough clay and silt to clog the interstitia they will not contain interstitial animals.

Fig. 12 shows the distribution of the nematodes. It is evident that the importance of nematodes increases in finer sediments, and in sands with median grain sizes below about 120 μ they constitute nearly 100 % of the micrometazoans present. These findings are in accordance with those of Wieser (1959). Also Jansson (1968a) demonstrated that the importance of nematodes in sandy beaches increases in finer sands. Nematodes are also common in clays and muds where they are often more numerous than in sands (Wieser & Kanwisher, 1961).

On a locality in Askö Harbour the sediment consists of fine, but poorly sorted sand (Mdφ: 170 μ, QDφ: 1) and with 13.5 % clay. The interstitia are thus completely clogged in this sediment which is covered by a thick layer of organic detritus containing a fauna of ciliates, turbellarians, rotifers, nematodes, etc. Below this layer, however, only nematodes were found (Fig. 67).

These observations all show that the nematodes are burrowers in the sediment. In coarse sediments with large interstitia they probably lack mechanical support for locomotion.

Finally, Fig. 13 shows the total number of micrometazoa per cm^2 and the percentage of macrodasyoids, turbellarians and harpacticoids. Representatives of these groups become scarce or absent in sediments finer than 310 μ, and harpacticoids were only found in small numbers in sands finer than 200 μ. No representatives of these goups are burrowers and are thus restricted by pore size. Some groups (chaetonotoids, rotifers, tardigrades, archiannelids, and oligochaetes) were found too sporadically in the 13 localities to draw any conclusions, but none were found in sediments finer than 175 μ.

It is not uncommon to find localities where clay is covered with sand and a larger or smaller zone with the two layers mixed. In such localities the vertical distribution of the fauna is influenced by the zonation of the sediment as shown by samples from Vellerup Vig (Fig. 70) where the fauna is restricted to the upper few cm due to the presence of glacial clay at some depth below the sediment surface.

Fauré-Fremiet (1950a, 1951b) studied the distribution of interstitial ciliate species in sands with different grain sizes. He found a different composition of the fauna in different sediments. In fine sands there were mainly species with a high degree of morphological and other kinds of adaptation (ribbon or thread like shapes, thigmotactic behaviour) and these forms he called "microporal". Species confined to coarser sands did not possess striking morphological features and were called "mesoporal". Still other species were found to have "microporal features" but lived also in coarse sands. These were named "euryporal". Finally, Fauré-Fremiet mentions species occurring in various sediments including all kinds of sand.

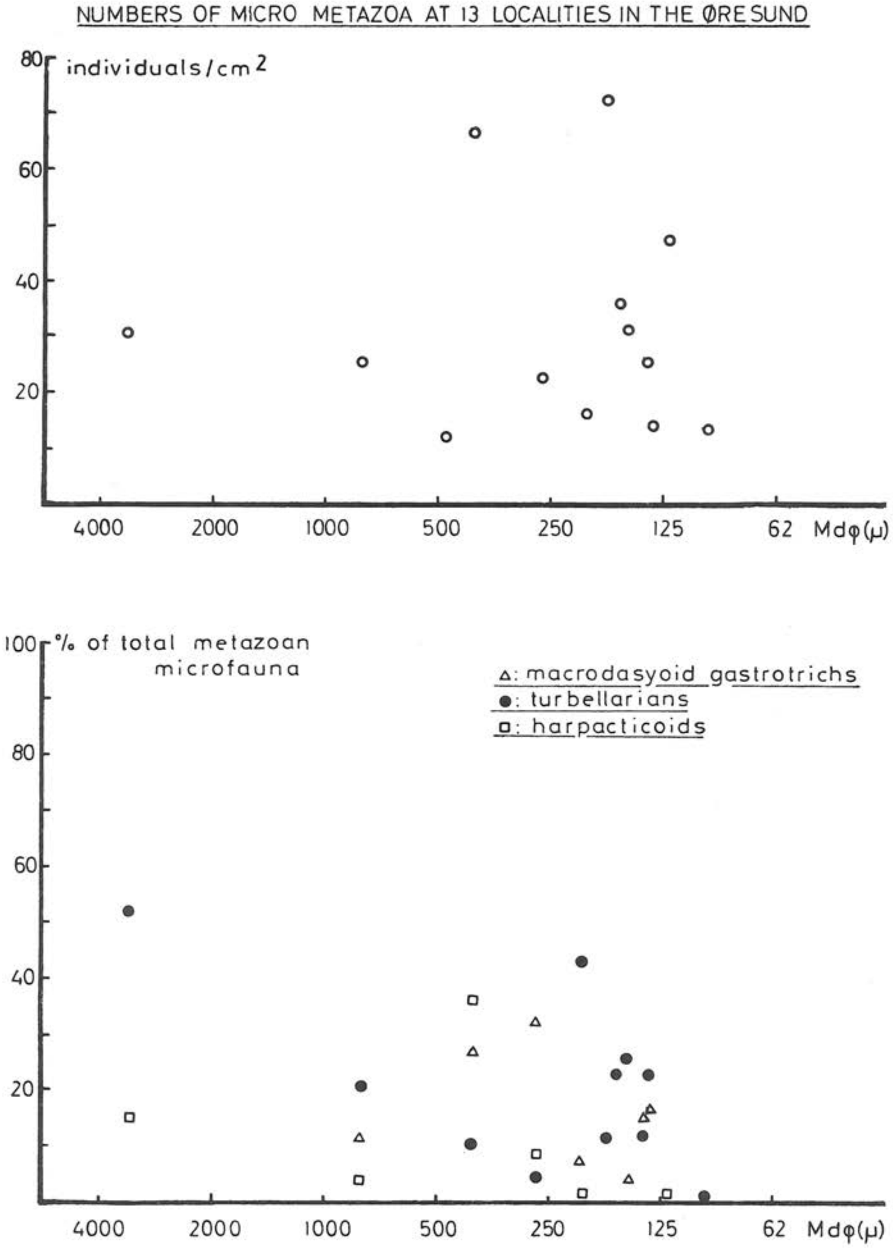

FIG. 13. The occurrences of all micro metazoa and of three taxonomic groups of micro metazoa in sands of different median grain sizes in 13 localities in the Øresund.

This classification does not seem to be a lucky one, since it is based on a mixture of morphological and ecological criteria. In spite of this, several authors (Dragesco, 1960; Agamaliev, 1967; Raikov, 1962; Petran, 1967) tried to classify a large number of sand ciliates into micro-, meso- and euryporal forms even though Dragesco is aware of the arbitrary nature of the classification and the fact that the classified species often occur in a "wrong sediment".

Many of the forms classified as microporal occur not only commonly in coarse sand but also in detritus, algal mats and masses of bacteria. This is for example the case with *Urostrongylum caudatum* classified as microporal by Fauré-Fremiet. The *Tracheloraphis* spp carefully classified as belonging to one group or another by the various authors often occur in detritus.

Different authors are often not in accordance when classifying the species. Thus, *Condylostoma remanei* is "microporal" according to Fauré-Fremiet (1951 b) and Petran (1967), "mesoporal" according to Dragesco (1960) and "euryporal" according to Agamaliev (1967). It may actually occur in nearly any marine environment and its morphology is in no way typical for an interstitial ciliate. The different classifications are probably due to accidental findings of the species in sediments of one type or another by the different authors.

Dragesco, Fauré-Fremiet and other authors classify some *Remanella* spp as "microporal" and others as "mesoporal" presumably because some species were also found in coarser sands, even though all *Remanella* spp are similar in their morphology. However, the microporal forms were originally defined on a morphological basis.

Also, these authors – being interested mainly in taxonomy and morphology – have to some extent neglected the many small species (*Uronema, Cyclidium, Cristigera,* small hypotrichs) which, though often occurring in large numbers in all sorts of sands, do not possess special morphological features.

Clearly, any ecological classification of protozoa should mainly be based on quantitative data since many species are omnipresent in small numbers.

What remains is the fact that many ciliate species are remarkably well adapted to life in small interstitia and that they mainly or exclusively occur in fine and medium sands. These forms nearly all belong to a few taxa (Loxodidae, Amphileptidae, Geleiidae, and a few others). In addition a large number of other species occur mainly or occasionally in or on sand and in environments like algal mats, detritus, masses of sulphurbacteria, etc. In the finest sands some of the larger representatives of the latter group are absent.

The distribution of all species is largely governed by other factors than grain size and a complete description of the faunal composition of the ciliate communities will therefore be given separately in Chapter 6.

4. INFLUENCE OF SALINITY AND TEMPERATURE ON THE DISTRIBUTION OF CILIATES

Salinity and temperature are usually considered to be among the most important factors governing the distribution of marine animals (see Muus, 1967; Remane & Schlieper, 1958). This, however, is not true when marine benthic ciliates are considered.

Most freshwater ciliates do not penetrate into high salinities, as could be expected when other limnic organisms are considered; in the field a salinity of 2-5‰ is the limit for most forms (Ax & Ax, 1960). However, a large number of forms have under experimental conditions been adapted to relative high salinities. For example, Frisch (1940) adapted *Paramecium caudatum* to 44 % sea-water (= ca. 15‰ S) and Yocom (1934) adapted *Euplotes patella* to 80-85 % sea-water. Several other experiments of similar nature have been made, for further references see Ax & Ax (1960) and Noland & Gojdics (1967). Yet, freshwater ciliates do not normally occur in the sea at salinities above 5‰, though Bick (1964) studying successions of freshwater organisms on cellulose in different salinities, found a relatively high number of freshwater ciliates in salinities between 5 and 21‰. In these experiments competition from marine ciliates was absent.

The euryhalinity of marine ciliates is even more striking. As noted by Kahl (1933): "Viele typische Salzwasserformen sind auffallend euryhalin und finden sich ohne Degenerationserscheinungen in sehr schwachen Salzstellen ein."

A number of forms are completely euryhaline. Thus Oberthür (1937) found that *Frontonia marina* may live in salinities varying from 0 to 125‰!

Ax & Ax (1960) have made the most comprehensive experimental study of the tolerance of marine ciliates to various salinities. These authors placed sediment samples from the Bay of Kiel into containers with water of different salinities and followed the development of populations of various species. The method will not always give the total tolerance range as the species may fail to develop for other reasons than the salinity conditions (competition, lack of food) but their results have given significant information.

Out of 51 marine species 7 multiplied in salinities ranging from 0 to 40‰, another 10 in salinities above 3‰, 7 in salinities above 5‰ and 14 in salinities above 8‰. The remaining 13 species multiplied at salinities above 12 or 15‰. It should be mentioned that the inoculate was added directly to water with the salinity to be tested; a slower adaptation would probably have given even larger salinity ranges for a number of species. Indeed, some species are actually found in wider ranges in the field than found in the experiments of Ax & Ax.

The fully euryhaline species are: *Loxophyllum setigerum, Uronema marina, Prorodon discolor, P. teres, Paramecium calkinsi, Cyclidium elongatum,* and *Frontonia marina.* To this list should be added *Litonotus lamella* and *Cyclidium citrullus* studied by Bick (1964). A parallel study on freshwater ciliates enabled Ax & Ax to demonstrate a "brackish-water minimum" of species around 5‰ though this is not very pronounced. They consider, as also Dietz (1964), that the existence of genuine brackish-water ciliates is most improbable. The fact that a few species until now have only been found in brackish water is due to the relatively intense studies of ciliates in the Baltic Sea and in estuaries carried out by many authors (for references see below).

In the field the distribution of ciliates in relation to salinity was studied by Bock (1960), Dietz (1964), Kahl (1928), and Webb (1956). These studies all show that salinity is relatively unimportant for the distribution of ciliates and that a typical marine ciliate fauna is developed even in dilute brackish water.

It is striking that a large number, if not the majority, of ciliate genera comprise both fresh- and sea-water species; it suffices to mention common genera like *Euplotes, Metopus, Paramecium, Frontonia* and *Lacrymaria.* Often members of the same genus occupy similar niches in the sea and in freshwater.

Salinity can only to a small extent explain the distribution in the localities studied during the present investigation. Salinities in the areas studied range from 6‰ at Askö to about 30‰ in the deeper parts of the Øresund. (The sulphuretum studied at Frederikssund had only a salinity of about 1‰, and in this locality only few totally euryhaline species were found; see Chapter 6, p. 126).

A few distributions must, however, be attributed to salinity conditions. *Spirostomum teres* and *Climacostomum virens,* found only at Askö, are typical (but the only) freshwater elements of this locality. Bick (1964) found the former species in salinities below 6‰ in succession experiments, and Kahl (1930-35) classifies both species as freshwater forms which may occur in brackish water.

More interesting is, however, the distribution of some groups characteristic of marine sands, viz Loxodidae, Geleiidae and Trachelocercidae. The latter two are not represented in freshwater, while the family Loxodidae is represented by *Loxodes* not found in the sea where *Remanella, Ciliofaurea* and *Ketrophorus* belong. Generally, there are relatively few freshwater forms morphologically well adapted to the interstitial life in fine sands, the mesopsammon here being to a higher degree constituted by forms normally occurring in other habitats.

Among the species studied by Ax & Ax (1960) a *Trachelocerca* (*Tracheloraphis*?) sp. was found to adapt to 3‰ and *Tracheloraphis* spp were commonly found during the present investigation at Askö. Ax & Ax found that *Geleia fossata* did not occur in salinities below 12‰.

Although the Askö localities were not optimal for *Remanella, Kentrophorus* and *Geleia* their absence was striking. In very similar Nivå localities they are nearly always present in smaller or larger numbers. Ciliates of these genera have been observed in the Bay of Kiel, the salinity of which is not very different from that of the upper layers of the Øresund, but to my knowledge they have not been found at lower salinities. Finally, the absence of some of the species (*Remanella gigas, Geleia nigriceps, Ciliofaurea* spp) in the shallow water localities in the

Øresund and their constant presence in localities deeper than about 5 m may be due to their salinity requirements. In the autumn 1968 I found *Remanella gigas* commonly in a sandy beach north of Frederikshavn (northern Jutland). Here the salinity of the surface water is much higher (about 30‰) than of the surface water of the Øresund.

Thus it is probable that the Geleiidae and the Loxodidae are rare examples of relatively stenohaline marine ciliates.

There is no evidence that temperature controls the distribution of marine benthic ciliates, which are all cosmopolitic. It is sufficient to recall the similarities of the ciliate faunas found in the White Sea (Raikov, 1962), the Caspian Sea (Agamaliev, 1967), Africa (Dragesco, 1965a), Norway (Fjeld, 1955), Florida (Borror, 1963b), Cape Cod (Fauré-Fremiet, 1951b), the Baltic Sea (Bock, 1952, 1952-53; Fenchel & Jansson, 1966; Kahl, 1930-35), inner Danish waters (Fenchel, 1968a, the present paper), an English estuary (Webb, 1956), the Black Sea (Petran, 1967), the west coast of France and the Mediterranean Sea (Dietz, 1964; Dragesco, 1960, 1963a; Fauré-Fremiet, 1950a), and a Californian salt marsh (Kirby, 1934). In no case has a benthic marine ciliate species in a convincing way been demonstrated to be endemic to any part of the world not even under extreme climatic conditions or geographic isolation.

Fenchel (1968b) studied the growth of cultures of different marine ciliates at different temperatures. It was found that all species survived at temperatures from 0° C to a little below 30° C. With one exception all species tested multiplied at temperatures above 4° C, one also at 0° C and all had their temperature optimum between 20 and 30° C (in one case determined more accurately to 27° C). It was further suggested that all species after a period of adaptation will multiply at 0° C.

Thus these experiments also indicate that temperature is not a limiting factor for the distribution of these animals and that they do not show any genetic adaptation to local climatic conditions. (It should be mentioned, however, that one freshwater ciliate, *Neobursaridium gigas,* is known to be distributed in tropical regions only. Recently Dragesco (1968b) has shown that in cultures this form only multiplies in the temperature interval 22-26° C).

(After completing the manuscript the author has together with dr. C. C. Lee, University of Miami, studied the effect of temperature on cultures of an undescribed ciliate isolated from the Antarctic pack ice. In spite of long adaptation periods this ciliate survives only in temperatures below about 17° C and reproduces only in the interval between —2 and 10° C, the optimum being around 5° C. A related ciliate isolated in Florida survives in the interval between 0 and about 45° C and reproduces in the interval between 5 and 41° C, the optimum being 29° C.)

5. ENERGY FLOW, MICROBIAL PROCESSES AND VERTICAL STRUCTURE OF THE SEDIMENT ECOSYSTEM

General considerations

The ecosystem constituted by the sediment and its flora and fauna receives energy from two sources: light and imported organic matter.

Taylor (1964) found that 10 % of the light at the surface of a sandy sediment penetrates to about 1.5 mm depth and about 1 % to 3 mm depth. He also found that benthic diatoms have a high photosynthetic efficiency at low light levels so that down to 3 mm depth the diatoms will be above their compensation point. The sand studied by Taylor was fine with a relatively large silt fraction (about 5 %) so it is probably that light in coarser sand would penetrate somewhat deeper, but photosynthesis does probably not occur below 5 mm.

Organic material may be autochthonous (corpses of the flora and fauna) thus not adding to the energy of the system or allochthonous, dead plankton organisms, live plankton caught by filter-feeders and remains of the macrovegetation; the latter being without doubt the most important source of organic matter in shallow water sediments. The leaves of large algae (*Fucus, Laminaria, Enteromorpha,* etc.) and of flowering plants (*Zostera, Ruppia*) are only to a very small extent consumed by animals. The dead plants are buried into the sand where they constitute a visible part of the sediment. Darnell (1967) has recently given a review of organic detritus in estuarine sediments.

The remains of plants may probably to some extent be utilized by the animals either directly or after a mechanical decomposition, and a large number of animals living in or on the sediment have been designated as detritus feeders (see e.g. Muus, 1967; Perkins, 1958; Remane, 1951; Renaud-Debyser & Salvat, 1963; Webb, 1956; all including discussions on the micro- and meiofauna).

However, increasing evidence indicates that the detritus in itself – mainly consisting of nitrogen-poor and indigestible cellulose, lignin, etc. – makes a rather poor food for most animals.

Most instructive are the experiments of Newell (1965). He demonstrated that the faecal pellets of the detritus feeding gastropod *Hydrobia* contain rather high amounts of organic carbon but very low amounts of organic nitrogen. After some time, however, the contents of organic nitrogen increased and that of organic carbon decreased somewhat in the faecal pellets due to microbial activity. When the faecal pellets which had been stored for some time were offered to the gastropods they were eaten again. New faecal pellets were analysed and it could be shown that the gastropods utilized the organic material containing nitrogen but that there still were considerable amounts of organic carbon in the pellets. Thus the snails utilized the bacterial flora on the detritus, while they were unable to utilize the nitrogen-poor plant remains of the detritus.

Bick (1964) showed in laboratory successions on cellulose that bacteria were always first to utilize the organic material and the bacteria were then utilized by the protozoa.

Detailed analyses of the food of the representatives of some microfauna groups (ciliates: Fenchel, 1968a; rotifers: Thane-Fenchel, 1968) have shown that practically no species of these groups feed on detritus. Out of about 90 species of ciliates studied by Fenchel (see Table 1) only a few species (*Sonderia* spp, *Discocephalus*, and a few others) were occasionally found with detritus in their feeding vacuoles and usually in very small amounts (these cases have not been included in Table 1). Possibly, these feeding habits have the same significance as swallowing of sand-grains by ciliates, i.e. utilization of attached microflora.

Practically all ciliates feed on other organisms; diatoms, flagellates, and other algae, other ciliates, bacteria, and sometimes small metazoa. It is characteristic that several ciliate species feed mainly on injured or dead animals, while none were found to have specialized on plant detritus.

Likewise Thane-Fenchel found no detritus feeding rotifers in marine and brackish water sediments.

Thus it must be concluded that the bacterial activity, and probably also the activity of fungi, is very important for the decomposition of organic matter and for the utilization of the energy of these materials by animals.

In aerobic environments the organic matter may be fully utilized by bacteria and by animals consuming the bacteria, i.e., the organic compounds will be fully oxidized to CO_2, H_2O, NO_3^-, etc., which do not contain any potential chemical energy.

However, except for very coarse sediments exposed to strong water currents, only a few mm to several cm thick surface layer is aerobic overlaying an anaerobic environment.

Under these conditions the limiting factor for the utilization of the potential energy of organic material is the availability of hydrogen acceptors. Heterotrophic bacteria may, by various types of fermentation processes, utilize organic compounds as hydrogen acceptors while at the same time oxidizing other organic substances thus producing, besides CO_2 and H_2O, reduced compounds such as lactate, alcohols, H_2S, NH_3, etc. Other anaerobic bacteria may utilize certain inorganic compounds as hydrogen acceptors for the oxidation of organic material. Thus SO_4^{--} may be reduced to H_2S, NO_3^- to NH_3, CO_2 to CH_4 and H_2O to H_2. The relative importance of these processes is dependent on the availability of the various hydrogen acceptors and the thermodynamic efficiency of the processes. As discussed later sulphate reduction plays a dominant role in the sea.

The reduced compounds – inorganic and organic – still contain potential energy which may be utilized by chemoautotrophic or heterotrophic organisms in the presence of oxygen and by photoautotrophic organisms in the presence of light.

Organisms are considered as chemoautotrophic if they do not require any organic compounds and obtain all their energy by oxidation of inorganic substances (H_2S, Fe^{++}, H_2, NH_3, NO_2, etc.) and the energy thus obtained is used to reduce CO_2 to carbohydrates. Organisms oxidizing reduced, simple organic compounds (hydro-carbons, fatty acids, alcohols, etc.) are considered heterotrophic. But the distinction is not sharp since some heterotrophs may use the obtained energy to reduce external CO_2, while others utilize inorganic compounds but also require organic substances. Besides, it is a matter of convention what is considered an organic or an inorganic compound.

All true chemoautotrophs are bacteria, while many bacteria and several protophytes use simple organic matter as substrate (Hutner & Provasoli, 1951; Lewin, 1963; Thimann, 1963; Wood, 1965).

By photoreduction is understood the ability to utilize reduced compounds for the reduction of CO_2 to carbohydrate in a photosynthetic process instead of using H_2O as in normal photosynthesis. The purple and green sulphurbacteria utilize H_2S, $S_2O_3^{--}$ and S, and some forms may also use H_2 or reduced organic compounds as hydrogen donor in this process, and the Athiorhodacea utilize organics or hydrogen. Also a number of protophytes utilize reduced organic material or hydrogen for the reduction of CO_2 (Thimann, 1963; Hutner & Provasoli, 1951; Wood, 1965). Nakamura (1937) states that *Oscillatoria* and the diatom *Pinnularia* can utilize H_2S for photoreduction.

Some of the energy contained in the reduced compounds may disappear from the ecosystem; H_2S and CH_4 may escape to the atmosphere or be oxidized abiologically in contact with O_2.

All these processes must necessarily result in a vertical zonation of the sediment ecosystem due to the one way supply of oxygen and light. Deepest in the sediment anaerobic decomposition will occur. Above the anaerobic zone, chemoautotrophic bacteria and aerobic heterotrophs utilizing the end products of anaerobic decomposition are found. If the anaerobic zone is sufficiently close to the sediment surface photoreduction occurs, often below a carpet of photosynthetic algae. This is possible because the compensation point for photoreduction is lower than for photosynthesis and since photosynthetic bacteria can utilize other wavelengths of light than can algae (Thimann, 1963).

Closest to the surface photosynthesis takes place.

Before discussing the organisms responsible for these processes and their significance for the microfauna in detail, methods which may be used to localize and quantify the processes going on in the sediments will be discussed.

The vertical distribution of substances such as O_2 and H_2S, the contents of organic material, the respiration and photosynthesis of the undisturbed sediment and the oxygen uptake of anaerobic sediments can be used as indicators. Valuable information could be obtained by studying the reduction-oxidation properties at various depths in the sediments. All the energy producing biological processes in the sediments are redox processes involving a variety of organic and inorganic hydrogen acceptors and donors, and it has been shown empirically that

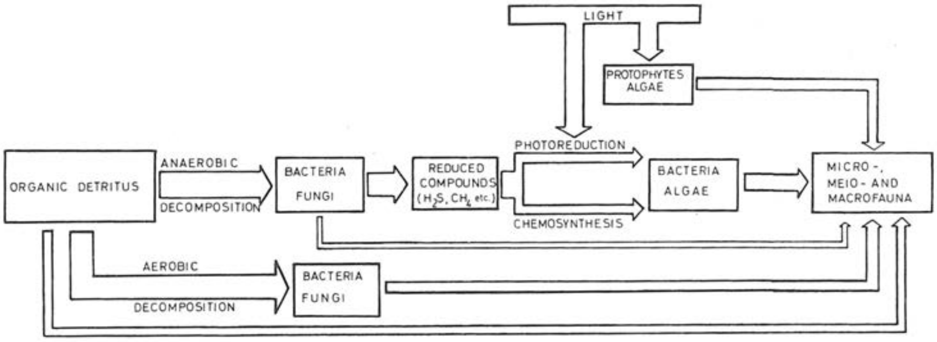

FIG. 14. Schematic representation of the energy flow through the sediment ecosystem.

several microorganisms are influenced by the redox potential and in their turn influence the redox potential of their environment.

The redox properties of sediments, their chemical interpretation and factors influencing the redox potentials of sediments will therefore be discussed first.

Fig. 14 shows in a diagrammatic way the energy flow through the sediment ecosystem.

Measurements of redox potentials and the redox-profile in sediments

The following introductory considerations on the theory of redox potentials are based on Hewitt (1950), Jørgensen (1945) and ZoBell (1946) to which the reader is referred for more detailed information and discussion.

If we consider a system consisting of a substance present both in oxidized and reduced form, for example a solution of ferric and ferrous iron, the following redox reaction may take place:

$$\mathrm{Fe^{++}} \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftharpoons}} \mathrm{Fe^{+++}} + \mathrm{e^{-}}$$

if another system capable of donating or accepting electrons is present. If the mass action law is applied:

$$\frac{C_{\mathrm{Fe}}{}^{+++} \cdot C_{\mathrm{e}}{}^{-}}{C_{\mathrm{Fe}}{}^{++}} = k$$

An inert electrode, for example a platinum wire placed in the solution may be considered as an electron reservoir; an exchange of electrons between the electrode and the system will take place and an electrode potential will be formed. This electrode potential will be a measure of the reduction-oxidation conditions of the system, i.e. the ratio of the concentrations of the oxidized and the reduced forms. The potential is thus a measure of the intensity of the oxidation -reduction condition but not of its capacity.

If a reference electrode is included in the circuit the potential can be measured. If the reference half-cell is a normal hydrogen electrode the measured potential is called Eh. In practice a saturated calomel electrode is used as a reference and its potential when referred to the hydrogen electrode (about +244 mV at room temperature) is added to the measured potential in order to find the value of Eh.

By thermodynamical considerations it can be shown that

$$\mathrm{Eh} = \mathrm{Eo} + \frac{\mathrm{RT}}{\mathrm{nF}} \log_e \frac{\mathrm{C_{ox}}}{\mathrm{C_{red}}} \qquad \text{or (at around 20°C)}$$

$$\mathrm{Eh} = \mathrm{Eo} + \frac{0.06}{\mathrm{n}} \log_{10} \frac{\mathrm{C_{ox}}}{\mathrm{C_{red}}} \qquad \text{(volts)}$$

where R, T, n and F are the gas constant, the absolute temperature, the number of electrons involved in the reaction, and Faradays constant, respectively. $\mathrm{C_{ox}}$ and $\mathrm{C_{red}}$ are the concentrations of the oxidized and the reduced form, respectively. Eo is a constant characteristic of each redox system. It can be seen from the equations that Eh = Eo when a system is half oxidized, i.e., the concentrations of the oxidized and the reduced form are equal. It can also be seen that Eh is not changed by a dilution of the system.

The equations are only valid at constant pH. In systems where protons are involved the relation between Eh and pH are complex depending on the dissociation constants of the involved compounds or ions. In many common systems the Eh decreases about 60 mV per pH unit increase.

Eh is analogous to pH in several respects. The latter is a measure of proton activity, the former a measure of electron activity.

Measurements of redox potentials play a great role in physical chemistry and biochemistry where known redox systems are studied. In addition redox measurements are used in a number of diciplines as an empirical indicator in studies of solutions containing unknown redox systems: bacterial cultures, soils, food products, and marine and freshwater sediments (for general references see Hewitt, 1950; Jørgensen, 1945, and Baas Becking et al., 1960).

Two fields of study are relevant for the present investigation: the redox conditions of sediments and the influence of microorganisms on the redox conditions of their environment. The redox conditions of sediments have been studied by several authors: Baas Becking et al. 1957, 1960; Berner 1963, 1967; Biggs, 1967; Emery & Rittenberg, 1952; Fenchel & Jansson, 1966; Gorham, 1958; Hayes et al., 1958; Mortimer, 1941-42; Pearsall & Mortimer, 1939; Teal & Kanwisher, 1961; and ZoBell, 1946). The redox potentials of sea-water were studied by Cooper (1937). The relationship between microorganisms and environmental redox conditions was studied by Baas Becking & Wood (1955), Baas Becking & Mackay (1956), Bahr & Schwarz (1956), Gunkel & Oppenheimer (1963), Hewitt (1950), Siebert & Schwarz (1956) and Taga (1967).

Two factors, temperature and pH, will influence the value of Eh. Temperature will influence the redox potential as well as the potential of the reference electrode. Since all my measurements – with few exceptions – were made at room temperature (20-25°C), a range in which Eh will change less than 10 mV, the effect of temperature was neglected.

Several authors (e.g. ZoBell, 1946; Mortimer, 1941-42; Siebert & Schwarz, 1956) consider the Eh of sediments to decrease 60 mV for each unit increase of pH and therefore corrected their Eh readings to pH 7.

ZoBell (op. cit.) demonstrated that the mentioned relation between Eh and pH is actually found in strongly reducing muds. It has since been shown (see p. 54) that the system $S^{--} \rightleftharpoons S_{\mathrm{rhmb}} + 2e^{-}$ controls the redox potential of strongly reducing sediments and this system behaves as predicted concerning pH. In most cases, however, the redox system controlling the Eh of sediments is still not known and as stressed by Baas Becking et al. (1960). Eh should not be corrected according to pH since their interrelationship in complex systems cannot be predicted. In accordance with this the rH concept (see Hewitt, 1950, or Jørgensen, 1945) which is a measure incorporating Eh and pH should not be used on complex systems.

The instrumentation used for Eh measurements during the present investigation was described in Chapter 2. Measurements were either made by pushing the Pt-electrode through an undisturbed sediment core taking readings at each cm or so or measurements were made in the 2-3 cm high core segments used for extracting the microfauna (see Chapter 2). The influence of atmospheric oxygen was found to be negligible in the bottom of the 3 cm high cylindrical containers used for the core segments since the Eh remained constant for hours even in strongly reduced sediments. This is due to the slow diffusion rate of dissolved gasses in water-saturated sediments.

The reproduceability of redox measurements in sediments was discussed by ZoBell (1946) and Hayes et al. (1958). ZoBell found that readings could be repeated within 10-20mV. Hayes et al. found a poor reproduceability in lake sediments, i.e. a scatter of ± 50 mV. They demonstrated that a large Pt surface gave better reproduceability and that the poising capacity was of importance for the reproduceability, which became much lower when the samples were diluted. They pointed out that electrodes may push some oxidized material down into the reduced zone which may result in an overestimation of the oxidized layer. This is certainly of significance when working in muds where the oxidized layer is only few mm thick but probably less so in sands.

Hayes et al. therefore proposed that instead of making potentiometric Eh measurements a copper wire could be pushed vertically into the sediment. In the oxidized zone the wire would become tarnished and in the reduced zone it would stay clean unless H_2S is present in which case it becomes black from copper sulphide. While this method may be used under some circumstances it is inpracticable in most marine localities and it is certainly a time consuming way to obtain data. Also, I had no difficulties when measuring Eh comparable to those experienced by Hayes et al.

When measuring redox potentials in sediments two things pose problems: inhomogeneity of the sediment and drifting readings.

The inhomogeneities in redox properties are common in some sediments. The sediment adjacent to worm burrows, for example, are more oxidizing than the surrounding sediments while bits of decaying algae or animals locally produce reducing conditions in an oxidized sediment. The Eh/depth curves may therefore differ over a distance of only 1 cm.

In one respect potentiometric measurements of pH differ from redox measurements, as the latter show a considerable drift in readings with time. A few examples are shown in Fig. 15. In most cases readings become constant (within 10-20 mV) after about 10 min but in a few cases no well defined potential could be read 40 min after electrode insertion (example II in Fig. 15). In practice readings were made when drifting had stopped or become very small. If readings still drifted strongly after 10-15 min the measurement was rejected. This, however, occurred rarely. Measurements in sediments or water with high H_2S contents drifted slightly or not at all and the same was the case with sediments in contact with aerated sea-water. Greatest difficulties were encountered with slightly reducing samples and when the poising capacity could be expected to be small, i.e. clean sands.

The drift is undoubted related to poising capacity of the sediment and to surface area of the electrode. Thus, less drift would probably have been experienced with larger electrodes. Nevertheless, mainly relatively small electrodes were used in order to facilitate the passage through sediments with minimal disturbance of the zonation. Probably, some redox systems of sediments come only very slowly or never into equilibrium with the electrode and this may also explain drifting readings.

At any rate most homogeneous samples give readings which may be repeated within 10-20 mV though in some sediments reproduceability is considerably poorer.

The Eh of clean sea-water ranged between +400 and +470 mV in accordance with the findings of Cooper (1937). In the Askö Harbour and in Nivå Bay values as low as +330 mV were often found in accordance with Baas Becking et al. (1957) who found values between +300 and +350 mV in estuarine waters.

A typical sediment redox-profile is as follows: At the surface the sediments usually have values of Eh close to the value of the supernatant water. Deeper in the sediment Eh decreases slowly to values of +200 to + 300mV. Still deeper in the sediment Eh decreases strongly usually within few cm or even mm to values between +100 mV and —100 mV. At greater depth Eh may decrease further to values between —180 and —200 mV. Thus a well defined "redox discontinuity layer" is usually developed. This layer may under certain circumstances be situated

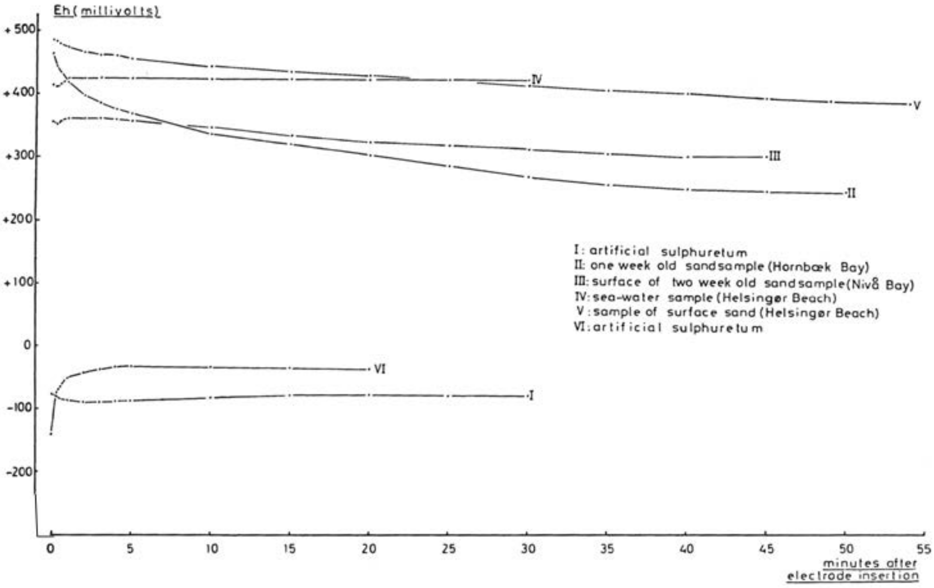

FIG. 15. Drift in the Eh readings with time in 6 natural and artificial sediments.

very close to the sediment surface. As has been shown by Mortimer (1941-42) and by Sorokin (1964, 1965) the redox discontinuity layer may, in stagnant lakes and in the Black Sea, be situated in the water above the sediment. Such conditions are naturally rare in the sea but in a sulphuretum partly in connection with the sea I have measured values of Eh of —190 mV during the night in the water above the sediment. Baas Becking et al. (1957) report one finding of negative Eh in estuarine water.

In a few cases two discontinuity layers seem to be present. Some sediments are oxidized throughout or Eh does not fall below +100 to +200 mV. In a few localities Eh increases again at some depth.

Examples of redox-profiles are given in Tables 2-3 and in several graphs in this and the following chapter.

In the following, the significance of Eh measurements for classifying localities, for predicting the vertical distribution of the microflora and fauna and for under-

TABLE 2. The redox-profile (mv) measured by two different Pt-electrodes in a core from the Nivå Bay.

Depth (cm)	0	1.5	3.5	5.5	7.5	9.5	11.5	13.5	15.5
Electrode I	+403	+202	+220	+220	+166	+136	+28	—140	—134
Electrode II	+370	.	+241	+178	+172	+136	+40	—128	—134

standing of the biological processes in sediments will be discussed. Here is only referred to Fig. 16 which shows the redox-profiles and the vertical distributions of three species of ciliates in samples from the Nivå Bay and Helsingør Beach.

Influence of heterotrophic and autotrophic microorganisms on the redox conditions of their environment

It is well known that in cultures, heterotrophic bacteria tend to reduce their environment. This was studied in detail by Hewitt (1950) and by other workers cited therein. It is due to the fact that when the supply of oxygen is exhausted fermentation processes take place, i.e., reduced organic compounds, for example lactate, are released in the culture fluid. Fermentation takes place to some extent even under aerobic conditions and aerobic cultures may become rather reducing. When conditions have become anaerobic and reducing, anaerobic bacteria may reduce the environment further; cultures of some forms may attain very intense reducing conditions (around —400 mV) for example cultures of *Clostridia* which produce hydrogen.

Heterotrophic bacteria utilizing inorganic electron acceptors (denitrifying bacteria, sulphate reducing bacteria, etc.) are also anaerobes producing reducing conditions.

Baas Becking & Wood (1955) studied the Eh/pH characteristics of cultures of *Desulphovibrio*. This organism is not active at redox potentials above +110 mV and different cultures attained electrode potentials between +100 and —400 mV. The high potentials were undoubtedly due to the presence of iron compounds reacting with the H_2S produced. The very low potentials cannot be due to H_2S. They occurred in cultures containing steel wool and are therefore probably due to hydrogen not consumed by *Desulphovibrio*. This organism may, in addition to organic substances, oxidize hydrogen by reducing SO_4^{--} in which case it is an anaerobic chemoautotroph (cf. Baas Becking & Wood, 1955).

For further references on the effect on Eh by heterotrophic bacteria see Hewitt (1950) and Baas Becking et al. (1960). Here it is sufficient to emphasize that heterotrophic bacteria reduce their environment. This is probably also the case with animals, especially anaerobic forms, but evidence is lacking, and in this respect animals are probably of little importance when compared with bacteria. Baas Becking & Mackay (1955), however, demonstrated that the green alga *Enteromorpha* reduces its environment in dark (to —80 mV) due to production of dimethyl sulphide.

In contrast to heterotrophic organisms the autotrophs tend to oxidize their environment. Photosynthetic organisms produce oxygen which may oxidize reduced compounds directly or through the activity of chemoautotrophs. Chemoautotrophic organisms and organisms performing photoreduction also oxidize their environment by utilizing reduced compounds.

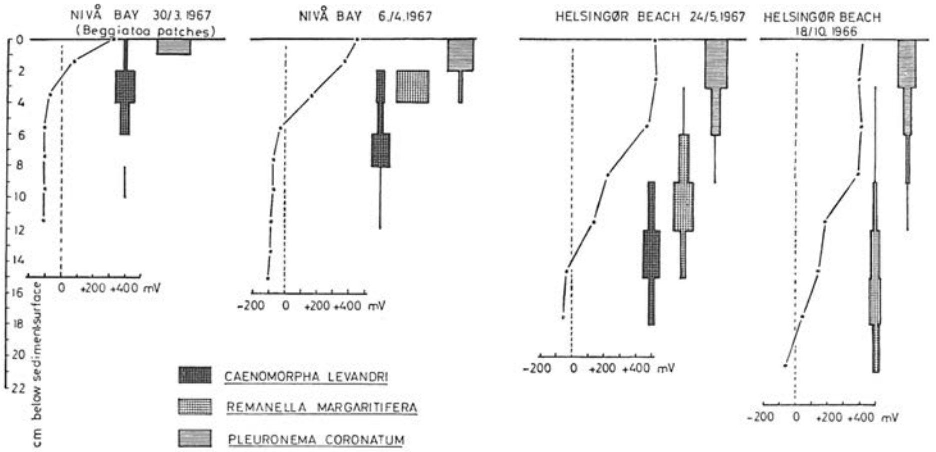

FIG. 16. The redox-profile and the vertical distribution of three species of ciliates in four core samples.

Experimental evidence from cultures has been given by Baas Becking & Mackay (1955) for algae (*Enteromorpha*), and for *Thiobacillus* and photoautotrophic sulphurbacteria by Baas Becking & Wood (1955).

Bahr & Schwarz (1956) studied the redox conditions of cultures of the filamentous white sulphurbacteria *Beggiatoa* and *Thiothrix* and found that these organisms form a film with different values of Eh above and below the film or "bacterial plate". The reproduction of the redox measurements in this paper is not, however, in accordance with convention; apparently the authors have interchanged the connections of the electrodes with their potentiometer and have not added the potential of the reference electrode. If this is kept in mind it was found that the substrate below the bacterial plate was strongly reducing and that conditions were more oxidizing above it. This agrees with the fact that the organisms in question oxidize H_2S to elemental sulphur with oxygen diffusing downwards in the culture.

Further evidence of the effect of microorganisms on environmental redox conditions comes from my own field observations and experiments given later in this chapter and in Chapters 6 and 9.

Chemical interpretation of the redox conditions in sediments

For the chemistry of the aquatic environment Eh is, like pH, an integrative measure, i.e. a measure of the state of a larger or smaller number of soluble elements, compounds and ions. Thus, the measured electrode potential may be due to the presence of several redox systems which may or may not be in equilibrium, and to irreversible processes. Under these conditions it could be criticised that the

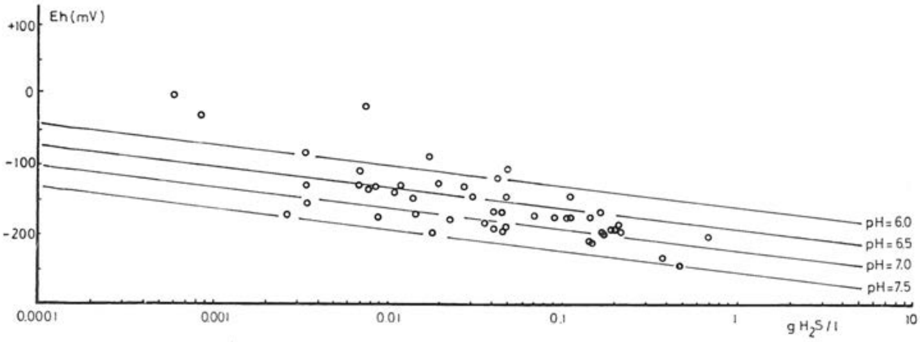

FIG. 17. The relation between H_2S contents and Eh in interstitial water and the theoretical values of Eh at different pH values.

measured potentials are called redox potentials, a concept which is clearly defined in physical chemistry. However, the term is widely used in other disciplines (technical biochemistry, bacteriology, etc.) so that it may be used as an empirical measure in spite of incomplete chemical interpretation.

Certain information, however, is available. Several authors (e.g. Baas Becking et al., 1960; ZoBell, 1946) ascribed very reducing conditions (i.e. negative potentials) in sediments to the presence of sulphide. This was supported by the fact that these sediments always smell of H_2S. Baas Becking et al. (1957) found that in certain inland waters, where sulphate is absent and sulphate reduction therefore cannot occur, very low potentials were not observed.

In Fig. 19 vertical distributions of total hydrogen sulphide ($H_2S + HS^- + S^{--}$) expressed as g H_2S /l interstitial water are shown together with the redox profiles. It is clearly seen that the strong decrease of Eh is correlated with the presence of H_2S.

Berner (1963, 1967) studied the relation between sulphides and Eh in marine sediments. He suggested that Eh of H_2S-containing sediments is controlled by the system

$$S^{--} \rightleftarrows S_{rhmb} + 2e^-$$

or at the prevailing pH rather

$$HS^- \rightleftarrows S_{rhmb} + H^+ + 2e^-$$

Assuming the presence of a saturated solution of elemental sulphur in the interstitial water, Eh would be dependent on the sulphide activity and Berner calculated that at 25° C

$$Eh = 475 + 29.5\ pS^{--}\ (mV)$$

It was further shown that in a number of natural and artificial sediments there was good agreement between the predicted and the actual relation between sulphide activity and Eh.

This relation was also examined during the present investigation. When the

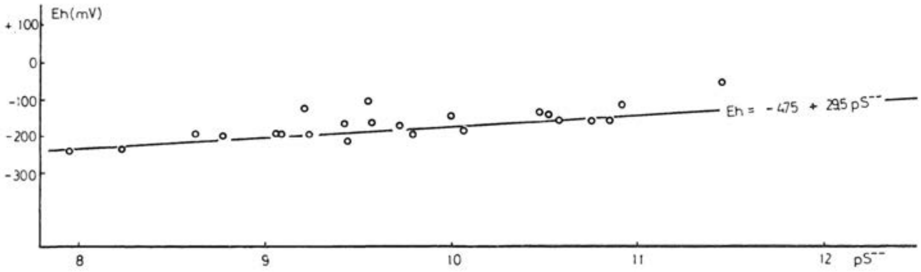

FIG. 18. The relation between Eh and pS^{--} in interstitial water and the theoretical relation.

relation between total H_2S and Eh is studied a scatter in the results could be expected since the pS^{--} is dependent on pH. In Fig. 17 the relation between Eh and H_2S contents in a number of natural and artificial sediments and in water samples taken in accumulated, decomposing seaweed is shown. The theoretical relation between H_2S and Eh at different pH values, which may occur in marine sediments, is also shown. It can be seen that the majority of the measurements fall within the expected range.

In 23 cases pH, Eh and H_2S contents were measured simultaneously and the sulphide ion activity could therefore be calculated. The relation between pS^{--} and Eh is given in Fig. 18 and shows good agreement between the predicted relation and the measured values.

Thus it can be concluded that the Eh of strongly reducing sediments is mainly controlled by the H_2S contents. Other compounds, however, may also play a role, for example organic sulphur compounds and hydrogen. Extremely low potentials in sediments (for example —500 mV measured by ZoBell, 1946) occasionally mentioned in the literature cannot be explained by the presence of sulphide, but may be due to the presence of hydrogen.

During the present investigation values below —250 mV were never measured in natural sediments.

Higher potentials cannot be due to the sulphide system. Attention has been given to an iron system as a poising agent in the aquatic environment. Pearsall & Mortimer (1939) and Mortimer (1941-42) found potentials below about +200 mV (at pH 7) associated with the occurrence of ferrous iron, above this value of Eh insoluble ferric compounds are found. This is in accordance with my own observations; at potentials below +200 mV the yellowish-brownish colour, characteristic of oxidized sediments and due to the presence of ferric iron, is replaced by a greyish colour.

Any equation for the process has not, however, been proposed.

Obviously also a large number of other compounds and ions may contribute to the formation of electrode potentials, such as nitrogen compounds and organic compounds.

The system controlling the redox potential of sea-water not poised by any other system originating from the sea-bottom is not known either. Cooper (1937) showed that if the reversible oxygen electrode controlled the redox potential, the Eh of sea-water in equilibrium with the atmosphere would be 300 to 400 mV higher than is actually the case. Baas Becking et al. (1960) showed that changing the oxygen tension in clean sea-water did not change Eh significantly.

Vertical distribution of some inorganic compounds and the pH-profile in sediments

As shown, the contents of H_2S controls the redox potential of strongly reducing sediments and as discussed elsewhere this compound constitutes an important source of energy for the bacterial flora and thus indirectly for the fauna of the sediments. In addition it is known to be strongly toxic to aerobic organisms and its quantitative distribution in sediments is therefore of interest.

As mentioned elsewhere H_2S will at pH 6.5-8.5 mainly be present as HS^-, while molecular H_2S and especially S^{--} occur in very small amounts. At atmospheric pressure a saturated solution of H_2S is of about 0.1 M (= 3.4 g/l). In sediments considerable amounts may become fixed as ferrous sulphides which are responsible for the black colour of the sulphide layers.

Hydrogen sulphide is produced by anaerobic decomposition of sulphur-containing proteins and by sulphate reduction. In the sea where sulphate is abundant the latter source is the most important according to ZoBell (1963a). This also explains the decrease of sulphate in reducing sediments (Biggs, 1967; Gunkel & Oppenheimer, 1963).

While numerous quantitative measurements of H_2S in the free water of stagnant lakes and basins have been taken (see ZoBell, 1963a; Sorokin, 1964, 1965) measurements of H_2S in interstitial water and especially its microdistribution are few. In free water (Black Sea, stagnant lakes and basins) the H_2S contents rarely exceeds 20 mg/l, and it is often less than 10 mg/l. In marine sediments concentrations may be higher. ZoBell (op. cit) states – without reference – that interstitial water may be saturated but so high concentrations were never found during my investigations.

Thamdrup (1935) measured the H_2S contents of interstitial water in the Danish Waddensea. His values do not exceed 7 mg/l. However, the sampling method employed (digging a hole in the sand flat and collecting the water draining into the hole) will give an average value for many different horizons in the sediment and considerable amounts may have been lost to the atmosphere or have become oxidized prior to analysis.

Berner (1963, 1967) studied the sulphide ion activity with the Ag-AgS electrode in various marine sediments. When pH is known the contents of total H_2S may be calculated from his data.

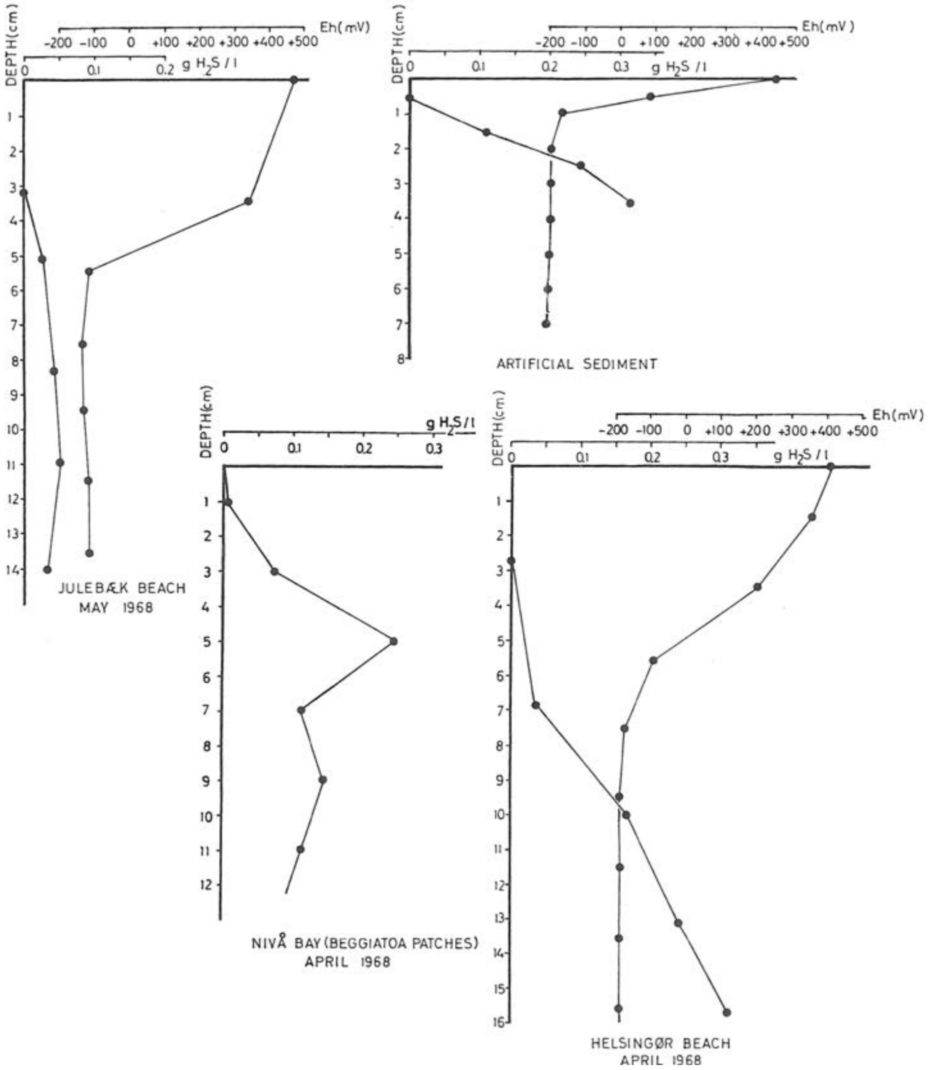

FIG. 19. The redox-profile and the vertical distribution of H_2S in four sediment samples.

During the present investigation the highest amounts of H_2S were found in the sediments of a sulphuretum in Nivå Bay, which consist of a mixture of decomposing *Zostera* and sand and are covered by a dense layer of purple sulphur-bacteria. There is usually a water layer of about 20 cm above the sediment. Twelve cm below the surface 740 mg H_2S/l interstitial water has been found. In this locality H_2S is also found in the free water above the sediment in calm periods during night. Up to 20 mg/l has been found in the morning.

Values of the same magnitude were found in similar localities in the Isefjord (Munkholmbroen) and at Frederikssund.

In water-covered piles of decomposing drift-weed covered with patches of sulphurbacteria at Vellerup Vig the water 20-30 cm below the surface contained from about 3 to 143 mg H_2S/l.

In Fig. 19 the vertical distributions of H_2S in some sediments are shown. It was found that in sandy sediments the amounts of H_2S very rarely exceeded 600 mg/l but values around 300 mg/l were common. In a sheltered Nivå locality, where patches of *Beggiatoa* and other white sulphurbacteria are found on the sediment, H_2S is detectable up to the sediment surface. The maximum found at about 5 cm depth was associated with the presence of a layer of decomposing *Zostera*.

In other localities H_2S first appears at some depth below the sediment surface.

The example shown for Helsingør Beach is not typical for the greater part of the year. Through most of the winter H_2S is absent; in other seasons of the year it is normally detectable at some depth but rarely in such high concentrations and so close to the surface as in the shown example. The conditions at Julebæk Beach and in the Nivå locality change less through the year.

At other Nivå localities (not shown in Fig. 19) H_2S is absent at least to 1 cm below the surface.

In samples from deeper water (Hornbæk and Ålsgårde transects) the H_2S contents were not measured. Based on Eh readings and the odour it can be stated that H_2S was present below some depth at most stations except those (Ålsgårde, 15 and 20 m; Hornbæk, 5 m) with coarse clean sand which was oxidized throughout. However, it seems that the concentration of H_2S was generally lower than in many shallow-water localities.

Another gas which is obviously of extreme importance for the ecology of sediments is oxygen. As is the case with H_2S, quantitative studies on the microdistribution of oxygen in sediments have rarely been made. In beaches the oxygen contents of interstitial water were studied by Brafield (1964), Gordon (1960) and Jansson (1966 a, b, 1968 b). All authors could demonstrate anaerobic conditions in interstitial water though Brafield as well as Jansson usually found small amounts present. Brafield even found some oxygen (about 3 % sat.) in the "black layers". (The ferrous sulphides apparently oxidize slower than H_2S and due to their low solubility Eh may be positive in the upper parts of the black layers. Thus the black layers may extend somewhat closer to the surface than the layers containing free H_2S and which are completely anaerobic).

Kanwisher (1962) studied oxygen profiles in marine sediments with a polarographic oxygen electrode. He was not able to demonstrate oxygen below 1 cm depth except in open beaches with a surf where oxygen was found several cm below the surface. In a salt marsh with clayey reducing sediments and a high content of organic matter oxygen was confined to the upper 2 mm. Thamdrup

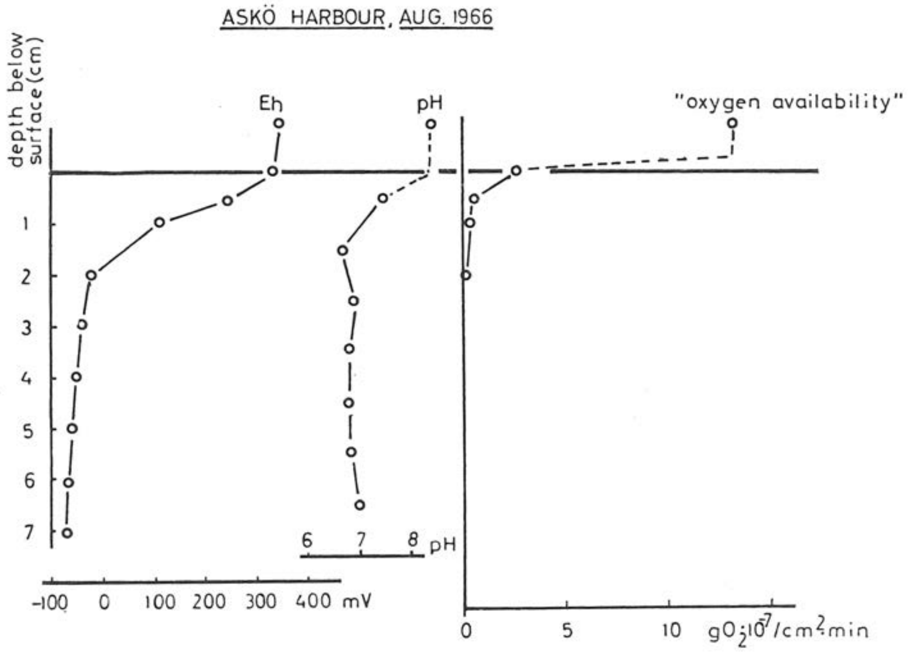

FIG. 20. The Eh and pH-profiles and the vertical distribution of "oxygen availability".

(1935) collecting samples by the same technique used for H_2S found values of oxygen contents ranging from 0 to 2.7 mg/l in the interstitial water of the Waddensea. Where the contents of H_2S exceeded about 3 mg/l oxygen was totally absent.

Actually great technical difficulties are involved in the study of oxygen in interstitial water. Sampling interstitial water with a syringe as done by Brafield and by Jansson is only possible in very shallow water or in air-exposed sediments and a risc of contaminating the sample with atmospheric oxygen is always present. Membrane covered polarographic electrodes disturb the zonation, may carry oxygenated water with them into the sediment and their membrane is easily ruptured. Also, these devices require some stirring of the water and are not very accurate when very small amounts of oxygen are to be measured.

During the present investigation a naked polarographic Pt electrode (see Chapter 2 for discussion) was used. It can hardly be calibrated to measure oxygen contents with any great precision since it is very sensitive to other factors such as pore size and turbulence. Besides, it is sensitive to electrode poisoning. However, since turbulence is absent, it will give a rough picture of the vertical distribution of oxygen. An example is shown in Fig. 20.

In all investigated sediments (excluding beach sands washed by the surf) the oxygen contents decrease much already close to the sediment surface. The oxygenprofile is to some extent parallel to the redox-profile as could be expected and in strongly reducing sediments oxygen could not be detected in accordance with the findings of Kanwisher. Clearly, when H_2S is present in any appreciable amounts, oxygen will soon be removed. As shown later in this chapter, reduced sediments quickly take up oxygen in large amounts when exposed to the gas. It is thus clear that the strongly reducing sulphide zone constitutes an anaerobic environment.

Many features of the sediment ecosystem are analogous to those found in stratified lakes with an anaerobic hypolimnion. Quantitative measurements are easier in this environment than in sediments and it is reasonable to compare the systems.

Mortimer (1941-42) found some correlation between Eh and oxygen contents in stratified lakes and already the +200 mV isovolt was correlated with low levels of oxygen contents. Sorokin (1965) found that in the Lake Belovod oxygen was quite absent at redox potentials below O mV where H_2S became detectable.

It can be concluded that —1. With the exception of sediments washed or mixed by water currents the contents of oxygen decrease strongly with depth in the sediments and —2. Oxygen is absent in the H_2S containing layers.

The distribution of a number of other compounds is also of some interest. These include CO_2, CH_4, Fe^{++}, Fe^{+++}, NO^-_3 NO^-_2, and NH_3 which were not, however, studied during the present investigation. Nelson (1962) found CO_2, H_2S and NH_3 to occur in the proportions 100:10:1 in marine sediments. The smaller amounts of NH_3 when compared with H_2S is undoubtly due to the greater availability of sulphate than of nitrate as electron acceptor for anaerobic bacteria.

Mortimer (1941-42) showed that when Eh is lower than +350 mV, NH_3 dominates over more oxidized nitrogen compounds and that, as previously mentioned, iron occurs as Fe^{++} below about +200 mV and as insoluble Fe^{+++} in more oxidized environments. The presence of CH_4 in marine reducing sediments was demonstrated by Kanwisher (1962).

Profiles of pH were measured at several occasions. In completely oxidized sands pH is, like Eh, constant throughout and identical with that of the sea-water. In sediments where a reduction layer is developed the pH-profile is very characteristic (Fig. 20). Below the surface the pH decreases from the value of the sea-water (usually around 8) and shows a minimum at the level of the redox discontinuity layer (usually around 6.8). At greater depths pH increases again and may sometimes reach more than 8 in the sulphide layer.

This pH-profile has previously been described (see Wood, 1965). It seems reasonable mainly to ascribe it to the distribution of CO_2.

Dry organic matter was determined at various localities in the Nivå Bay. On the sand bars sheltering the bay it was less than 0.2 % which may be taken as characteristic of clean sand. Inside the sand bars the sediments contained 1-3 % dry organic matter. In the innermost part where sulphureta are developed the contents were not measured but they must have been considerably higher.

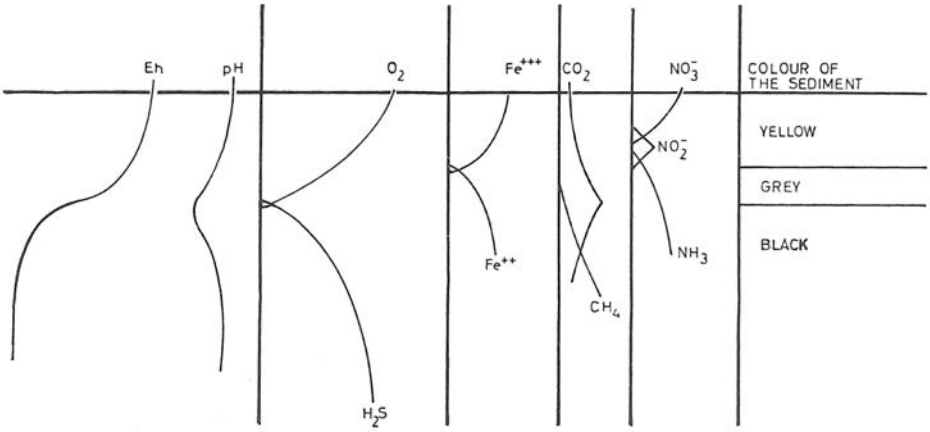

FIG. 21. Schematic representation of the Eh and pH-profiles and the vertical distributions of some compounds and ions in sediments.

The vertical distribution in the sediments was not studied in detail. In some localities (viz Helsingør Beach, Julebæk Beach) it is erratically distributed. In the sediments of Nivå Bay well defined layers of decomposing *Zostera* and *Fucus* leaves are often found and these layers are correlated with high contents of H_2S and low redox potentials.

The vertical distribution of some chemical factors in a "typical sediment" is shown schematically in Fig. 21.

Poising capacity and oxygen uptake of reduced sediments

The reducing capacity of marine sediments was studied by ZoBell (1946) who made redox titrations with different oxidizing agents such as ferric chloride solutions.

During the present investigation the oxygen consumption of reducing sediments in suspension was studied.

A core sample containing about 40 cm^3 sediment was emptied into flasks filled with 0.5 or 1 litres air saturated filtered sea-water. The flasks were closed with a rubber stopper fitted with a calibrated oxygen electrode. Air bubbles were avoided when closing the flask. The sediment in the flask was kept in suspension by a magnetic stirrer. After the end of the experiment the oxygen contents had usually fallen to 30-60 % of the initial value. The oxygen tension was read at regular intervals and the oxygen consumption of the sediment could be calculated.

Two examples are shown in Figs 22-23. Both represent reducing and sulphide containing sediments.

In comparison with the respiration experiments described later in this chapter, the initial oxygen uptake is about 100 times greater than the normal respiration of the undisturbed sea bottom having the same surface area.

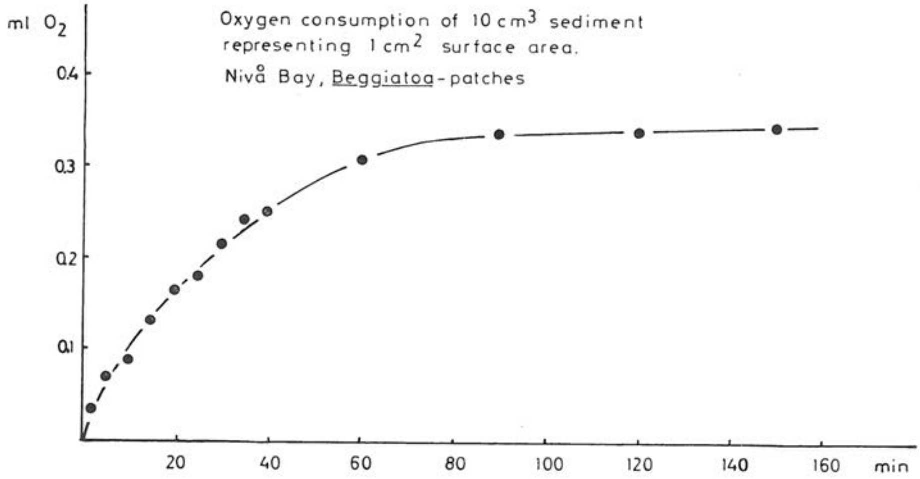

FIG. 22. Oxygen consumption of a reducing sediment.

This clearly demonstrates that —1. The undisturbed sulphide layer must be anaerobic, —2. The penetration of oxygen is a limiting factor for the biological or abiological oxidation of the reduced, energy containing compounds, and —3. The oxidation of reduced sediments may proceed abiologically in the presence of oxygen within relatively short time which explains the action of surf on the redox conditions of beaches (see below).

If the Eh of the suspension is measured during the experiment it is found that it increases from initial negative values to values between +200 and +350 mV. Thus some slightly reducing substances are not oxidized by oxygen during the experiments which lasted 1 to 3 hours (at room temperature).

From Fig. 22 it can be seen that a sediment cylinder 10 cm deep and 1 cm² in surface area from Nivå Bay will take up a little more than 0.3 ml O_2 or about 0.4 mg. From the same locality (but admittedly some weeks later) it was found that the same amount of sediment contains 0.55 mg H_2S.

If we assume that the oxidation of H_2S results in elemental sulphur and water, then 0.55 mg H_2S will require 0.26 mg or 0.18 ml O_2. Thus nearly about 2/3 of the oxygen consumption may be due to the oxidation of free H_2S, of course provided that the H_2S contents of the two samples were identical.

Oxidation of other reduced substances and bacterial respiration under the experimental conditions may explain the remaining oxygen uptake.

Factors controlling the redox conditions of sediments

It is certain that the oxidation of organic material by anaerobic bacteria is the primary cause of reducing conditions in the aquatic environment. It should therefore be possible to find a correlation between the redox conditions and the

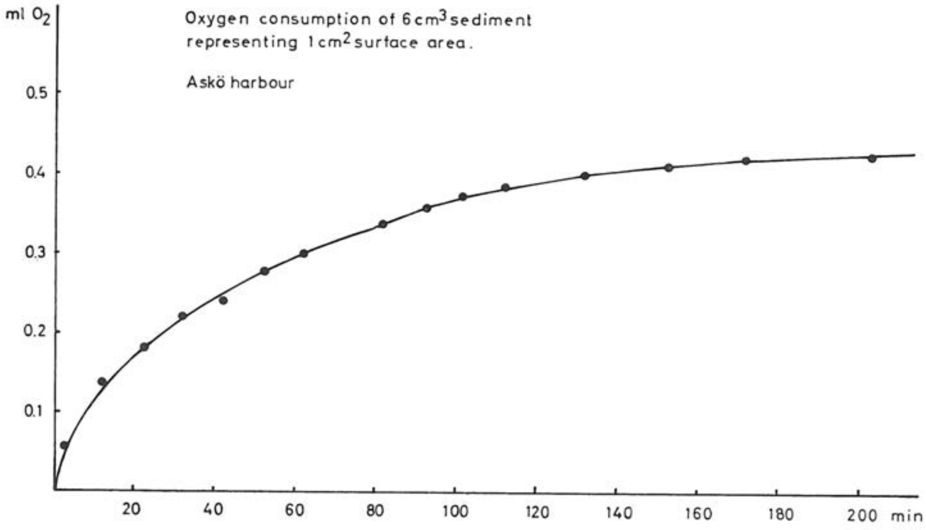

FIG. 23. Oxygen consumption of a reducing sediment.

contents of organic material in sediments. This is also the case. Baas Becking et al. (1960), ZoBell (1946) and other authors all agree that reducing conditions in sediments are associated with the presence of organic material. Dietrich et al. (1965) found a clear correlation between the two factors in the sediments of the North Sea.

Also during the present investigation a correlation was found between a high intensity of reduction and a redox discontinuity layer situated close to the surface and large amounts of organic material.

Another important factor controlling the depth of the redox discontinuity layer is the supply of oxygen in the sediment. This is again controlled by turbulence and grain size (and of course the oxygen contents of the water but this is rarely limiting in the sea).

Evidence for the significance of the grain size for the depth of the redox discontinuity layer was already discussed in Chapter 3.

Perkins (1957) demonstrated that the "black layer" was found deeper in the sediment in the winter and interpreted this as an effect of temperature as sulphide producing bacteria will be more active at high temperatures. This explanation does not seem too convincing since the activity of sulphide-oxidizing bacteria must also increase with increasing temperature. As shown later in this chapter conditions may in certain localities be quite opposite to those found by Perkins.

The Helsingør Beach (Fig. 24) is exposed to north east and not all winds will therefore affect the beach equally much by stirring the sediment through wave action. Also the water level will influence the degree of stirring by wave action.

During the windy period of September 1966 large amounts of drift-weed (mainly *Fucus* and *Zostera*) were mixed with the sand. During the following calm period in October and November anaerobic conditions were soon prevailing followed by sulphide production, and the reduction layer moved closer to the surface. The few storms in the beginning of December did not influence the beach very much, but late in that month the sediments were exposed to a heavy surf resulting in a complete oxidation of the sediment. The Danish winters are windy and consequently the sediments become regularly oxidized by wave action. During shorter calm periods (middle of February, middle of March to middle of April) a tendency towards reducing conditions can be seen, but the evolving systems were completely destroyed by two violent storms at the end of February and the end of April, respectively. The low water temperatures during winter probably also resulted in a relatively slow evolution of reducing conditions. The last storm in April again brought in large amounts of drift-weed which then resulted in reducing conditions prevailing throughout the relatively calm summer period. At the end of the summer, however, conditions slowly became more oxidizing which may in part be due to biological oxidation.

Thus on this rather exposed beach the redox conditions are mainly controlled by irregular import of organic matter and by wave exposure. – During 1967-68 redox conditions were rather similar to those in 66-67, reducing conditions prevailed during summer and especially during autumn and spring, and oxidizing conditions during winter.

As discussed previously, photoautotrophic organisms oxidize their environment in light and reduce it in dark. This was demonstrated by Baas Becking & Mackay (1956) for the green alga *Enteromorpha*. Purple sulphurbacteria exhibit the same effect. In a glass jar the bottom was covered with reducing mud from Nivå Bay and the jar filled with sea-water, containing *Chromatium* and *Lamprocystis*, collected in the same locality. The glass was kept in the dark for 12 hours when the water in the middle of the jar contained 16 mg H_2S/l and had a redox potential of about —180 mV. The jar was then kept in daylight and the Eh and the contents of H_2S measured every hour. After three hours H_2S could not be detected and Eh had increased to +196 mV. The glass was then placed in the dark again and one hour later the contents of H_2S had increased to 9 mg/l and the Eh decreased to —176 mV.

The effect on a sediment was tested in a laboratory experiment. In a 10 l glass aquarium a sandy sediment from Nivå Bay enriched with peptone and $CaSO_4$ had been kept for about 2 months. The sediment was covered with about 3 cm water. At the sediment surface a typical zonation was present, i.e. a layer of purple sulphurbacteria covered with a dense layer of blue-green algae and diatoms. Some white sulphurbacteria were also present. Three Pt electrodes were placed in the tank, one 6 mm above the surface, one resting on the surface and one 9 mm below the surface and these electrodes were kept in the same position during the experiment.

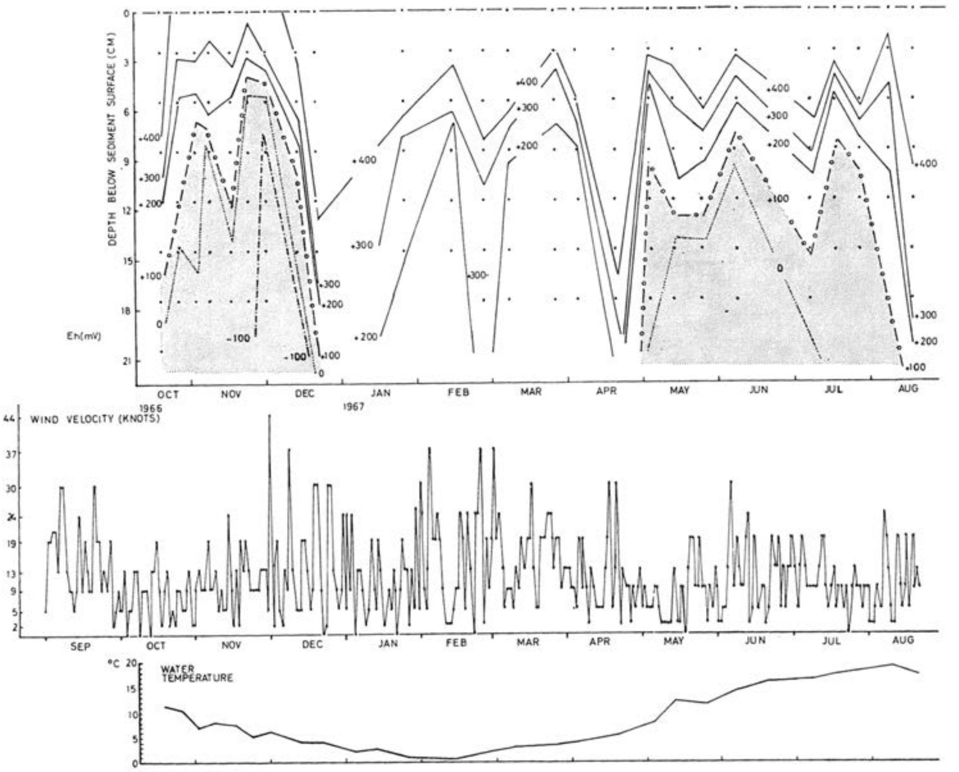

FIG. 24. Redox conditions at various depths in the fixed Helsingør Beach locality (water depth about 0.5 m) from October 1966 to August 1967; the shaded areas indicate values below +100 mV and fall approximately together with the black layers indicating ferrous sulphides and formation of H_2S at some depth below this isovolt; wind velocities at noon at a local lightship and water temperatures at the station are also indicated.

The result is shown in Fig. 25. At the beginning of the experiment the tank had been exposed to daylight (1-3 kilolux) for 10 hours. It was then placed in the dark. The Eh of the supernatant was unaffected, but at the surface a decrease of the Eh was observed already after one hour, and after 4 hours the Eh had decreased from +435 to +213 mV; at 9 mm depth it fell from – 116 to – 183 mV in 17 hours. When exposed to light the potentials quickly increased again.

The vertical displacement of the redox discontinuity layer was accompanied by vertical migrations of sulphurbacteria. After the dark period white clouds consisting of *Thiovolum* and *Macromonas* were observed at the surface of the sediment, but within two hours after the return of light they disappeared into the sediment again.

In the field the effect could be expected in localities where reducing conditions prevail close to the sediment surface. In such a locality in Nivå Bay, where the surface is dominated by blue-green algae and purple bacteria, redox-profiles

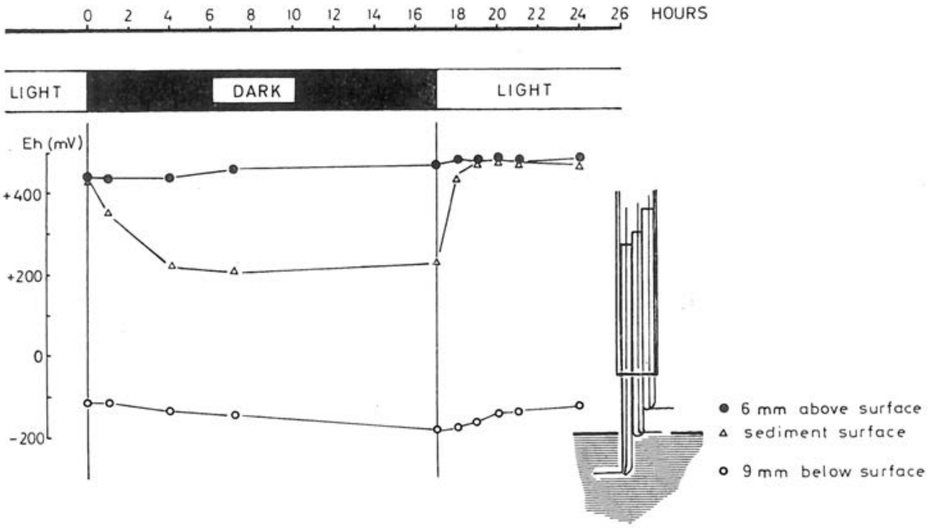

FIG. 25. The influence of light on the redox conditions of an artificial sediment.

were measured in the field at sunrise (04^{20}) and in the afternoon (14^{20}) on a sunny day in August 1968. At sunrise the Eh of the surface was 0 to +10 mV, in the afternoon it had increased to about +300 mV. Already 5 mm below the surface the redox conditions remained rather stable, i.e. strongly reducing. Thus any vertical migration due to this phenomenon would be restricted to the upper 5 mm of the sediment. The vertical migration of white sulphurbacteria (*Beggiatoa, Thiovolum, Macromonas*) was clearly visible. At sunrise the bottom was white from these bacteria, later in the day they disappeared into the sediment leaving the algae on the surface.

Sorokin (1965) demonstrated that the redox discontinuity layer of a stagnant lake moves downwards by several metres during day time.

The phenomenon could also be expected to effect the sediments on a year basis since the photoautotrophic organisms are the only ones affected by light.

In a sheltered locality in Nivå Bay where the sand is practically never stirred by water turbulence the surface is dominated by patches of white sulphurbacteria from autumn to spring. In summer these are to a great extent covered or replaced by photosynthetic sulphurbacteria and algae. The redox-profile was studied six times during the period October 1966 to July 1967 (Fig. 26). It can be seen that in late autumn the Eh of the surface was below +200 mV and in June it was above +400 mV (all readings were made between 09^{00} and 11^{00}). Thus in this locality conditions are opposite to those at the Helsingør Beach and this must be an effect of light.

Finally, it should be mentioned that the activity of burrowing animals (*Mya, Nereis, Arenicola,* etc.) may play a role for the oxidation of the sediments. An

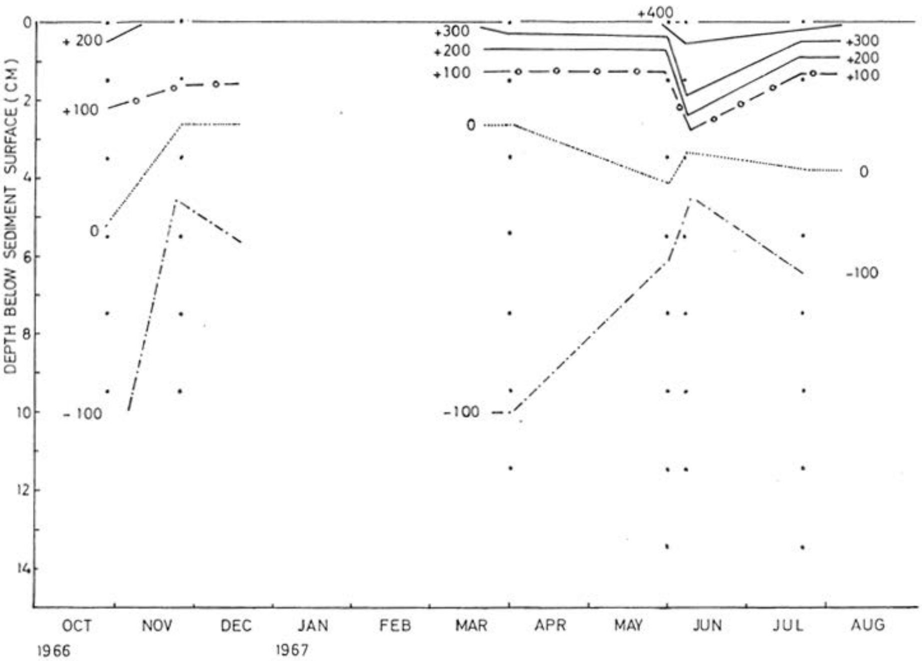

FIG. 26. Redox conditions at various depths in the sediment of a Nivå Bay station in the periods October-November 1966 and April-July 1967.

oxidized microzone is always seen around the siphons of lamellibranchs and the burrows of polychaetes. The effect of this, however, is probably limited to the immediate vicinity of the animals. *Nereis* and *Mya* occur abundantly in the above mentioned Nivå locality where reduced conditions may prevail close to the surface. In laboratory sediments the presence of the gastropod *Hydrobia* seems to prevent or repress the growth of dense mats of blue-green algae thus probably increasing the rate of oxidation of the sediments.

Vertical and horizontal distribution of some representatives of the microflora

DIATOMS. These algae constitute a significant part of the food for the microfauna. Of the 84 species or genera of ciliates listed in Table 1 (p. 24) 15 feed exclusively on diatoms and for another 31 forms diatoms are an important part of the diet. Also a number of other representatives of the microfauna feed mainly or exclusively on diatoms and their distribution is therefore of considerable interest.

As is apparent from Chapter 6 a large number of diatom-feeding ciliates often or always occur in the aphotic zone of the sediments several cm below the surface. Earlier workers (see Grøntved, 1960, for references) stated that diatoms only occur at or close to the sediment surface, but increasing evidence shows that this

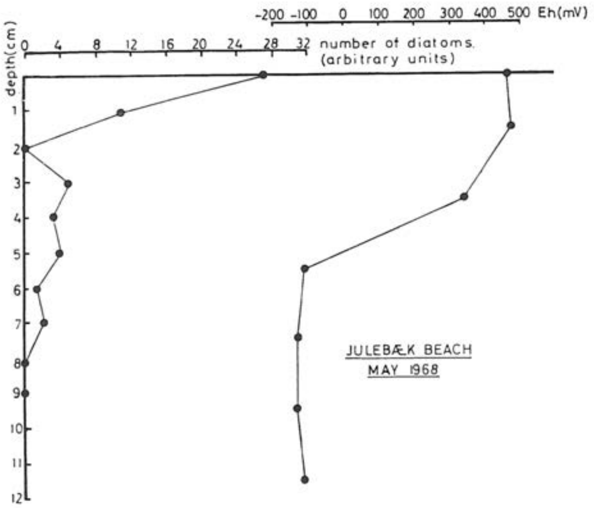

FIG. 27. The vertical distribution of diatoms and the redox-profile in the Julebæk Beach.

is not the case. Grøntved (1962), Meadows & Anderson (1968), Oppenheimer & Wood (1962), and Steele & Baird (1968) all found significant numbers of living diatoms in the deeper layers of the sediment. Fauré-Fremiet (1951 c) demonstrated diurnal vertical migrations of diatoms in a tidal flat.

The vertical distribution of diatoms was studied at some localities. The methods employed were described on p. 22 in Chapter 2 and the results are shown in Figs 27-28. In all localities significant numbers of diatoms could be found several cm below the surface in the aphotic zone. There is no tide in the studied localities. In Julebæk Beach a clear maximum of diatoms was found at the surface but considerable numbers were found down to 6-8 cm. In Nivå Bay large numbers also occurred at the surface but the maximum was sometimes found at 1-2 cm depth and large numbers were found at greater depths even in the sulphide layers.

In the Helsingør Beach the diatom flora is always rather poor close to the surface and largest numbers were found several cm below the surface. As in Nivå Bay relatively large numbers were also found in the sulphide zone.

It is a controversial question whether diatoms may live heterotrophically in nature. Lewin (1963) and Lewin & Lewin (1960, 1967) demonstrated that a number of diatoms can live heterotrophically in cultures in the dark utilizing lactate or glucose as substrates. Out of 24 littoral, pennate diatoms 12 were able to grow heterotrophically. Species of the genus *Nitzchia* lacking chloroplasts were also described by these authors. Dark assimilation was also demonstrated for *Nitzschia* and for *Phaeodactylum* in the presence of glucose and of peptone by Seki (1967).

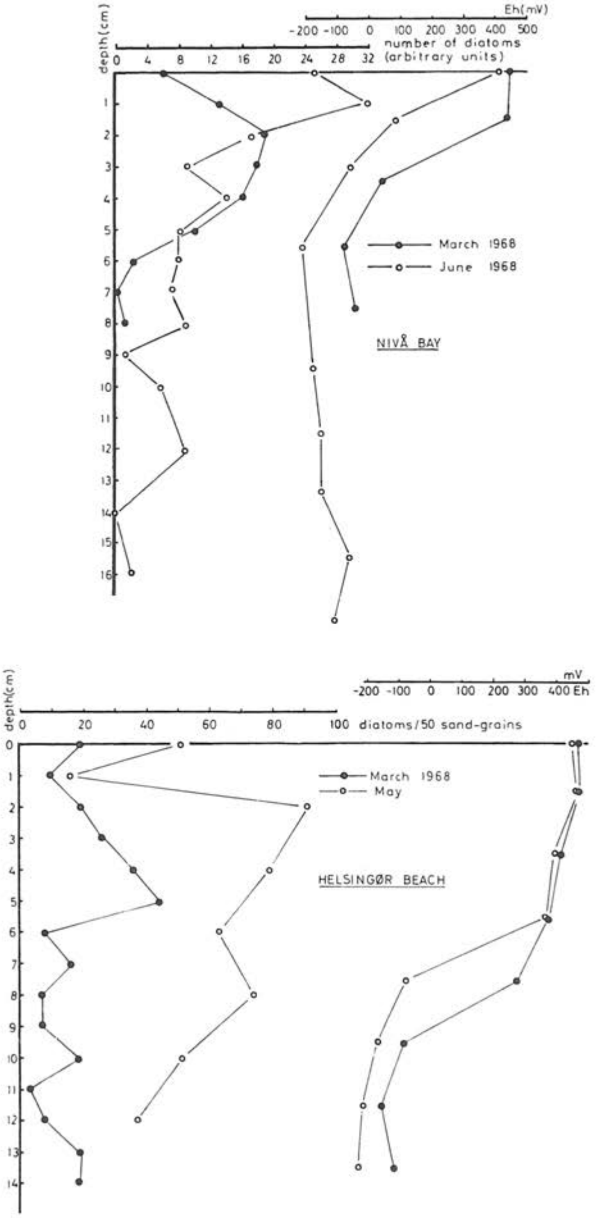

FIG. 28. The vertical distributions of diatoms and the redox-profiles in two samples from the Nivå Bay and two samples from the Helsingør Beach.

On the other hand, Munro & Brock (1968) could not demonstrate uptake of labelled acetate and glucose by diatoms.

Steele & Baird (1968) and Grøntved (1962) believe that diatoms become mixed with the sediment by wave action and that the diatoms are able to survive, but not grow, for long periods in the dark.

This explanation does not seem satisfactory in many cases. Fauré-Fremiet (1951 c) found that the diatom *Hantzschia* can move at a rate of 40 μ/sec = 14 cm/hour. It is further known that some species of diatoms collect at the surface of mixed sand samples within a few hours. It therefore seems improbable that the diatoms should stay for long periods in the deeper strata if they were dependent on light.

In the Helsingør Beach the sediments are mixed periodically but in May 1968 conditions had been calm for long periods as is apparant from the well developed reduction layer. Julebæk Beach and Nivå Bay sediments are very rarely mixed and then probably only the upper 1-2 cm are effected. Diurnal migrations may well play a role but this was not investigated (all samples were taken in the morning between 09^{00} and 11^{00}).

The mixing of the surface sands would probably make possible some photosynthesis at depths which would be aphotic under calm conditions, but this must play a small role since diatoms are always scarce in more exposed sands.

Thus it remains tempting to consider some of the diatoms to be at least facultatively heterotrophic in the field. The finding of living green diatoms on bottom deposits of the deep sea (Wood, 1966) also supports this view.

It would be interesting to study whether different species of diatoms occur at different depths in the sediment.

Grøntved (1962) showed that at least some of the diatoms of the deeper layers of the sediments were able to carry out photosynthesis and added this production to his "rates of potential production" of the sediments.

No quantitative enumerations were carried out during the present investigation. Grøntved (1960) found up to 15×10^6 specimens / cm^2 in the sediments of some Danish fjords. This probably refers to localities very rich in diatoms. Grøntved (op. cit.) and Steele & Baird (1968) agree that the diatom flora is poor in exposed localities where the sands are mixed. This is in accordance with my own observations. The relatively exposed Helsingør Beach has a poor diatom flora compared with for example the Julebæk Beach and the Nivå localities, all sheltered by sand bars, and localities at 5-10 metres depth in the Øresund. Especially the Julebæk Beach has an extremely rich diatom flora.

Diatoms were more abundant during spring and summer than in other seasons of the year in all localities. This is in accordance with the findings of Mare (1942) and Aleem (1950).

CYANOPHYCEANS. These plants play a more restricted role as food for ciliates. A few species of ciliates (*Chlamydodon mnemosyne, Frontonia algivora*) have specialized on filamentous forms which are also eaten more or less frequently by some other species (*Frontonia marina*, large *Sonderia* spp, *Diophrys scutum*). Unicellular cyanophyceans are eaten more or less frequently by a larger number of ciliates (see Table 1, p. 24).

Filamentous cyanophyceans were especially found in great numbers close to the surface of sediments with high contents of H_2S in sheltered localities (Nivå Bay, Isefjord, Askö Harbour), among sulphurbacteria and in mats of *Vaucheria*. Common genera are *Oscillatoria* and *Lyngbya*. They also occurred in large masses in artificial sulphureta. Here some colourless forms which also developed in the dark were observed.

In the tropics filamentous cyanophyceans are known to develop thick mats over reducing estuarine sediments (Conover, 1962). Something similar was often observed during the present investigation in sheltered localities where sulphurbacteria were not dominating. Wood (1965) notes that the occurrence of many blue-green algae is associated with strongly reducing sediments.

Unicellular forms were also most abundant in the above mentioned type of environments but were also found in cleaner sands and in deeper water. Meadows & Anderson (1968) found such forms attached to sand grains.

For further information and references to the ecology of blue-green algae see Wood (1965).

PHYTOFLAGELLATES. As seen in Table 1 (p. 24) the phytoflagellates are extremely important as food for the ciliates and this is also the case with other representatives of the microfauna.

Dragesco (1965c) studied the flagellates of marine sands. He found representatives of coccolithophorids, euglenoids and especially dinoflagellates (*Exuviella, Amphidinium, Thekadinium, Polykrikos, Gymnodinium*) and estimated the population sizes of localities rich in dinoflagellates to be of the order 1000-2000 individuals /cm^2 representing about 2 g/m^2.

During the present investigation only the larger forms were investigated quantitatively, i.e. representatives of the dinoflagellate genera *Amphidinium* and *Gymnodinium* and representatives of some less important genera.

Dinoflagellates were found in nearly all localities but they were not always enumerated. Largest numbers – here mainly *Gymnodinium* – were found in sulphureta, thus about 6000/cm^2 were found in masses of purple sulphurbacteria in Nivå Bay. In the "*Beggiatoa* patch locality" 100 to 600/cm^2 were found. *Gymnodinium* is also very abundant in Julebæk Beach (1400/cm^2 have been counted). This flagellate seems to be photosynthetic and was found close to or at the surface of the sediment. At one occasion *Gymnodinium* was found to be concentrated between 2 and 4 cm depth in the Julebæk Beach. It is possible that these organisms carry out vertical migrations.

In other localities with cleaner sands mainly species of *Amphidinium* were dominating. Many are devoid of chloroplasts and they occur at considerable

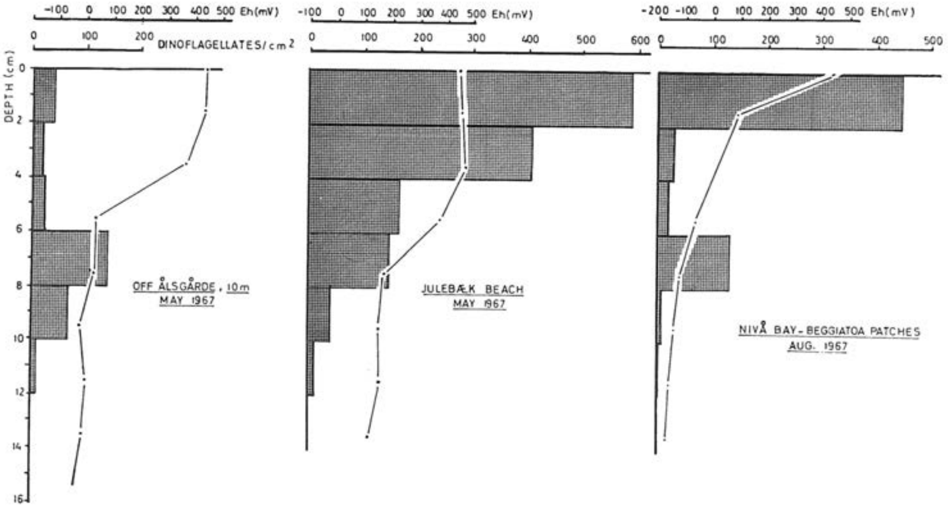

FIG. 29. The vertical distributions of large dinoflagellates and the redox-profiles in three core samples.

depths in the sediment. Numbers of these flagellates varied much. In the Helsingør Beach from less than 10 to more than 1000 have been found per cm^2 (Fig. 47). Some examples of vertical distribution of dinoflagellates are shown on Fig. 29.

Euglenoids were particularly common in sulphureta (*Euglena, Eutreptia*). In samples from Frederikssund the latter form was found in large concentrations in water containing about 40 mg H_2S/l. Euglenoids, green as well as colourless ones, were also found in cleaner sands though usually in small numbers.

Phytomonads were regularly found in sand samples as well as in the feeding vacuoles of ciliates. Neither phytomonads nor other small phytoflagellates were studied in any detail.

Desmidiaceans were present in the sediments of the brackish Askö Harbour where they were sometimes eaten by ciliates.

Vertical structure of the sediment ecosystem and its significance. Respiration and photosynthesis of undisturbed sediments

In an important paper Sorokin (1965) studied the role of chemosynthesis and bacterial biosynthesis in stagnant water bodies. He demonstrated the importance of the production of particulate organic matter by these processes based on the energy bound to reduced compounds of low molecular weight. In this way the endproducts of anaerobic decomposition are linked to the new production of organic matter. In another paper (Sorokin, 1964) the trophic significance of chemosynthesis in the Black Sea was demonstrated.

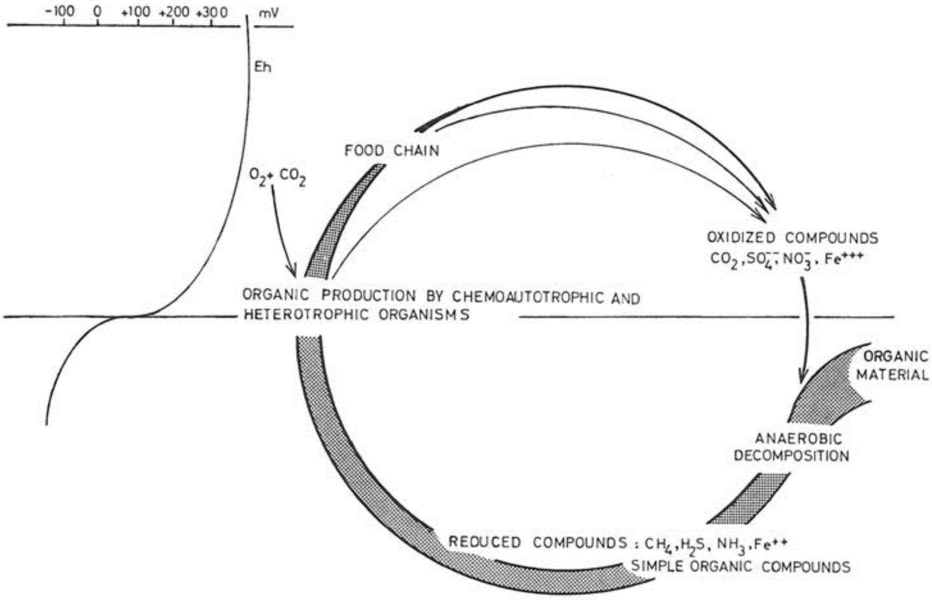

FIG. 30. Schematic representation of the microbial processes at the redox discontinuity layer in sediments.

Strictly speaking these processes should be considered as secondary production, but they resemble primary production since they use energy which is not accessible for higher life for the formation of particulate organic matter.

The true chemoautotrophs, oxidizing H_2, NH_3, H_2S, Fe^{++}, etc., obtain all their organic carbon by fixing external CO_2, but in addition Sorokin showed, by using C^{14}-carbonate, that bacteria oxidizing C_1-compounds (methane, methanol, formic acid) to a large extent (30-90 %) bind external CO_2, while bacteria oxidizing C_2-C_8 compounds utilize only 6-10 % external CO_2.

The significance of these processes was also studied in the "Volga Reservoirs" and in the Lake Belovod. Not only was the bacterial biomass and the bacterial biosynthesis maximal in the redox discontinuity layer, but also the populations of a number of animals (cladocerans, copepods, protozoans) feeding on the bacteria, which again derived their energy from reduced compounds, H_2, CH_4 and H_2S being most important. Finally Sorokin demonstrated an increase of $C^{14}O_2$ uptake in water samples bubbled with H_2 and CH_4 and measured the rate of $C^{14}O_2$ reduction by thiobacilli in water samples from the Black Sea after adding Na_2S.

It is clear from what has been said in this chapter that the model of Sorokin for stagnant lakes is highly relevant to marine sediments where anaerobic decomposition is important. Fig. 30 shows diagramatically the processes taking place at the redox discontinuity layer. Reduced low-molecular compounds diffuse up-

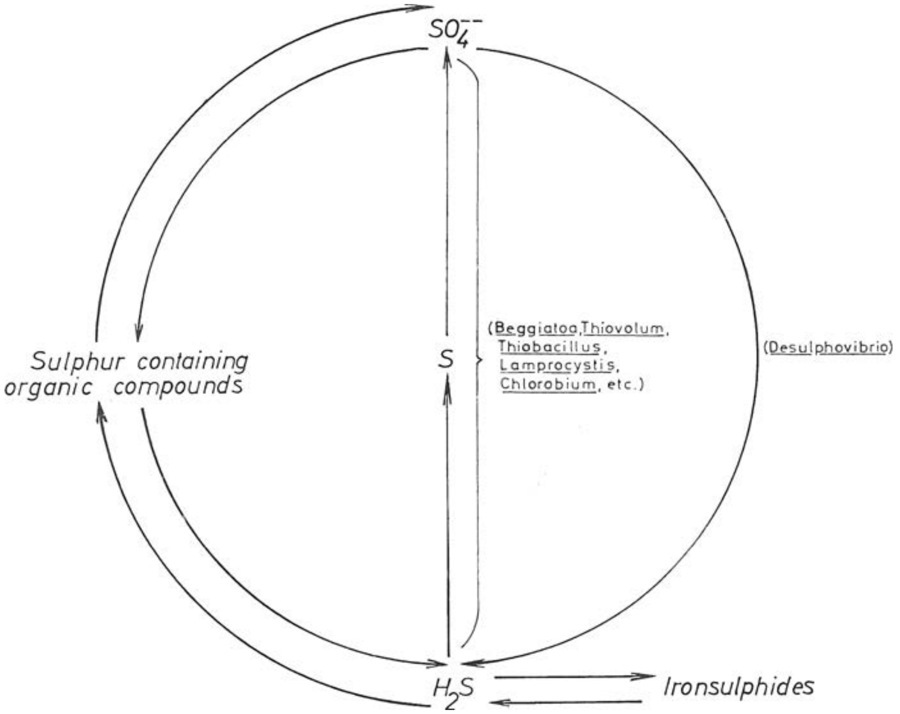

FIG. 31. Schematic representation of the sulphur cycle.

wards. When oxygen becomes available bacteria oxidize these compounds whereby —1. The redox potential increases and, —2. CO_2 is incorporated in the bacterial biomass which forms the base of new food chains. The oxidized end products of aerobic decomposition (CO_2, NO_3^-, SO_4^{--}, etc.) are utilized by anaerobic decomposers as electron acceptors and these compounds thus act as energy carriers from the anaerobic to the aerobic zone.

It should be mentioned that a few chemoautotrophs are anaerobic. Thus *Thiobacillus dinitrificans* obtains energy by oxidizing H_2S and at the same time reducing NO_3^-, and *Desulphovibrio* may oxidize H_2 by reducing SO_4^{--} (Baas Becking & Wood, 1955).

Due to the high productivity of bacteria in the redox discontinuity layer it could be expected that large numbers of animals also occur here and as shown in Chapter 6 this is also the case.

If the reducing layers are sufficiently close to the surface to receive some light, photosynthetic bacteria, notably the purple sulphurbacteria, will be present. They use light as well as reduced compounds as energy sources for reducing CO_2 to organic matter. They may occur deeper than photosynthetic plants due to a lower compensation point and are often found at 1-2 cm depth.

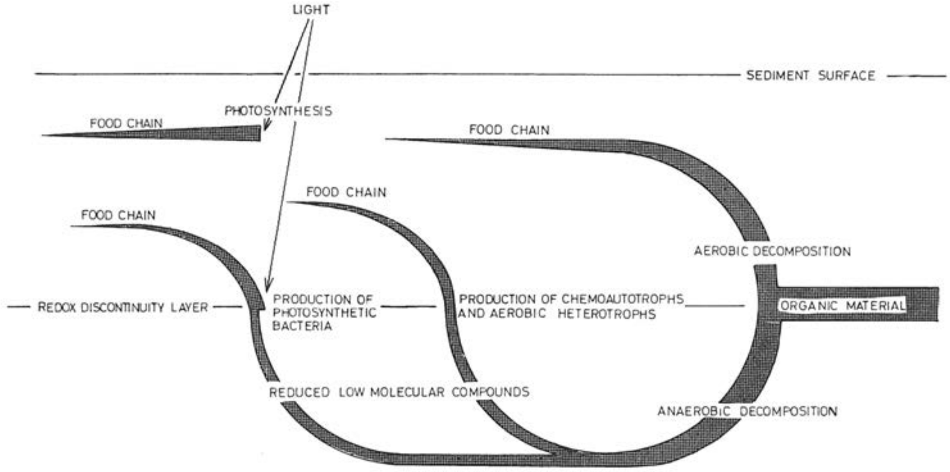

FIG. 32. Energy flow in the sediment ecosystem with schematic localization of the various processes.

The green sulphurbacteria require lower Eh (higher H_2S concentration) according to Baas Becking & Wood (1955). In the field I have never observed them in numbers great enough to be visible, but in laboratory experiments they often occur in large numbers below the purple bacteria.

These processes involve a number of elemental cycles of which – next to the carbon cycle – the sulphur cycle (Fig. 31) is without doubt the most important one. The cycles of energetic interest are those involving change of oxidation while for example the phosphorus cycle is not relevant in this connection since only P^{5+} occurs in the cycle.

The anaerobic processes are of importance from other points of view than the energetic one. Thus the production of sulphides liberates phosphate bound to insoluble complexes in oxidized sediments which is of significance for plants (see Chapter 9, and for discussion and references also Golterman, 1967).

The trophic significance of the end products of anaerobic decomposition is – as mentioned above – demonstrated by the occurrence of a rich fauna at the redox discontinuity layer but also, as shown in Chapter 6, by the importance of chemoautotrophs and photoautotrophic bacteria as food for the fauna. Thus in some estuarine sediments between 25 and 50 % of the ciliates feed on purple and white sulphurbacteria. In addition other chemoautotrophic bacteria, which cannot be identified as such in the feeding vacuoles of ciliates, must play a role.

Also other animals feed on sulphurbacteria. Thus Thane-Fenchel (1968) mentions several rotifers feeding on these bacteria.

During the present investigation it was observed that naidid oligochaetes often feed on sulphurbacteria in the Nivå Bay. The oligochaetes are again eaten by fishes in the bay so that H_2S actually enters the food-web of man.

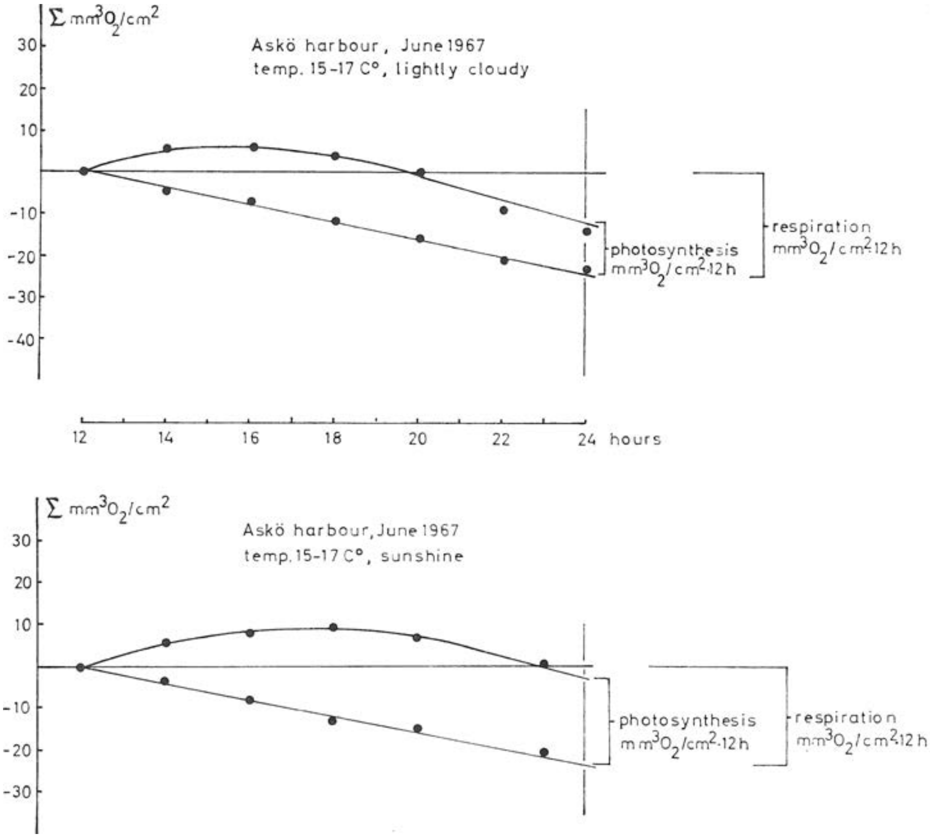

FIG. 33. Respiration and photosynthesis in a sandy sediment in the Askö Harbour in lightly clouded weather and in bright sunshine.

The total energy flow of a sediment is shown schematically in Figs 14 and 32.

It remains to discuss the importance of photosynthesis compared to the import of organic matter. This was only investigated in a few preliminary experiments in the Askö Harbour where the proportion between oxygen uptake and oxygen production of the undisturbed sediments was measured.

The experiments took place at the end of June, and Askö is situated only about 100 km south of 60° N, so photosynthesis could take place at least from 03^{00} to 20^{00}. The locality is sheltered and there is a high concentration of diatoms on the surface of the sediments. The water depth is 20-30 cm.

In each experiment two aquaria (volume about 10 l and surface area 546 cm^2) with a hole in the bottom closed with a stopper, were inverted filled with water and pushed 10 cm into the sediment. One aquarium was covered with metallic foil. The experiments were started at noon and terminated at midnight. The oxygen contents were measured every second hour with a calibrated oxygen electrode

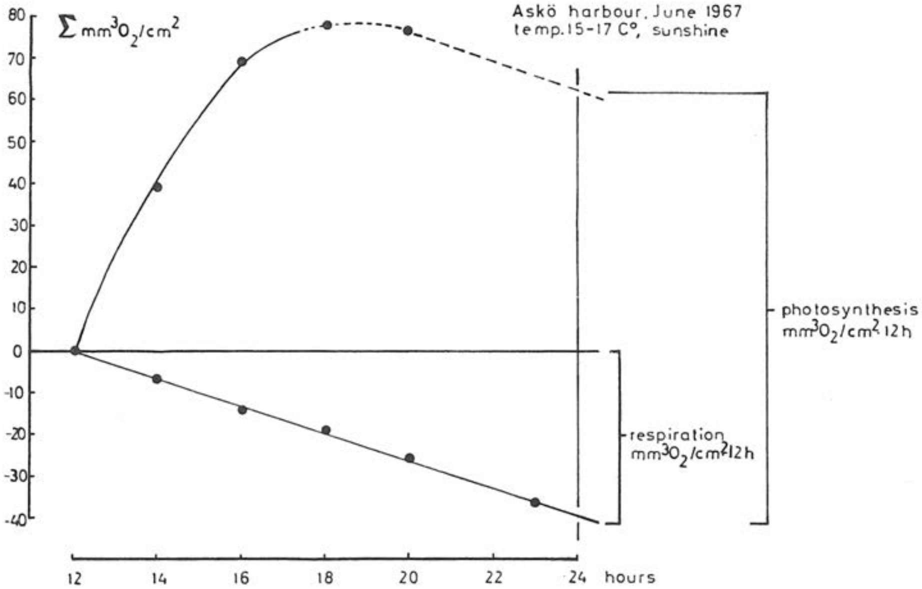

FIG. 34. As Fig. 33, but a few stones with growth of *Cladophora* were added in the dark and in the light experiment.

and the water stirred. Oxygen consumption and production in the water were measured in dark/light bottle experiments. They were so low that they would change the results with less than 10 % and could, therefore, be ignored. Water temperatures varied between 15 and 17.5°C during the experimental period.

Two experiments are shown in Fig. 33. The results are given as the cumulative oxygen production in the two tanks in mm^3 O$_2$.

When the sky was slightly clouded photosynthesis was about half the respiration. Given as mg organic carbon /m^2/24 hours the photosynthesis was 262 mg C and respiration 508 mg C. When the sky was clear throughout the day the value of photosynthesis was found to be 428 mg C and that of the respiration 492 mg C. Other experiments gave results in accordance with these. Measurements on an organic detritus layer among *Phragmites* in the vicinity of the sandy locality studied, gave somewhat higher results for both photosynthesis (555 mg C) and respiration (780 mg C).

In all cases respiration was higher than photosynthesis. To the respiration of the bottom should be added that of the macrobenthos (gastropods, small fishes) which were abundant in the locality but removed prior to the experiments.

The locality and the period of the year should be considered as optimal for photosynthesis and it seems justified to conclude that even in shallow water the bottom sediments in the main constitute a heterotrophic ecosystem which, for a great part, is based on imported organic matter. The main function of the sediments is the decomposition of organic matter and secondary production based on this matter.

Fig. 34 shows the results of an experiment similar to those described above and made in the same locality, but in each aquarium a few stones with growth of the green algae *Cladophora* were included. Respiration and photosynthesis of the underlying sediment is not subtracted. This primitive experiment cannot, of course, give any quantitative data, but it does indicate that the important producers of the shallow water regions are the representatives of the macrovegetation which in turn feed the sediment ecosystem with dead organic matter.

6. MICROFAUNA COMMUNITIES, THEIR COMPOSITION, VERTICAL ZONATION AND TROPHIC STRUCTURE

It is not possible to make a clear-cut classification of the microfaunal communities of the different localities. Tentatively they are classified into —1. The "sublittoral sand microbiocenosis", —2. The "estuarine sand microbiocenosis", and —3. The "sulphuretum". In addition reference will be given to a few other less studied communities, viz the splash zone of beaches, gravels, carpets of *Vaucheria*, and detritus covering non-capillary sediments.

The sublittoral sand microbiocenosis is found in clean (i.e. poor in detritus and silt) fine to medium sands which are permanently submerged. A reduction layer is usually found but always covered with a usually several cm thick oxidized zone. This type of sea-bottom covers large areas off the Danish coasts from 0.3 to about 20 m depth. The macrofauna assemblages in these localities are either *Macoma* communities or, in deeper water, *Venus* communities (see Chapter 2). This type of biotype is in the present paper examplified by the Hornbæk and Ålsgårde transects, localities off the Helsingør Beach and the Julebæk Beach.

By the "estuarine sand microbiocenosis" is meant the microfaunal assemblages found in or on sandy sediments of fjords, lagoons and sheltered shallow bays. Due to the large production of macrovegetation the content of organic matter is high and in connection with lentic conditions this results in reducing conditions close to the sediment surface. The sediments are often rich in detritus and silt. The macrofauna assemblage characteristic of such areas is designated a *Cardium lamarcki-Hydrobia ventrosa* community (Muus, 1967; see Chapter 2).

This type of biotope is in the present investigation examplified by the Nivå localities, Vellerup Vig (Isefjord) and the Askö Harbour. The term "estuarine" is used in its broad meaning, i.e. sheltered areas such as lagoons, fjords, shallow water bays with more or less brackish water as adopted by Green, 1968. In the strict meaning, i.e. a river mouth with fluctuating salinity due to the tide, none of the areas investigated in the present study qualify as estuaries.

By the term "sulphuretum" Baas Becking (1925) designated biotopes quite dominated by the sulphur cycle. This characteristic biocenosis which is dominated by various sulphurbacteria, is brought about by strong H_2S development of anaerobic decomposers, especially *Desulphovibrio*. Reducing conditions prevail throughout the sediment and often, at least periodically, in the supernatant water. Sulphureta are found in connection with accumulations of drift-weed in sheltered localities.

Sulphureta were studied in Nivå Bay and in the Isefjord-Roskildefjord area (Vellerup Vig, Munkholm Bridge, Frederikssund).

It is obvious that there is no sharp limit between the three types of biotopes. Thus the Julebæk Beach locality could with justification be classified as "estuarine" and the sediments in the cove in the northern part of the Nivå Bay could be designated a sulphuretum since the reduction layer often reaches the sediment surface resulting in patchy growth of sulphurbacteria on the surface.

The "sublittoral sand microbiocenosis"

Before discussing the individual localities a "generalized sand microbiocenosis" will be described (Fig. 35).

In the oxidized zone the great majority of the metazoans are found, i.e. representatives of turbellarians, gastrotrichs, nematodes, harpacticoids, tardigrades, rotifers, and archiannelids.

The turbellarians are probably mainly carnivorous though some forms (e.g. *Mecynostomum auritum*) feed on diatoms. Turbellarians are abundant in nearly all localities except those with very fine sand. The gastrotrichs are represented by macrodasyoids as well as chaetonotoids though the former are more abundant (*Turbanella, Cephalodasys,* etc.) They seem mainly to feed on diatoms. Macrodasyoids are strictly confined to the oxidized layer and may be extremely numerous where diatoms are abundant.

The nematodes are most numerous in fine sands (Chapter 3) where they often dominate the metazoan microfauna. Not many details on their feeding biology are known. Different forms eat detritus, bacteria, sulphurbacteria, other animals, and protophytes (Perkins, 1958).

Harpacticoids, archiannelids, tardigrades, and rotifers are generally less numerous. The three former groups are strictly confined to the oxidized layer and harpacticoids and archiannelids are not found in very fine sand.

The harpacticoids living in sand seem mainly to feed on bacteria. Archiannelids were, during the present investigation, mainly represented by *Protodrilus* spp. Tardigrades were represented by *Batillipes mirus* which feeds on protophytes and is frequently found in connection with a rich growth of diatoms.

Rotifers were rarely found in clean sands. Specimens of *Encentrum marinum* may be found abundantly in connection with a rich growth of protophytes (flagel-

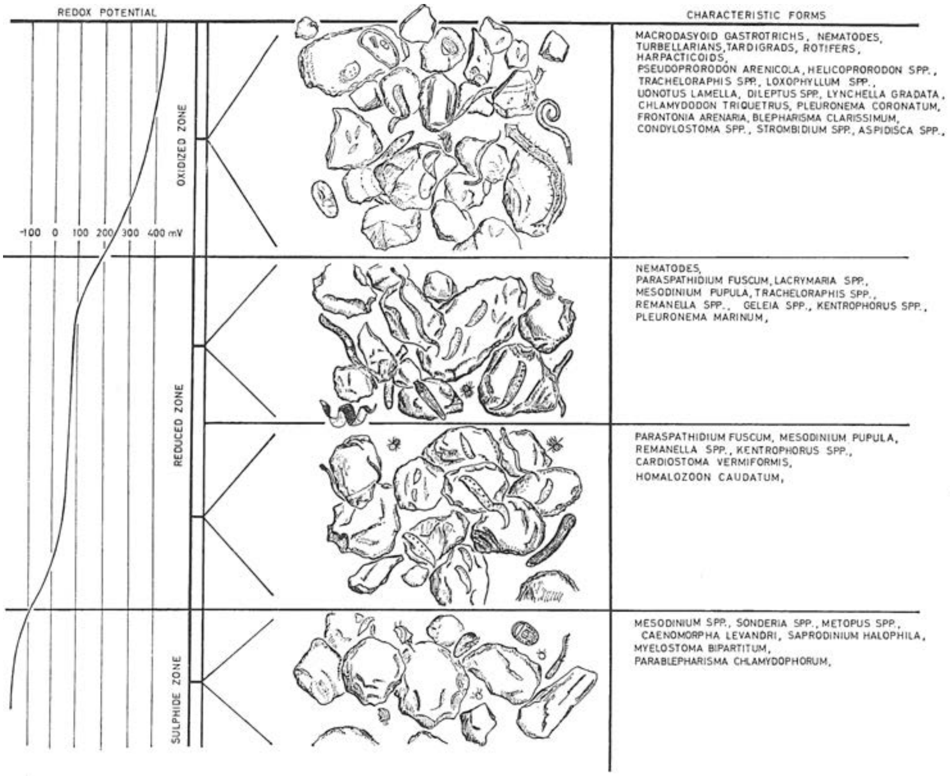

FIG. 35. Schematic representation of the vertical zonation of sublittoral sands.

lates, diatoms) on which it mainly feeds (Thane-Fenchel, 1968). More rarely specimens of a *Proales* sp. and the bacteria-feeding *Colurella* spp were found.

The oxidized zone of sands contains a large number of ciliate species characteristic of this zone. Several feed exclusively on diatoms (Fig. 36). Among them species of the oligotrich genus *Strombidium* (*S. sauerbrayae*, *S. latum*) are nearly always present. The cyrtophorines, *Chlamydodon triquetrus*, *Chilodontopsis elongata*, and *Lynchella gradata* are also frequent; they feed mainly on large diatoms. The frontoniids are mainly represented by *Frontonia arenaria;* the larger *F. marina* is rare in clean sands except in very coarse sands, it belongs more to the estuarine biocenosis. The trichostome *Discotricha papillifera,* which was found unfrequently, also belongs to the diatom feeders of the oxidized zone. Among them should also be mentioned a *Blepharisma* sp. (This species is related to *B. clarissimum,* which mainly occurs in estuarine sands, but differs by being smaller and more flattened, by a relatively longer oral zone and by possessing a much larger undulating membrane.)

A number of forms in the oxidized zone feed on diatoms, flagellates and other ciliates. To these belong the *Tracheloraphis* spp (also found in the reduced zone)

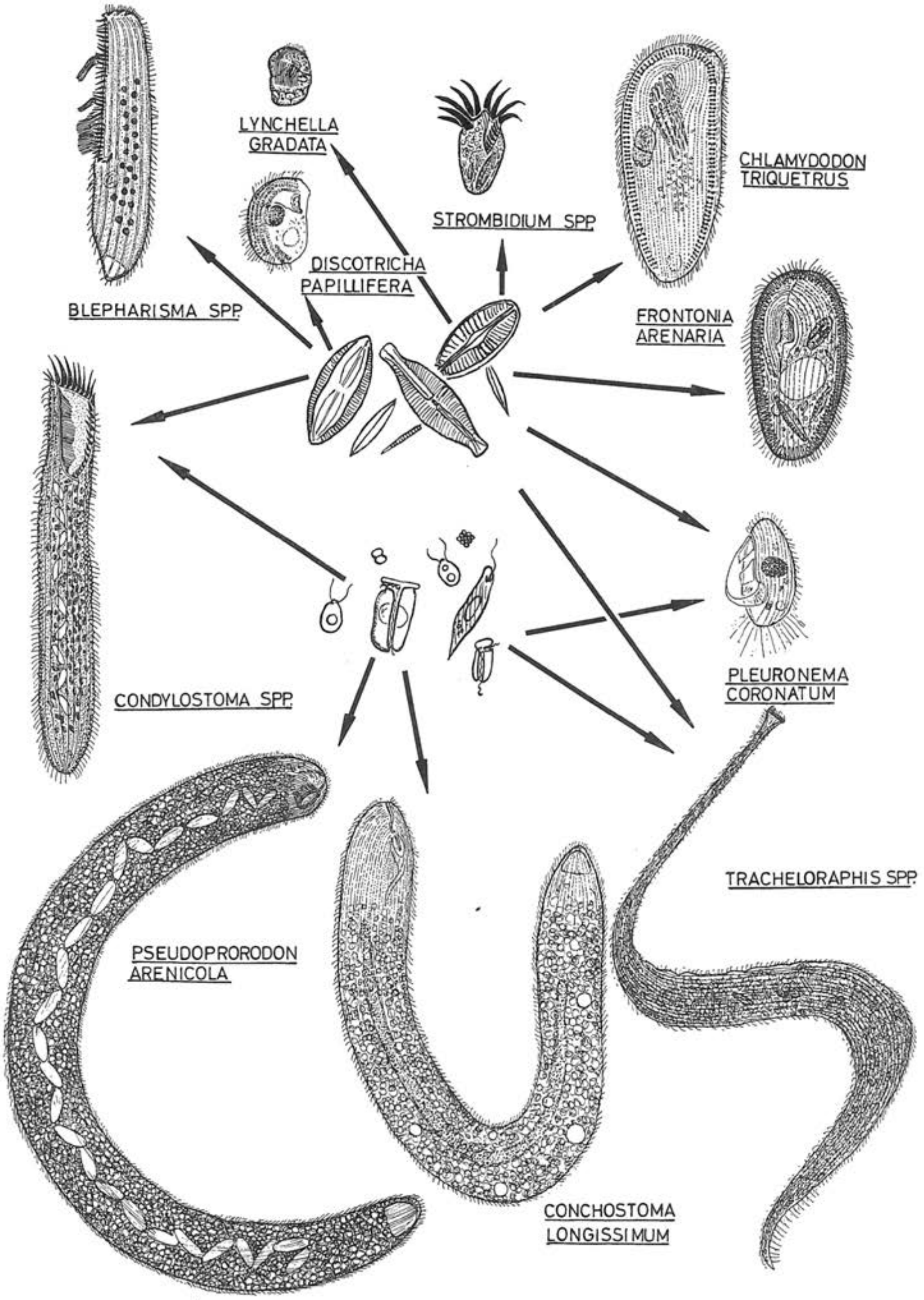

FIG. 36. The most important herbivorous ciliates in the surface layers of sublittoral sands.

and the *Condylostoma* spp, *Discocephalus ehrenbergi* and *Swedmarkia arenicola* feed on diatoms and on dinoflagellates. The *Coleps* spp (*C. pulcher, C. tesselatus*) may occur in the oxidized zone as well as in the deeper reduced layers. They are histophages, predators and herbivores at the same time. *Paraspathidium fuscum* has a similar feeding biology and may also occur in reduced layers. The *Prorodon* spp occur regularly but rarely in larger numbers in sands. They eat mainly diatoms and flagellates or may be histophagous (*P. discolor*). The two gigantic ciliates, *Pseudoprorodon arenicola* and *Conchostoma longissimum*, have both specialized on dinoflagellates. *Pleuronema coronatum*, which is also confined to the oxidized surface layers, eats small diatoms and flagellates in addition to bacteria.

The predators of the oxidized zone are forms such as *Loxophyllum* spp, *Hemiophrys* spp, *Litonotus lamella*, *Dileptus* spp, and the large *Helicoprorodon gigas* which all feed on ciliates. As mentioned the *Condylostoma* and *Tracheloraphis* spp also play a role as predators. The carnivorous *Lacrymaria* spp may occur in all zones of the sediment, but the large *L. marina* seems to be confined to the oxidized zone. The carnivores are shown in Fig. 37.

The bacterivorous ciliates of the oxidized layers comprise *Aspidisca* spp, oxytrichids (mainly *Holosticha* spp and *Trachelostyla pediculiformis*), *Pleuronema coronatum*, *Euplotes* spp and small hymenostomous species (Fig. 38).

Finally a number of species with poorly known biology are found in the oxidized zone. Among them the *Plagiocampa* spp should be mentioned. *Diophrys appendiculata* eats probably mainly bacteria (the diatom eating *D. scutum* is rare in clean sands).

The omnipresent and omnivorous *Uronychia transfuga* may also be present in the oxidized zone of sands.

In the underlying reduced layers of sand but above the sulphide zone, i.e. in the region of the redox discontinuity layer at potentials between 0 and ÷ 200 mV quite another fauna is found. Metazoans are rarer, only nematodes are still numerously represented usually together with a few turbellarians and rotifers.

Among the ciliates members of the family Loxodidae are dominating, especially species of *Remanella*, *R. margaritifera*, *R. rugosa* and *R. brunnea*, which feed mainly on diatoms and flagellates and more rarely on small ciliates and large bacteria (*R. rugosa* may feed on *R. margaritifera*), *R. gigas* which feeds on large diatoms, and *R. minuta* the food of which is not known. A number of rarer species are also found. Species of the genus *Ciliofaurea*, which mainly feed on dinoflagellates, are often found as are the species of the genus *Kentrophorus*. The feeding biology of the *Kentrophorus* spp is incompletely known, but they do sometimes contain tests of diatoms.

Another characteristic element of the fauna of the reduced layers are *Geleia* spp (*G. fossata, G. orbis, G. decolor, G. nigriceps* and some rarer species of which some are undescribed). *G. nigriceps* feeds on diatoms and flagellates, the other species mainly on other ciliates.

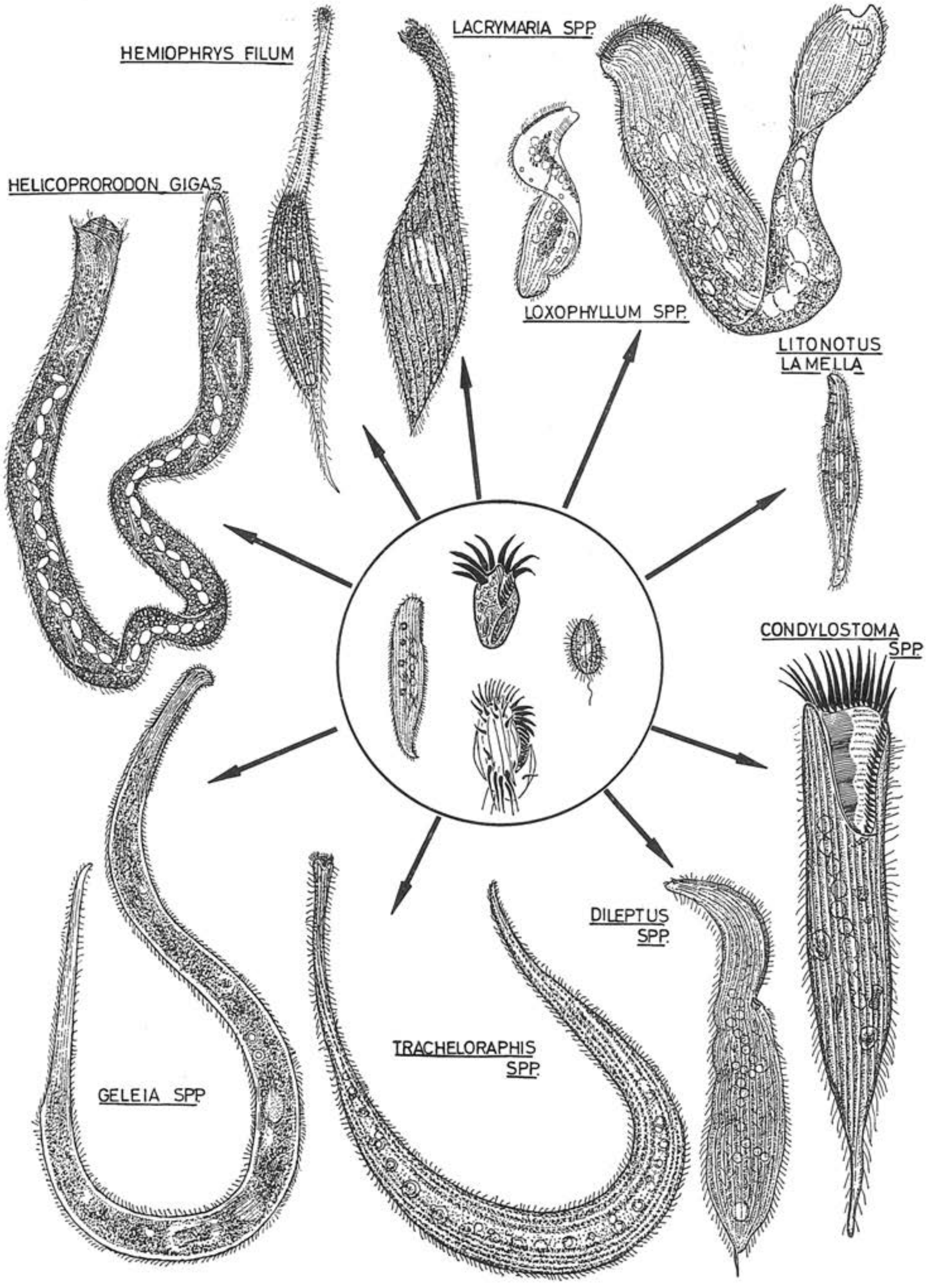

FIG. 37. The most important carnivorous ciliates in sublittoral sands.

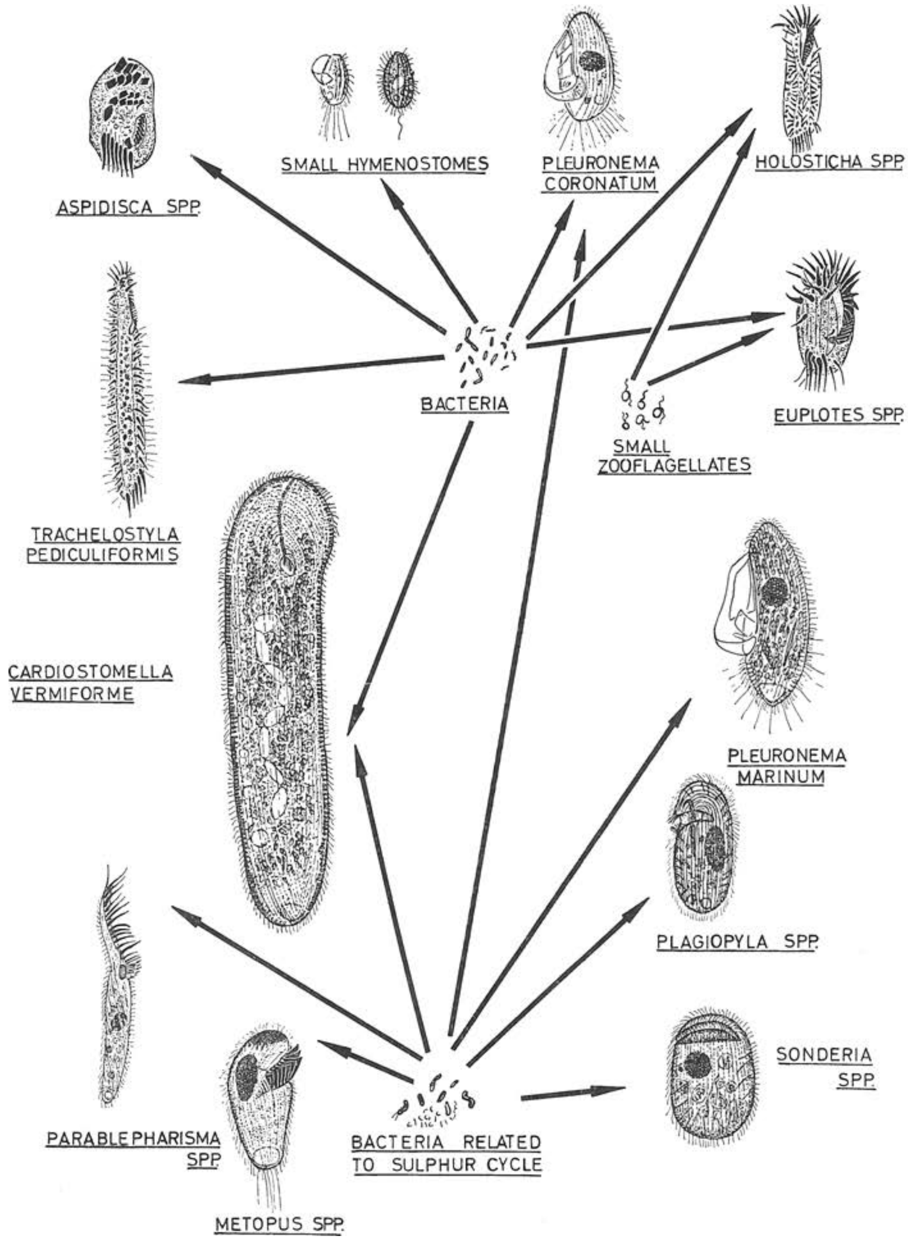

FIG. 38. The most important bacterivorous ciliates in sublittoral sands.

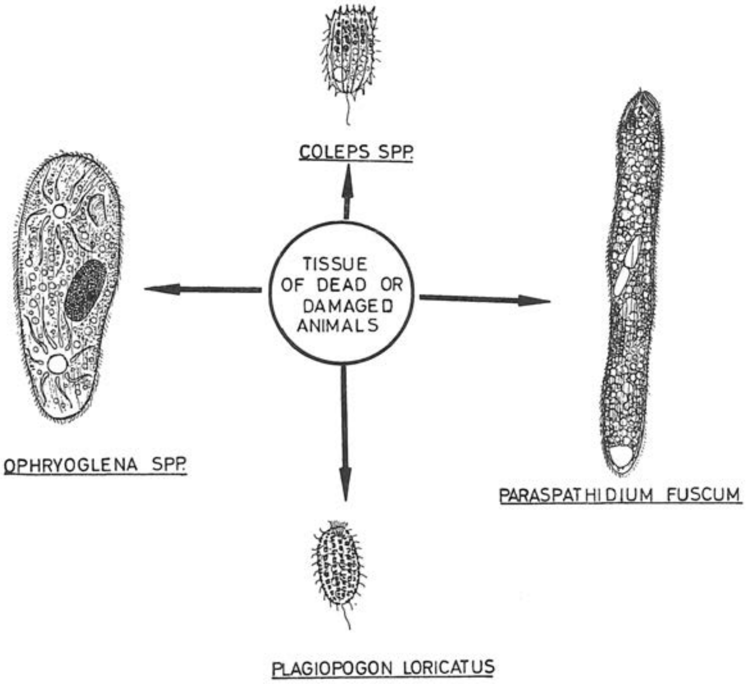

FIG. 39. Histophagous ciliates of sublittoral sands.

The genus *Tracheloraphis* is well represented in reduced sands. The spathidiid *Homalozoon caudatum*, which feeds on flagellates and the related *Paraspathidium fuscum* are also common. *Urostrongylum caudatum*, a diatom-eating oxytrichid, is sometimes found here. Characteristic for the zone is *Mesodinium pupula* probably feeding on protophytes. *Pleuronema marinum* replaces *P. coronatum* in the reduced layer. As its smaller relative it feeds on diatoms, flagellates and bacteria, not least sulphurbacteria, but may swallow somewhat larger objects. An undescribed cuplotid ciliate (Fig. 42,7) is frequently found in the reduced layer and so is the flagellate-eating and histophagous *Plagiopogon loricatus*.

The predators are mainly represented by the above mentioned *Geleia* and *Tracheloraphis* spp in addition to several *Lacrymaria* spp. The amphileptids are practically absent in reduced sands.

The bacterivorous ciliates are represented by small hymenostomes (*Uronema*, etc.), oxytrichids and *Euplotes* spp in addition to the above mentioned *Pleuronema marinum*. *Cardiostomella vermiforme* also feeds mainly on bacteria and also to some extent on small diatoms.

When a sulphide zone is developed deeper in the sediment a number of forms will be present in the vicinity of the redox discontinuity layer, i.e. species of *Plagiopyla*, *Metopus* and *Sonderia* which all feed mainly on sulphurbacteria and are also living in the sulphide zone. They will be discussed below.

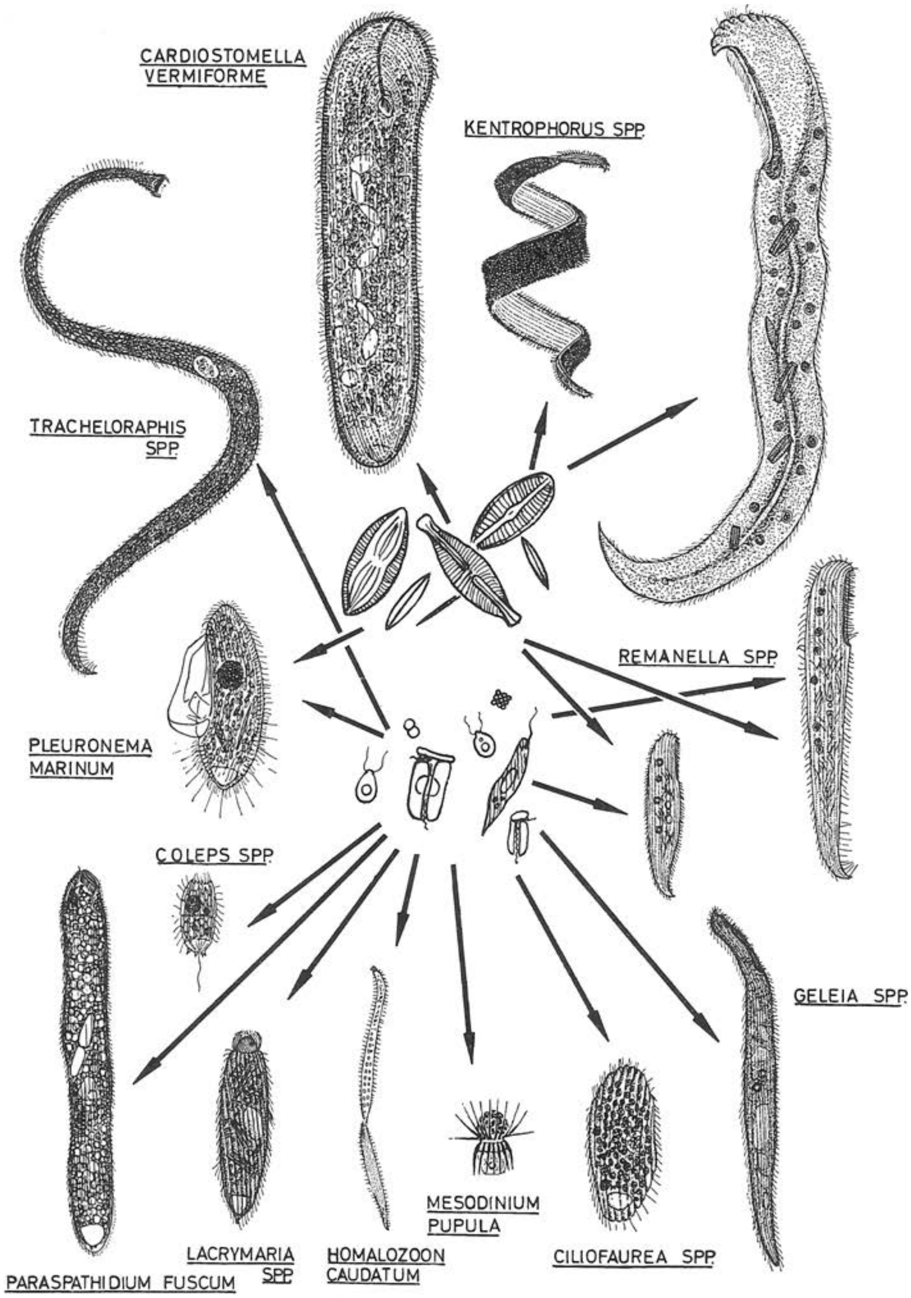

FIG. 40. The most important herbivorous ciliates of the reduced zone of sublittoral sands.

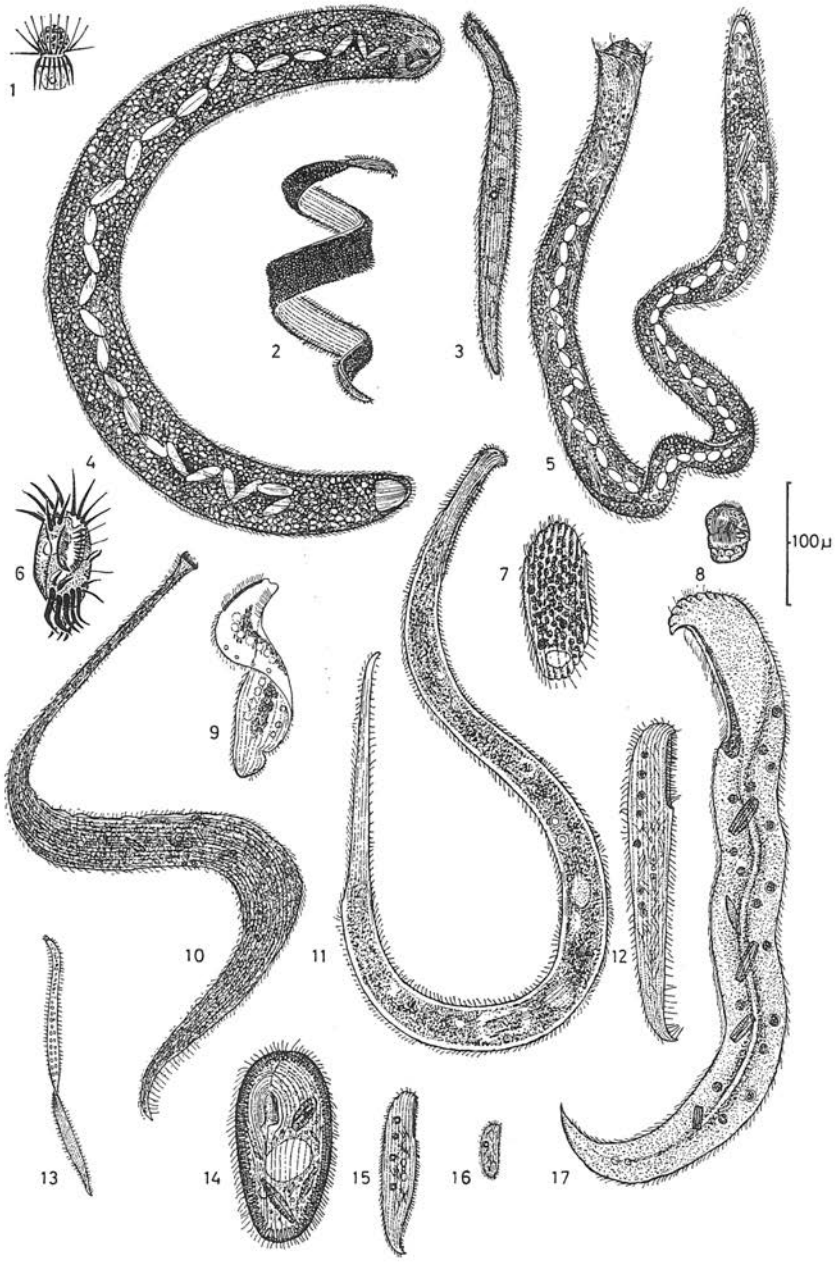

FIG. 41. Ciliates characteristic of fine sands. 1, *Mesodinium pupula;* 2, *Kentrophorus fasciolatum;* 3, *Geleia fossata;* 4, *Pseudoprorodon arenicola;* 5, *Helicoprorodon gigas;* 6, *Diophrys appendiculata;* 7, *Ciliofaurea mirabilis;* 8, *Lynchella gradata;* 9, *Loxophyllum kahli;* 10, *Tracheloraphis* sp.; 11, *Geleia orbis;* 12, *Remanella rugosa;* 13, *Homalozoon caudatum;* 14, *Frontonia arenaria;* 15, *Remanella margaritifera;* 16, *R.minuta;* 17, *R.gigas.*

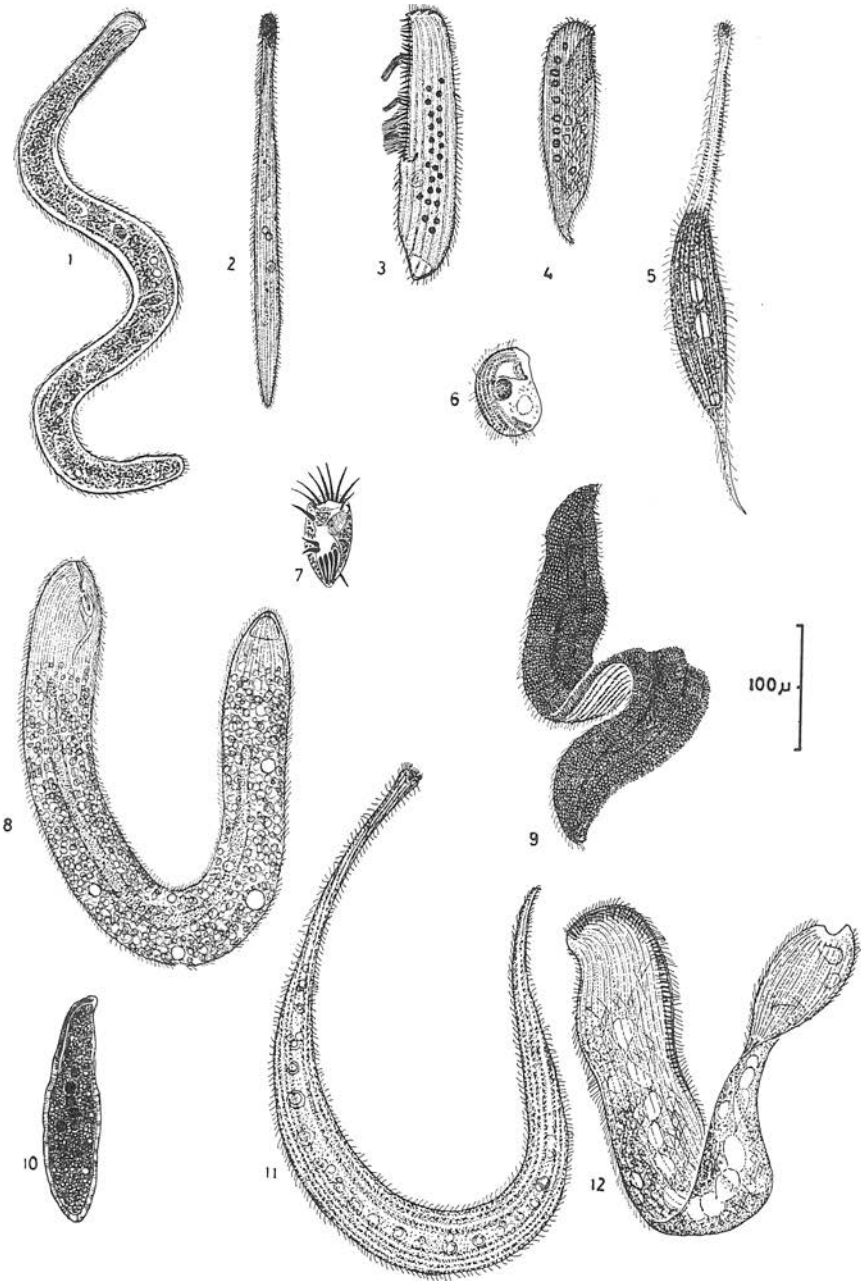

FIG. 42. Ciliates characteristic of fine sands. 1, *Geleia decolor;* 2, *G. nigriceps;* 3, *Blepharisma* sp.; 4, *Remanella brunnea;* 5, *Hemiophrys filum;* 6, *Discotricha papillifera;* 7, Euplotidae sp.; 8, *Chonchostoma longissimum;* 9, *Kentrophorus latum;* 10, *Loxophyllum variabilis;* 11, *Tracheloraphis kahli;* 12, *Loxophyllum vermiforme.*

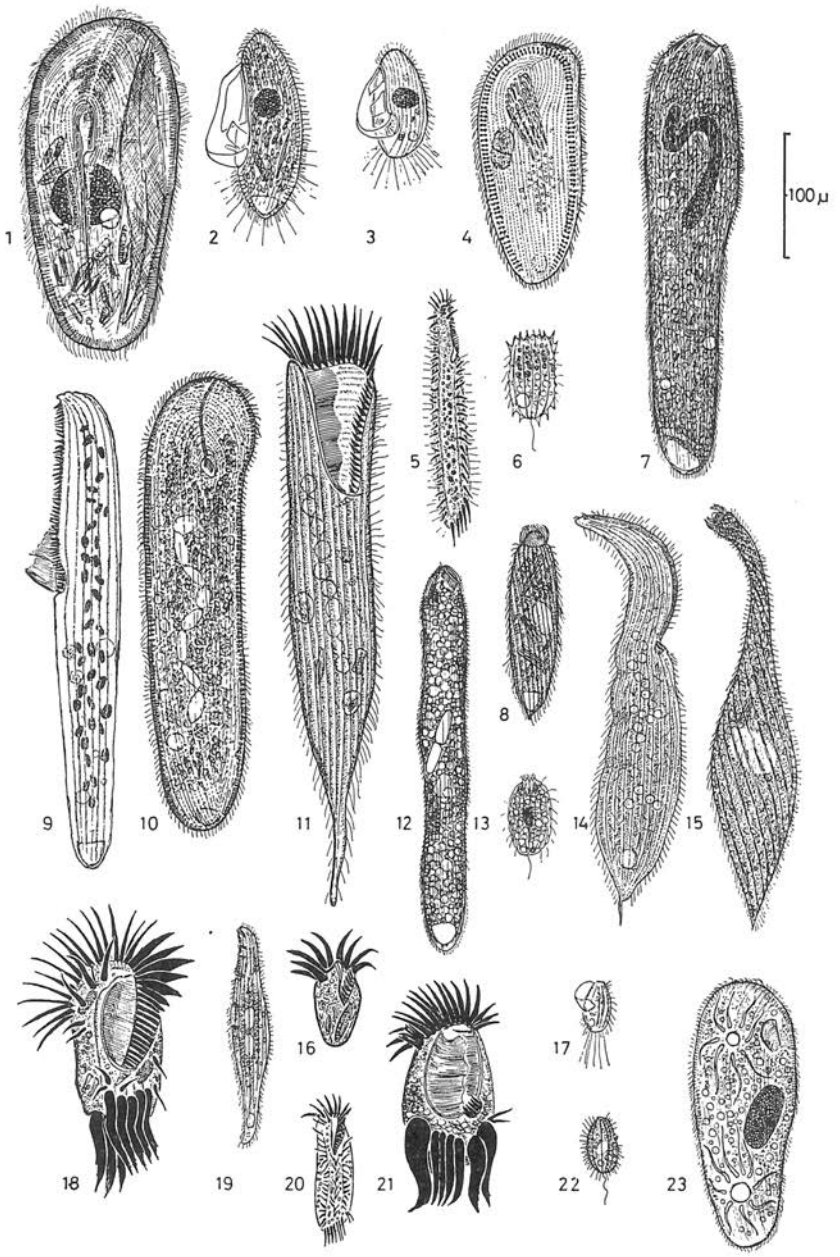

FIG. 43. Common benthic ciliates. 1, *Frontonia marina;* 2, *Pleuronema marinum;* 3, *P. coronatum;* 4, *Chlamydodon triquetrus;* 5, *Trachelostyla pediculiformis;* 6, *Coleps pulcher;* 7, *Prorodon morgani;* 8, *Lacrymaria delamarei;* 9, *Blepharisma clarissimum;* 10, *Cardiostomum vermiforme;* 11, *Condylostoma remanei;* 12, *Paraspathidium fuscum;* 13, *Plagiocampa* sp.; 14, *Dileptus* sp.; 15, *Lacrymaria marina;* 16, *Strombidium sauerbrayae;* 17, *Cyclidium* sp.; 18, *Diophrys scutum;* 19, *Litonotus lamella;* 20, *Holosticha arenicola;* 21, *Uronychia transfuga,* 22, *Uronema marina;* 23, *Ophryoglena* sp.

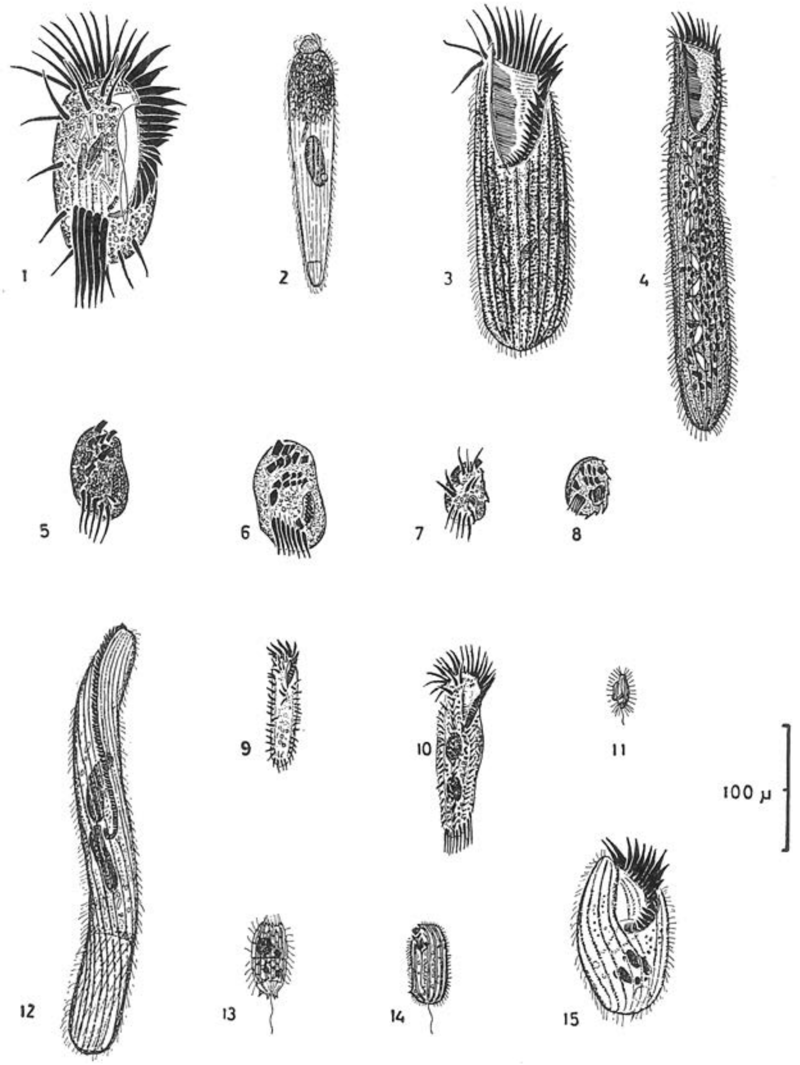

FIG. 44. Common benthic ciliates. 1, *Euplotes harpa;* 2, *Lacrymaria scuta;* 3, *Condylostoma patulum;* 4, *C. tenuis;* 5, *Aspidisca major;* 6, *A. mutans;* 7, *A. fusca;* 8, *A. pulcherrima;* 9, *Oxytricha marinum;* 10, *Holosticha alveolata;* 11, *Cyclidium citrullus;* 12, *Spirostomum teres;* 13, *Coleps tesselatus;* 14, *Platynema denticulatum;* 15, *Climacostomum virens.*

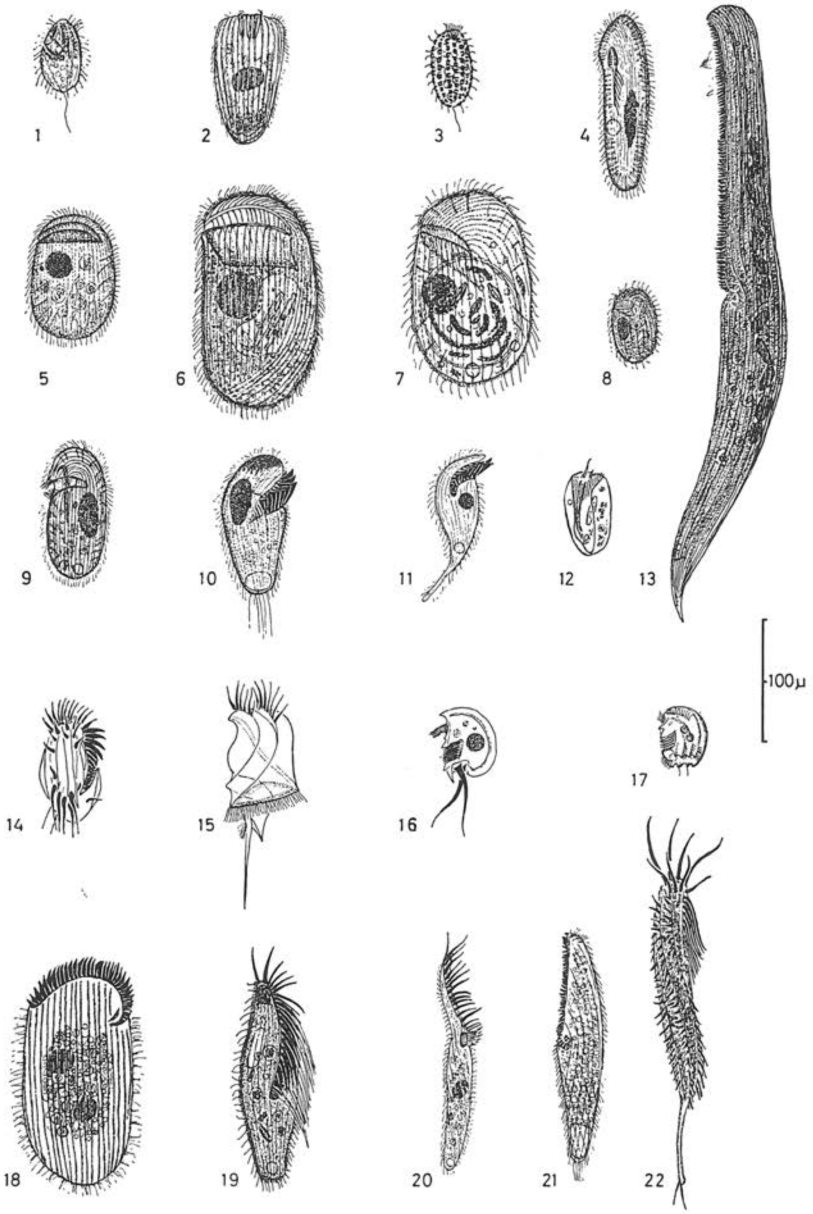

FIG. 45. Benthic ciliates, especially forms living near to or in sulphide containing environments. 1, *Cristigera media;* 2, *Prorodon discolor;* 3, *Plagiopogon loricatus;* 4, *Frontonia microstoma;* 5, *Sonderia vorax;* 6, *S. sinuata;* 7, *S. schizostoma;* 8, *S. cyclostoma;* 9, *Plagiopyla frontata;* 10, *Metopus contortus;* 11, *M. vestitus;* 12, *Trochiloides recta;* 13, *Gruberia* sp.; 14, *Euplotes elegans;* 15, *Caenomorpha levandri;* 16, *Myelostoma bipartitum;* 17, *Saprodinium halophila;* 18, *Peritromus faurei;* 19, *Parablepharisma pellitum;* 20, *P. chlamydopherum;* 21, *Blepharisma salinarum;* 22, *Urostrongylum caudatum.*

The fauna of the hydrogen sulphide zone is again rather different from that found in the above overlying strata. Some forms are, however, common to the sulphide zone and the zone with reducing but not H_2S containing sediments. These are the *Remanella* spp, *Mesodinium pupula, Cardistomella vermiforme, Pleuronema marinum, Plagiopogon loricatus,* sometimes *Kentrophorus* spp, and *Trachelostyla pediculiformis.* More characteristic for the sulphide zone are the *Metopus* spp (*M. contortus, M. vestitus, M. verrucosus*), the *Sonderia* spp (*S. vorax, S. cyclostoma* and some unidentified forms) and the *Plagiopyla* spp all feeding mainly on sulphurbacteria but also on other bacteria and protophytes.

Strictly confined to H_2S containing sediments are *Caenomorpha levandri, Saprodinium halophila, Myelostoma bipartitum,* and *Parablepharisma chlamydopherum* which all live on bacteria. An unidentified *Cristigera* sp and an undescribed *Mesodinium* sp are nearly always associated with H_2S containing sands. Several small unidentified hymenostomes (species of *Uronema, Cyclidium,* etc.) are often also found in the sulphide zone.

Having described a somewhat idealized sublittoral sand microbiocenosis we shall now discuss the individual localities.

HELSINGØR BEACH. As shown in Chapter 5, the locality off Helsingør Beach (see Figs 1, 3 and 4) at 0.2 to 0.5 m depth constitutes a rather unstable environment due to periodic strong wave action and periodic supply of drift-weed. It was shown that reducing conditions with H_2S production prevail in spring and autumn and to a somewhat smaller extent in summer, while the sediments are oxidized during long periods in winter due to the mixing of the sand by the surf (see Fig. 24). During calm periods the sulphide zone may be as close as 5 cm from the sediment surface but usually it is deeper (10-20 cm from the surface). It was shown that diatom growth is poor, especially at the surface of the sediment and this is probably due to the mixing of the surface sands. The median grain size of the sand is 200 μ.

The metazoan microfauna mainly comprises nematodes, turbellarians and macrodasyoid gastrotrichs, while harpacticoids, rotifers and archiannelids are rarer. The number of individuals were larger during the summer though representatives of most of the groups were found throughout the year (Fig. 46).

Nematodes were usually found in numbers between 1 and 10/cm^2, but up to 25/cm^2 were found in July (average for the year: 6.8/cm^2). Turbellarians occurred with the same frequency but more than 30/cm^2 were found in a period from July to August (average for the year: 6.7/cm^2). Gastrotrichs, mainly *Turbanella,* were found in numbers between 1 and 5/cm^2. A few specimens of a chaetonotoid were sporadically found. Rotifers and harpacticoids were too rare (less than 1/cm^2) to give a reliable quantitative picture of their occurrence.

In June 1968, a number of samples in total covering 30 cm^2, taken in order to allow of a better estimate of the numbers of micrometazoa, gave the following results: Nematodes: 19/cm^2, turbellarians: 7.6/cm^2, *Turbanella:* 1.4/cm^2, harpac-

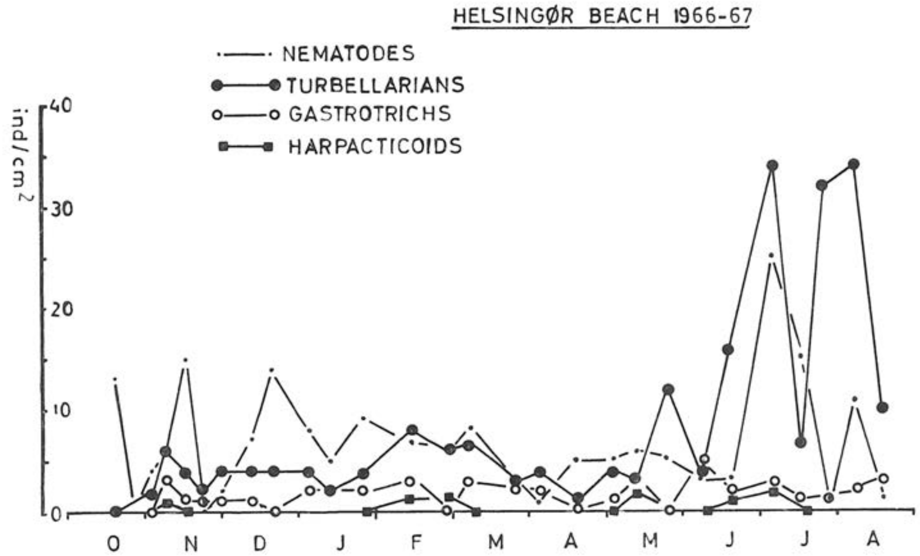

FIG. 46. The occurrences of nematodes, turbellarians, gastrotrichs, and harpacticoids in the Helsingør Beach from October 1966 to August 1967.

ticoids: 0.7/cm², oligochaetes (enchytraeids): 0.7/cm², *Protodrilus:* 0.03/cm², juvenile lamellibranchs: 0.06/cm² and the amphipod *Bathyporeia:* 0.3/cm².

The number of dinoflagellates (mainly *Amphidinium* spp lacking chromatophores) varied between 10 and more than 1000/cm² throughout the year (Fig.47). These fluctuations remain unexplained. Amoebae are rare: between 0 and 7/cm² were found.

The dominating animal group is, no doubt, the ciliates (679-4024 per cm², average for the year: 1695/cm²). Large populations are found throughout the year but the largest numbers occurred in autumn and in late spring (Fig.47) following the supply of drift-weed washed into the sand in early spring and early autumn.

The surface fauna of the Helsingør Beach is poor in species and this applies especially to the herbivorous forms which are also very poor in individuals. This is due to the shifting of the sediment and the poor growth of diatoms. The only diatom-feeding ciliates frequently encountered in the surface layers are *Frontonia arenaria* and *Pleuronema coronatum* which occur in large numbers in calm periods especially during summer (Fig.47), probably due to a richer growth of diatoms in that season. Other herbivores characteristic of the surface layers are unfrequent. *Blepharisma* sp., *Strombidium* spp, *Chlamydodon triquetrus, Discocephalus* sp, *Lynchella gradata* and *Condylostoma* spp were all regularly found in the surface layers especially during summer, but always in small numbers.

Conchostomum longissimum which is also confined to the oxidized layers was

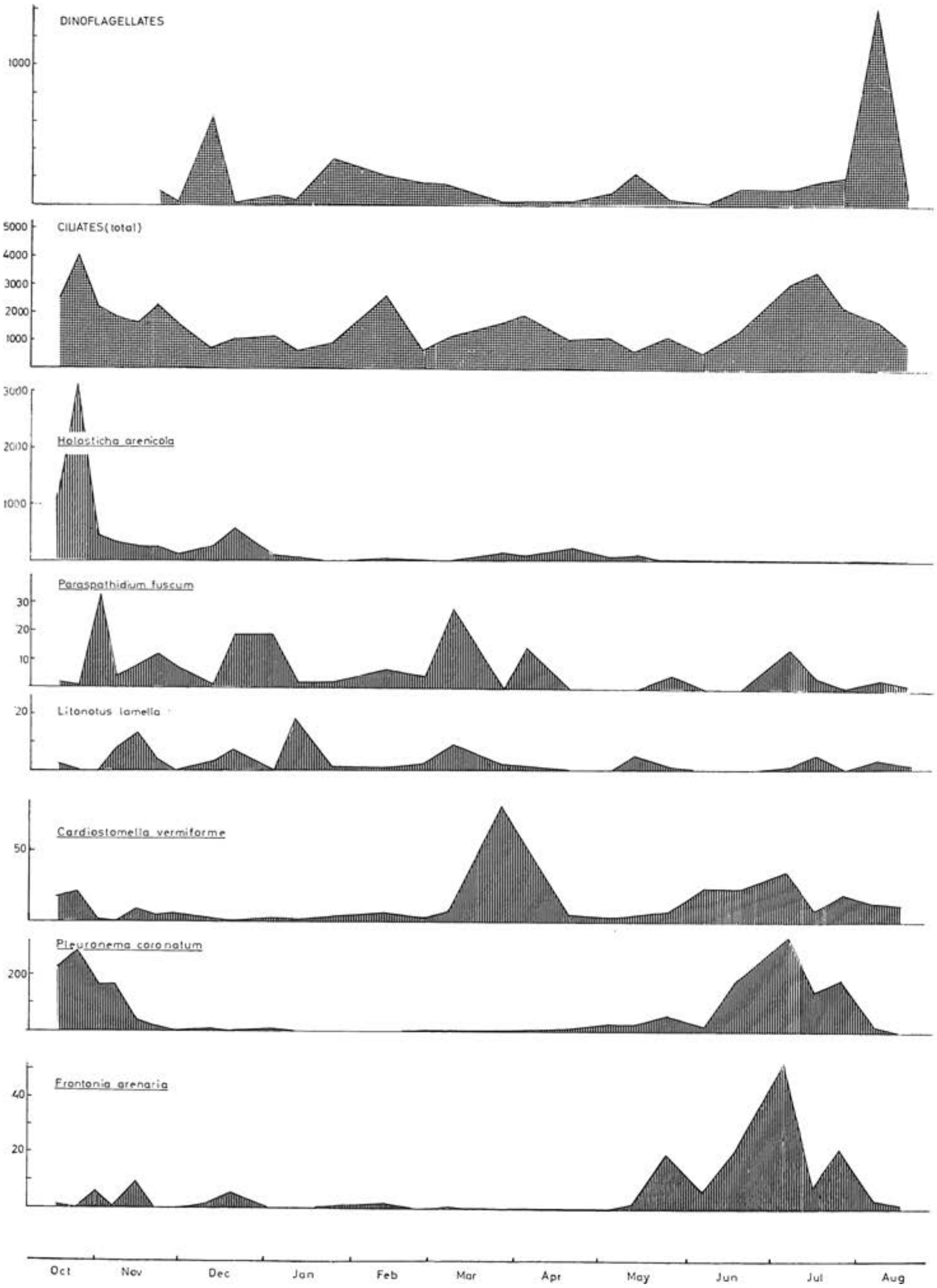

FIG. 47. The quantitative occurrences (ind./cm²) of large dinoflagellates and of ciliates in the Helsingør Beach from October 1966 to August 1967. (Redox conditions are shown in Fig. 48).

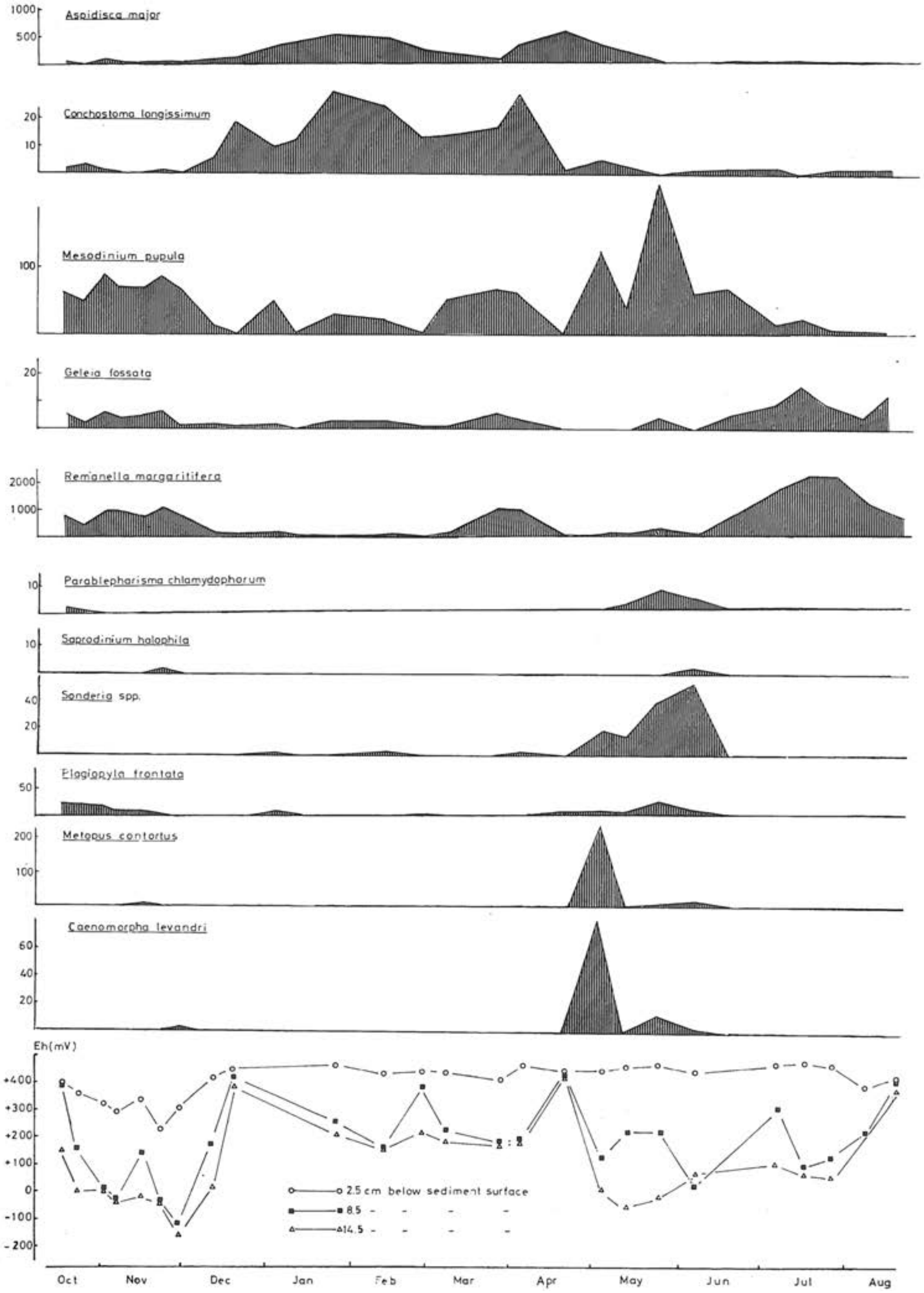

FIG. 48. The quantitative occurrences (ind./cm²) of two species confined to oxidized sediments, three species confined to reduced sediments and six species confined to sulphide containing sediments. The Helsingør Beach, October 1966 to August 1967.

found in largest numbers during the winter when the sediments were oxidized to a considerable depht.

The bacteria-feeding forms living in the oxidized surface layers are mainly represented by *Aspidisca* spp (mainly *A. major*) which occurred in large numbers when the sediments were oxidized throughout (Fig. 48). Under such conditions they were found at considerable depths in the sediment. When a reduction layer was developed they were confined to the surface layers and occurred in smaller numbers. Other bacterivorous ciliates were *Holosticha arenicola, Trachelostyla pediculiformis* and some other forms, which also occurred at greater depths in the sediment.

The predators of the surface layers are *Litonotus lamella, Loxophyllum* spp and *Hemiophrys filum.* Sometimes a *Dileptus* sp was found. These forms were, as the other inhabitants of the oxidized zone, found deeper in the sediments when these were oxidized to a greater depth.

In contrast to the surface a large number of species and individuals is found in the underlying reduced layer, especially around the redox discontinuity layer. The most common species are *Remanella* spp (especially *R. margaritifera* with more than 2000 individuals per cm^2 in July, 1967), the *Kentrophorus* spp, *Geleia fossata* and, more rarely, *G. decolor, Mesodinium pupula, Cardiostomella vermiforme, Tracheloraphis* spp, *Lacrymaria* spp, and *Paraspathidium fuscum.*

The depth at which these species are found depends on the redox-profile. At some occasions a considerable number of these forms may have been present at greater depths than those from which core samples were taken, i. e. when the sand was oxidized to greater depths.

Populations of *Mesodinium pupula, Geleia* spp, *Kentrophorus* spp and especially *Remanella* spp are much larger when reducing conditions prevail (Fig. 48).

The oxytrichid *Holosticha arenicola,* which feeds on bacteria and small zooflagellates living on decomposing seaweeds, became numerous soon after these had been accumulated in the sands (Fig. 47), especially in autumn 1966.

During autumn 1966 and May-June 1967 a sulphide zone developed due to accumulated drift-weed and calm weather. In accordance with this, species as *Metopus contortus, Caenomorpha levandri, Sonderia vorax, S. cyclostoma, Parablepharisma chlamydopherum, Saprodinium halophila, Myelostoma bipartitum, Mesodinium* sp., and *Plagiopyla frontata* appeared. For unknown reasons this fauna was better developed in the spring than in autumn (Fig. 48).

The occurrence of some other forms during the period from autumn 1966 to summer 1967 is shown in Fig. 47.

Figs 49-50 and Table 3 show the vertical distribution of some representatives of the microfauna and the total number of ciliates found in samples taken 18 October 1966 and 24 May 1967, respectively. At both sampling dates a sulphide zone was present but only in the May sample was the "sulphide fauna" fully developed. The typical zonation can be seen with the ciliate maximum (numbers

TABLE 3. Numbers of animals and values of Eh in a core sample from Helsingør Beach; fine sand (Mdφ: 200 μ, QDφ: 0.42); water depth: ca. 50 cm; temperature 11.5 °C; sample size: 1 cm²; 24 May 1967.

Depth (cm)	0-3	3-6	6-9	9-12	12-15	15-18	total
Mesodinium pupula	.	.	6	146	122	1	275
Remanella margaritifera	.	2	67	132	20	.	221
Tracheloraphis spp.	5	8	34	46	3	5	101
Aspidisca major	9	9	7	23	5	18	71
Pleuronema coronatum	40	17	1	.	.	.	58
Mesodinium sp.	.	.	.	.	5	47	52
Sonderia cyclostoma	.	.	1	21	9	3	34
Remanella brunnea	.	1	9	20	.	.	30
Lacrymaria sp.	2	4	12	5	5	.	28
Plagiopyla frontata	.	1	8	14	3	.	26
Frontonia arenaria	15	5	.	.	.	.	20
Remanella minuta	.	.	.	12	3	.	15
Homalozoon caudatum	.	.	.	10	3	2	15
Caenomorpha levandri	.	.	.	3	8	3	14
Metopus contortus	.	.	.	7	4	.	11
Holosticha arenicola	.	.	.	3	3	2	8
Aspidisca sp.	1	.	1	4	.	1	7
Parablepharisma chlamydophorum	.	.	2	4	1	.	7
Cardiostomella vermiforme	.	.	.	4	.	3	7
Remanella rugosa	.	.	1	3	2	.	6
Paraspathidium fuscum	.	.	2	3	.	.	5
Sonderia vorax	.	.	.	5	.	.	5
Pleuronema marinum	1	3	.	.	.	.	4
Geleia fossata	.	2	2	.	.	.	4
Strombidium sp.	.	.	.	4	.	.	4
Loxophyllum sp.	3	.	.	.	.	.	3
Kentrophorus latum	.	.	.	3	.	.	3
Blepharisma sp.	1	.	.	.	.	.	1
Litonotus lamella	1	.	.	.	.	.	1
Euplotidae sp.	.	1	.	.	.	.	1
Strombidium latum	.	1	.	.	.	.	1
Diophrys appendiculata	.	1	.	.	.	.	1
ciliate spp	1	1	1	.	.	.	3
small ciliate spp	12	5	15	54	22	45	153
total	91	61	169	526	218	130	1195
amoebae	.	1	1	.	2	.	4
dinoflagellates	12	25	2	1	1	1	42
nematodes	2	1	2	.	.	.	5
turbellarians							
Mecynostomum auritum	.	2	.	.	.	.	2
Archilopsis unipunctata	1	.	1	.	.	.	2
juvenile	.	.	5	3	.	.	8
total	3	3	8	3	.	.	17

depth (cm)	0	2.5	5.5	8.5	11.5	14.5	17.5
Eh (millivolts)	+454	+466	+442	+220	+154	−26	−32

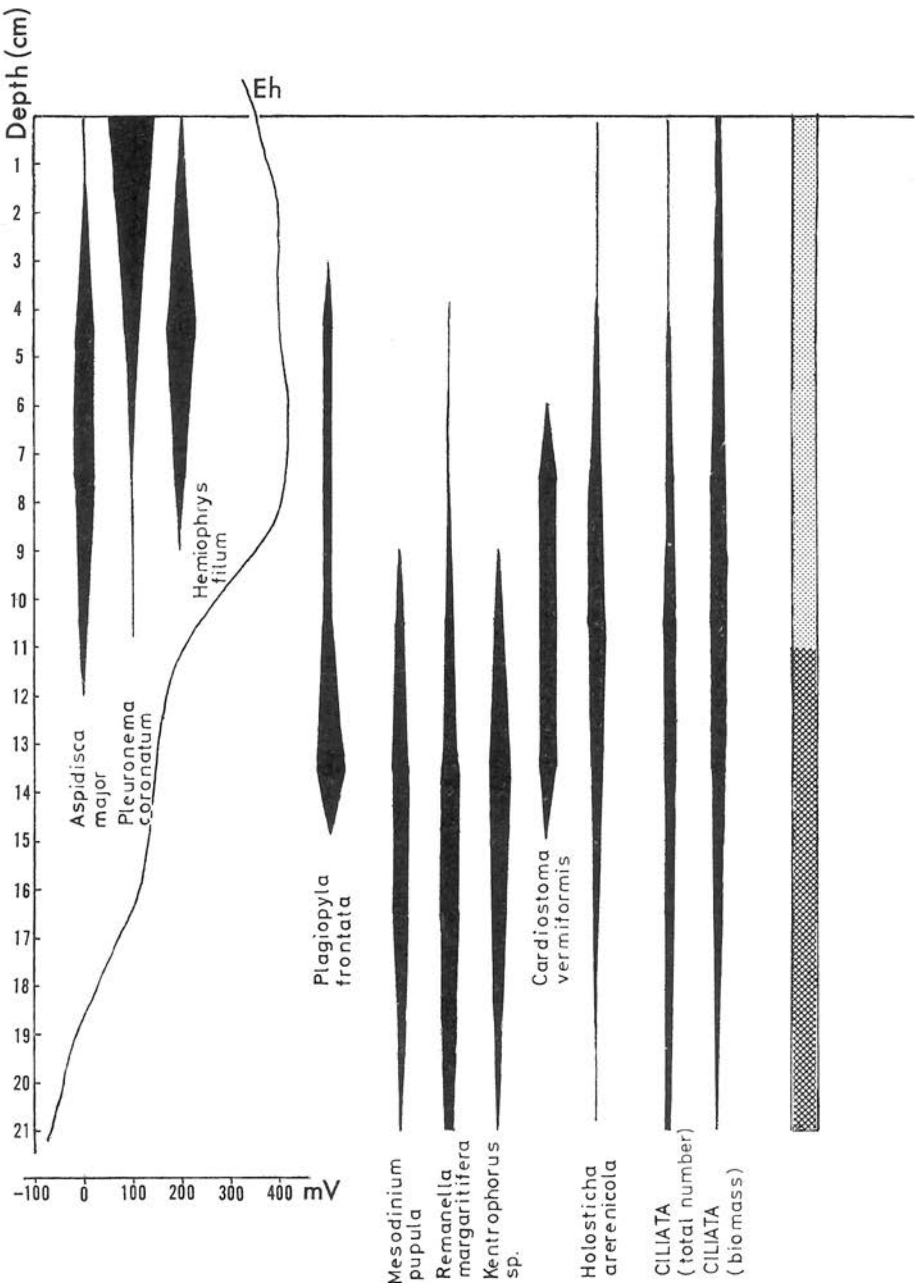

FIG. 49. Vertical distributions of some more common species in the Helsingør Beach and the redox-profile. 18 October 1966.

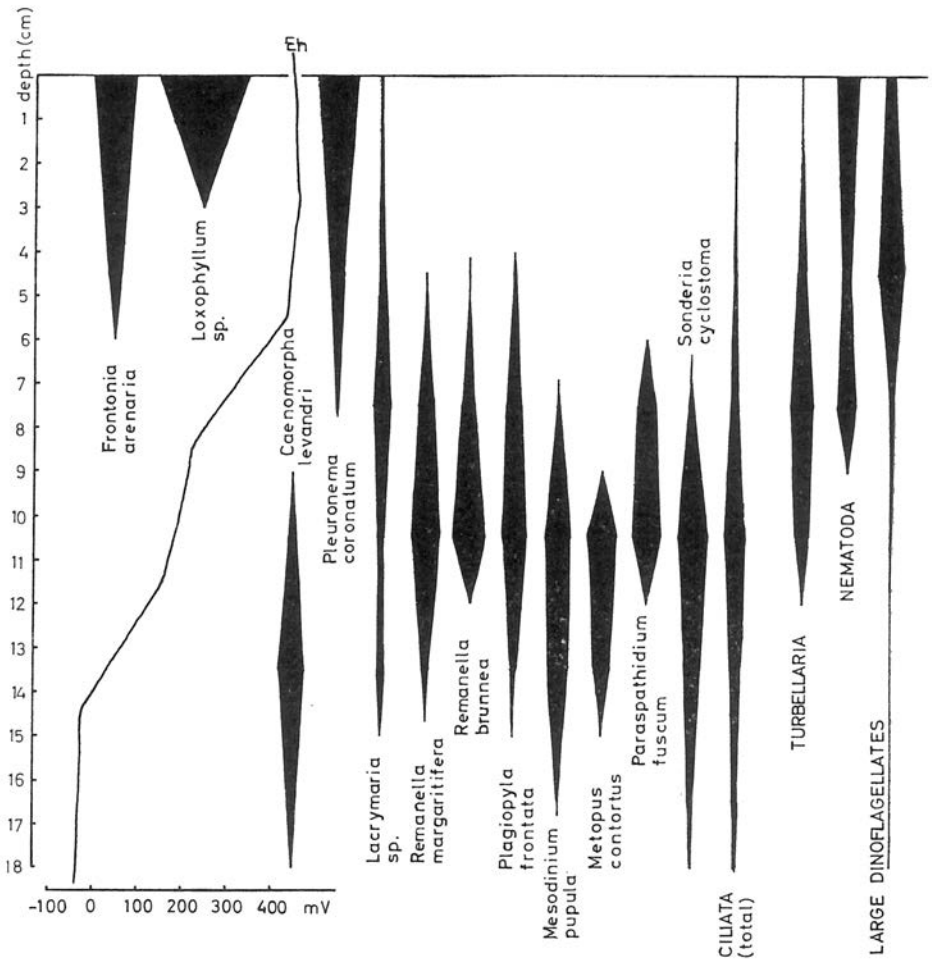

FIG. 50. Vertical distributions of some common forms and the redox-profile in the Helsingør Beach. 24 May 1967.

of individuals as well as biomass) found in the redox discontinuity layer. This is generally the case, and when the sediment becomes oxidized throughout, the zonation of the fauna breaks down. It deserves mentioning that some of the quantitatively most important diatom consumers among the ciliates (viz the *Remanella* spp) live far below the photic zone of the sediment.

Fig. 51 shows the percental occurrence of ciliates feeding on different items in the Helsingør Beach from autumn 1966 to summer 1967. The classification of the ciliates according to their food is based on Fenchel (1968a) and Table 1. This classification is not free from weak points since it may well be that a species changes its diet during the year. Since many species feed equally frequent on different items it was necessary to make classes such as: "those feeding on diatoms and

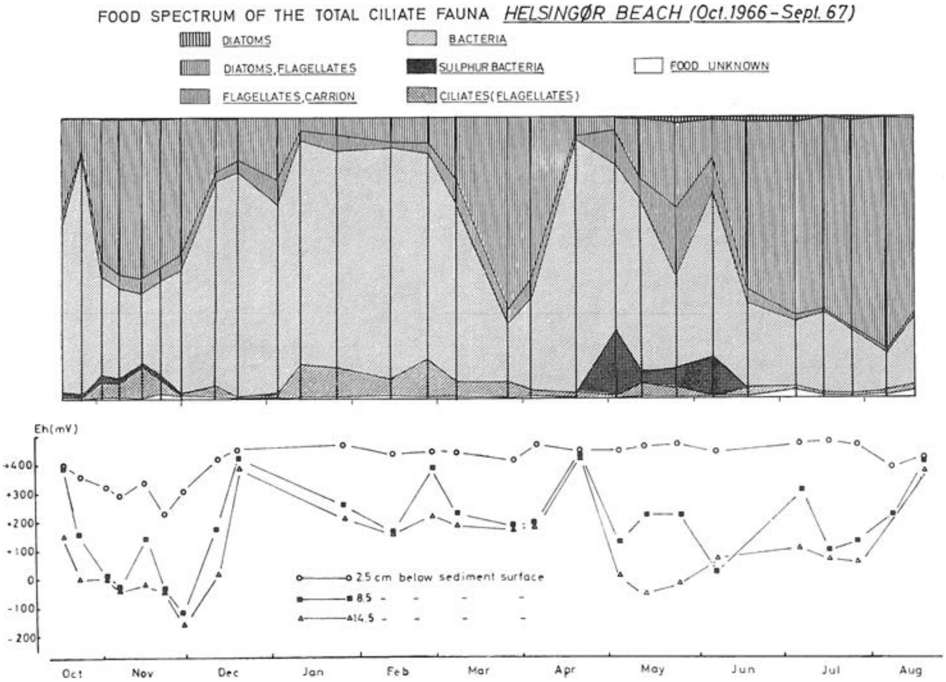

FIG. 51. The food spectrum of the total ciliate fauna and the redox conditions in the Helsingør Beach in 1966-1967.

flagellates" or "those feeding on flagellates and carrion" and there is a subjective factor in the classification. Unfortunately, it was only to a small extent possible to classify bacteria-feeding ciliates according to the kind of bacteria they eat. Only those preferably or exclusively eating the sulphurbacteria containing sulphur granules are readily recognized. Thus the group: "ciliates eating sulphurbacteria" only constitutes a part of those feeding on chemoautotrophic bacteria, the remaining are "hidden" among the "bacteria-eating forms" in the graph. This probably also applies to forms eating *Thiobacillus* which do not contain sulphur granules in their cytoplasm.

The following may be seen in Fig. 51: —1. Forms exclusively eating diatoms are rare in the beach and occur practically only in summer. —2. Predators constitute only a small part of the fauna, which could be expected as most ciliate predators feed on other ciliates and —3. Forms feeding on sulphurbacteria and presumably other chemoautotrophs occur in connection with the presence of reducing sediments.

ÅLSGÅRDE. The samples taken at 5 and 10 metres depth in the Ålsgårde transect resemble the idealized sand microbiocenosis previously described. In both samples a reduced zone is found 3-5 cm below the surface and at 8-10 cm depth

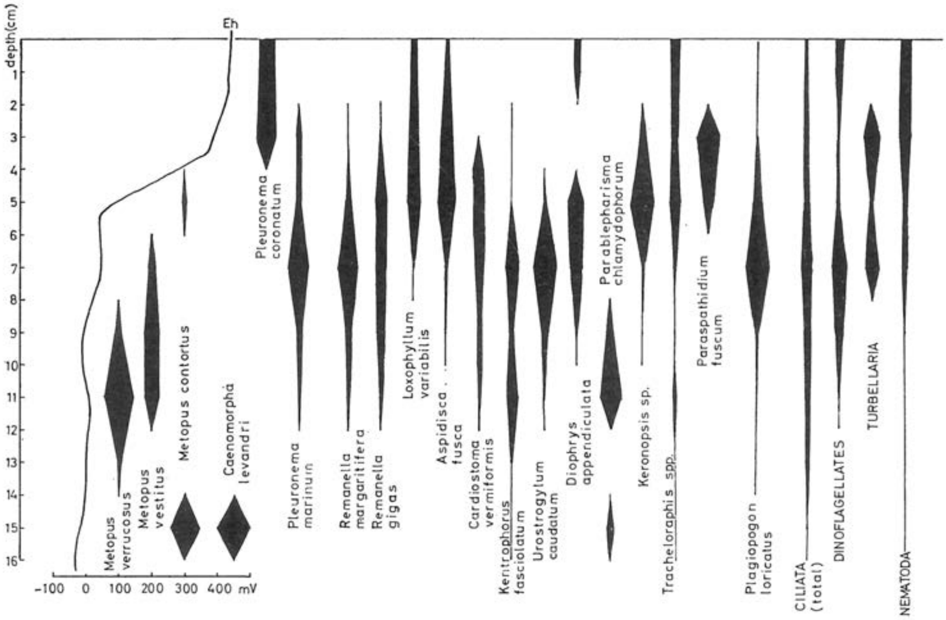

FIG. 52. The vertical distribution of some common forms and the redox-profile in the locality off Ålsgårde, 10 m depth. 9 May, 1967.

H_2S is present. (The 5 m sample was unfortunately not sufficiently long to give a complete picture of the fauna and many ciliates may have occurred deeper in the sediment. However, the hard sand on this locality made it impossible to obtain cores longer than 8-9 cm).

A typical surface fauna was found in both samples, though the diatom feeders were poorly represented in the 10 m sample (*Blepharisma* sp., *Frontonia arenaria* and *Pleuronema coronatum* in relatively small numbers).

The number of species is larger in these localities than in the Helsingør Beach locality. This may be attributed to the richer surface fauna and also the presence of a number of species which were never or only rarely found in shallow water localities in the Øresund, i. e. *Remanella gigas*, *Geleia nigriceps* and *Ciliofaurea* spp.

The metazoan microfauna comprised mainly nematodes and turbellarians. In addition *Batillipes mirus*, chaetonotoid gastrotrichs and harpacticoids were found. Qualitative samples have shown that macrodasyoid gastrotrichs also occur in these localities.

The vertical distribution of some representatives of the more important forms and of the total ciliate population in the 10 m sample is shown in Fig. 52. Here a total of 1441 ciliates, 292 dinoflagellates and 72 micrometazoa were found below 1 cm^2.

As is the case with the Helsingør Beach localities, the largest numbers of ciliates were found not at the surface but in the vicinity of the redox discontinuity layer. In the 5 m sample the largest numbers were found at the surface but an increase

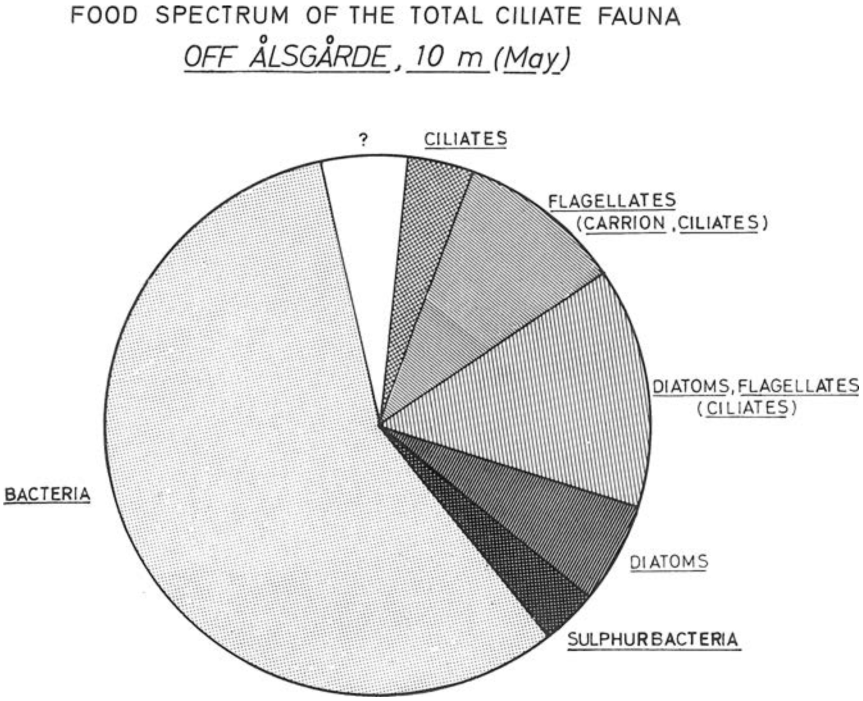

FIG. 53. The food spectrum of the ciliate fauna on 10 m depth off Alsgårde.

in numbers can be seen in the lowest part of the sample. The sample was not, however, deep enough to see the significance of this.

In Fig. 53 the relative numbers of ciliates eating different food items are shown for the 10 m locality.

In the Ålsgårde transect the sediments become coarser at greater depths. At 15 m the sediment is well sorted medium sand with very small amounts of silt and consequently the sediment is oxidized throughout. In accordance with the oxidized conditions and the relatively coarse sand the metazoan microfauna is rather varied (turbellarians, archiannelids, rotifers, ostracods, nematodes, chaetonotoids and especially harpacticoids and macrodasyoids), but the total number (67/cm^2) is not larger than in many localities with finer sands. Also the ciliate fauna is rather varied but the total number of individuals is small (171/cm^2) The ciliate fauna does not show the characteristic zonation (Fig. 54) and forms such as *Frontonia arenaria, Loxophyllum variabilis, Litonotus lamella,* and *Helicoprorodon gigas,* normally confined to the surface layers, occur here at considerable depth in accordance with the redox-profile. The presence of a few specimens belonging to species normally confined to reducing layers (*Remanella gigas, Geleia* spp, *Ciliofaurea* spp, *Urostrongylum caudatum*) in the deepest part of the sample indicate the presence of a reduced zone deeper in the sediment.

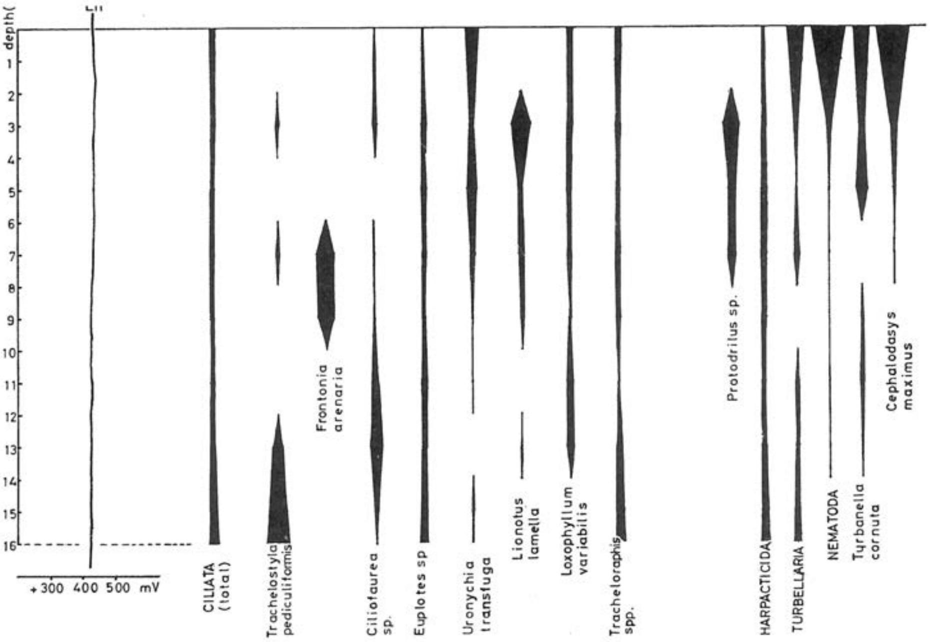

FIG. 54. The vertical distribution of some common forms and the redox-profile in the locality off Ålsgårde, 15 m depth. 17 May 1967.

At depths greater than 15 m in the Ålsgårde transect the sediment remains rather coarse but is more poorly sorted and contains increasing amounts of clay with increasing depth. Consequently a reduction layer is developed. The microfauna becomes poorer at greater depths undoubtedly due to clogging of the interstitia. At 20 m only 125 ciliates per cm² were found and metazoans were only represented by nematodes. At 25 m the sediment contained 4 % clay and here an interstitial fauna was absent. Only 4 ciliates confined to the upper loose layer and 16 nematodes were found below 1 cm² sediment surface.

HORNBÆK. Of the samples from the Hornbæk transect the one from 5 m depth will be treated separately. The sediment consists of a 15-17 cm thick layer of coarse sand below which fine sand is found. Unfortunately only the coarse sand could be sampled. This layer is oxidized throughout. The fauna is relatively poor (230 ciliates and 25 micrometazoa per cm²) and in accordance with the oxidized conditions no clear stratification of the fauna is evident. However, the larger number of ciliates and the presence of forms such as *Remanella margaritifera* and *Mesodinium pupula* in the lowest part of the core indicates that the underlying fine sediment is reduced.

The remaining localities of the Hornbæk transect have fine sands, and a reduction zone and H_2S is present though often at a considerable depth below the sediment surface. The sediments become finer with increasing depth in the transect.

The samples from 8, 11 and 15 metres contain a ciliate fauna rather rich in species and individuals (509 to 1074 ciliates per cm²) and a micrometazoan fauna mainly of nematodes, turbellarians and macrodasyoids. A typical vertical zonation is found.

At 20 and 22 m depth the sand is very fine (median grain size: 125 and 95 μ, respectively). Due to this the diversities and the population sizes of the faunas are small. At 20 m 152 ciliates and at 22 m only 42 ciliates were found per cm². The metazoan microfauna is mainly composed of nematodes. As characteristic of sediments containing significant amounts of silt and clay the fauna is concentrated closer to the surface than should be expected when the redox-profile is considered; this is probably due to the fact that the surface layers are more loosely packed allowing locomotion of the animals.

The different Hornbæk samples demonstrate an effect of grain size on the fauna. This was to some extent discussed in Chapter 3 but discussion on individual species was omitted.

In the 22 m sample with a median grain size of only 95 μ (this sediment could nearly be characterized as silt) *Kentrophorus* spp, *Coleps* sp. and *Trachelostyla pediculiformis* were found. In the 20 m sample (Mdφ: 120 μ) a richer fauna with among others *Tracheloraphis* spp, *Urostrongylum caudatum, Lacrymaria* sp., and *Geleia* spp is found. The only slightly coarser sand from 15 m (Mdφ: 135 μ) again contained the much richer fauna characteristic for fine and medium sands.

JULEBÆK. As discussed in Chapter 5 the locality off Julebæk Beach (Fig. 4) is characterized by being shallow (< 0.5 m) and sheltered, rather rich in organic matter and with an extremely rich growth of diatoms and flagellates close to the sediment surface.

A reduction layer containing H_2S is present in most places (Fig. 19).

As could be expected there is a large number of diatom-consuming ciliates in the surface layers of Juelbæk Beach (*Strombidium* spp, *Frontonia arenaria, Tracheloraphis kahli, Pleuronema coronatum, Condylostoma* spp and *Chilodontopsis elongata*). Common predators of the surface layers are *Loxophyllum* spp, *Tracheloraphis* spp and *Geleia orbis*. Deeper in the sediment *Remanella margaritifera* and *Mesodinium pupula* are common among other species. Where a sulphide zone is developed *Plagiopyla frontata* and other forms eating sulphurbacteria occur.

Fig. 55 shows the relative abundance of ciliates eating different food items. It is seen that more than 50 % of the ciliates belong to species exclusively or dominantly eating diatoms and an additional 20 % of the fauna eat flagellates and diatoms.

The metazoan microfauna comprises besides the nematodes and the turbellarians large populations of the gastrotrich *Turbanella cornuta* and also the tardigrade *Batillipes mirus* and chaetonotoid gastrotrichs. Qualitative observations have shown that the mainly herbivorous rotifer *Encentrum marinum* may also be common in this locality.

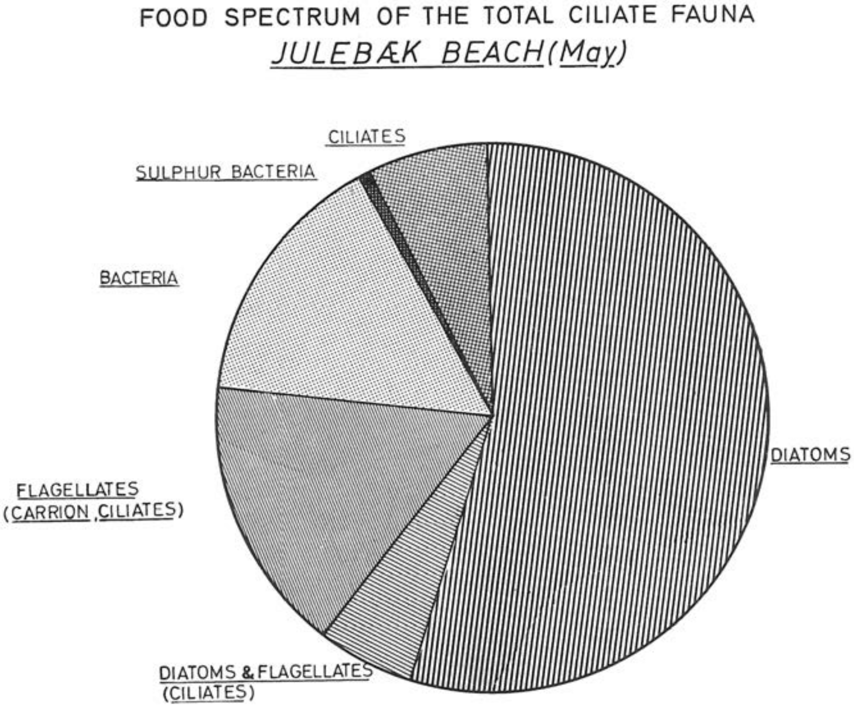

FIG. 55. The food spectrum of the ciliate fauna in the Julebæk Beach.

The "estuarine sand microbiocenosis"

The characteristic features of this biocenosis are mainly due to the reducing properties of the sediments close to the surface brought about by a large import of organic matter in combination with lentic conditions. A view on Figs 58-60, 65, 67-68, and 70 clearly shows the effect of this. The fauna, largely composed of the same species as the sublittoral sand biocenosis, shows the same pattern of zonation but compressed towards the surface in accordance with the redox-profile.

Several species are, however, characteristic of estuatine sediments. The occurrence of reduced sediments close to the surface results in growth of photoautotrophic bacteria on which certain ciliates have specialized as food.

Probably due to minerals, reduced carbon compounds and other reduced compounds released in large amounts by the anaerobic processes in the sediment (see Chapter 5) and also due to the lentic conditions a dense growth of blue-green and other algae is found. Often a stable structure consisting of sandgrains and algae is formed at or close to the sediment surface. This structure acts as a mechanical substratum and also as food for many representatives of the microfauna. A layer of purple sulphurbacteria is often found immediately below the algal felt.

Also felts of filamentous, white sulphurbacteria, especially *Beggiatoa,* may provide mechanical support and food for the microfauna.

The sediments are often rich in organic detritus and silt and they are often poorly sorted.

Before dealing with the individual localities we shall consider an idealized estuarine sand microbiocenosis (Fig. 56).

By far the greater part of the metazoan microfauna is confined to the surface layers; harpacticoids, ostracodes, oligochaetes, rotifers and turbellarians, though the three latter groups are sometimes found in small numbers in the deeper strata. The nematodes live regularly in the reduced zone and in the surface layers. Many of the micrometazoans are not really interstitial, living also on the sediment.

In the surface layers an extremely rich ciliate fauna is found. Important diatom eaters are the large brownish *Tracheloraphis kahli* (up to 586 individuals per cm^2 have been found), *Chlamydodon triquetrus, Frontonia marina, Pleuronema coronatum, Blepharisma clarissimum, Condylostoma patulum, Strombidium* spp, *Urostrongylum caudatum, Holosticha kessleri,* and *Diophrys scutum.* If reducing conditions prevail very close to or at the surface *Sonderia* spp. (*S. vorax, S. sinuata, S. schizostoma*), *Plagiopyla* spp and *Gruberia* sp., which mainly feed on sulphurbacteria but also on diatoms, may occur at the surface.

The most important consumers of blue-green algae are *Frontonia algivora* and *Chlamydodon mnemosyne* which have specialized on filamentous forms while also *Frontonia marina, Diophrys scutum, Sonderia schizostoma,* and *S. sinuata* feed on these algae. Unicellular blue-green algae are eaten by many of the species listed as diatom consumers above.

The purple and white sulphurbacteria close to the sediment surface are eaten by ciliates such as *Blepharisma salinarum, Sonderia* spp, *Peritromus faurei, Gruberia* sp., and *Plagiopyla frontata.* The filamentous white sulphurbacteria constitute the main part of the diet of *Sonderia schizostoma* and *Trochiloides recta,* and also other *Sonderia* spp may eat these bacteria.

Forms as *Trachelostyla pediculiformis* and other oxytrichids, small hymenostomes (*Cyclidium citrullus, Uronema* spp), *Pleuronema coronatum,* and *Aspidisca* spp are the most important bacterivorous ciliates of the surface layers.

Forms feeding on flagellates are *Condylostoma patulum, Tracheloraphis* spp, *Conchostoma longissimum, Prorodon morgani, P. discolor,* and *Paraspathidium fuscum.* The latter two are also histophages feeding on dead or living tissue of mechanically damaged or dying metazoans and protozoans. Both species may occur in the deeper strata of the sediment also. *Diophrys scutum* and *Frontonia marina* may, in addition to diatoms, eat flagellates.

Helicostoma notatum and *Ophryoglena* spp are present at the surface of many localities. Both feed exclusively on mechanically damaged (but living) invertebrates.

Common predators of the surface layers are the *Loxophyllum* spp, *Litonotus*

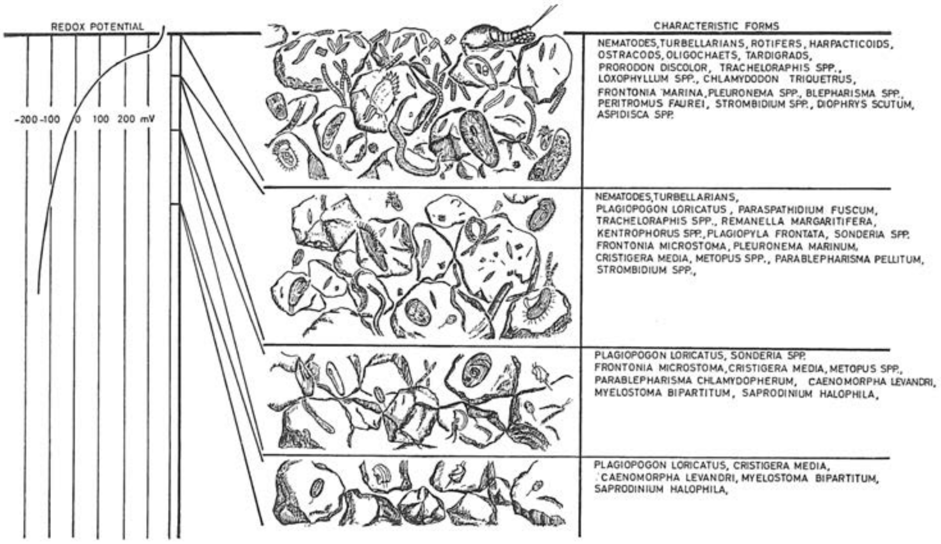

FIG. 56. Schematic representation of the vertical zonation of "estuarine" sands.

lamella and the *Lacrymaria* spp; also *Condylostoma patulum, Tracheloraphis* spp, and some dominantly herbivorous forms play a role as predators.

Below the surface where conditions are reducing the composition of the fauna changes. A number of forms (*Remanella margaritifera, Cardiostomella vermiforme, Kentrophorus fasciolatum, Pleuronema marinum,* and *Homalozoon caudatum*) occur in all the reduced layers of the sublittoral sand microbiocenosis. Also the *Tracheloraphis* spp, except *T. kahli, Strombidium* spp, *Paraspathidium fuscum,* and *Prorodon discolor* occur in the reduced layers of estuarine sands as do the *Sonderia* spp, the *Plagiopyla* spp and *Urostrongylum caudatum.* Characteristic of the reduced strata close to the sediment surface is *Parablepharisma pellitum* which mainly feeds on purple sulphurbacteria and to some extent on diatoms and unicellular blue-green algae. It is only found in reducing H_2S containing sediments but, being dependent on purple bacteria, is confined to the upper layers of the reduced zone. In the more oxidized surface layers *Blepharisma salinarum* and other forms are the main consumers of purple bacteria.

The reduced zone also harbours the *Metopus* spp (*M. vestitus, M. contortus* and an undescribed species) and *Cristigera media* all feeding mainly on sulphurbacteria. *Plagiopogon loricatus* is also mainly confined to the reduced layers; it feeds on flagellates and on carrion. *Mesodinium pupula* is not common in estuarine sediments but was regularly found in the Askö Harbour and more rarely in the Nivå Bay.

Deeper in the sediments, where the contents of H_2S are even higher, many of the above mentioned forms still occur, i.e. *Sonderia* spp, *Metopus* spp, *Plagiopogon loricatus, Cardiostomella vermiforme, Christigera media,* and *Plagiopyla* spp. In

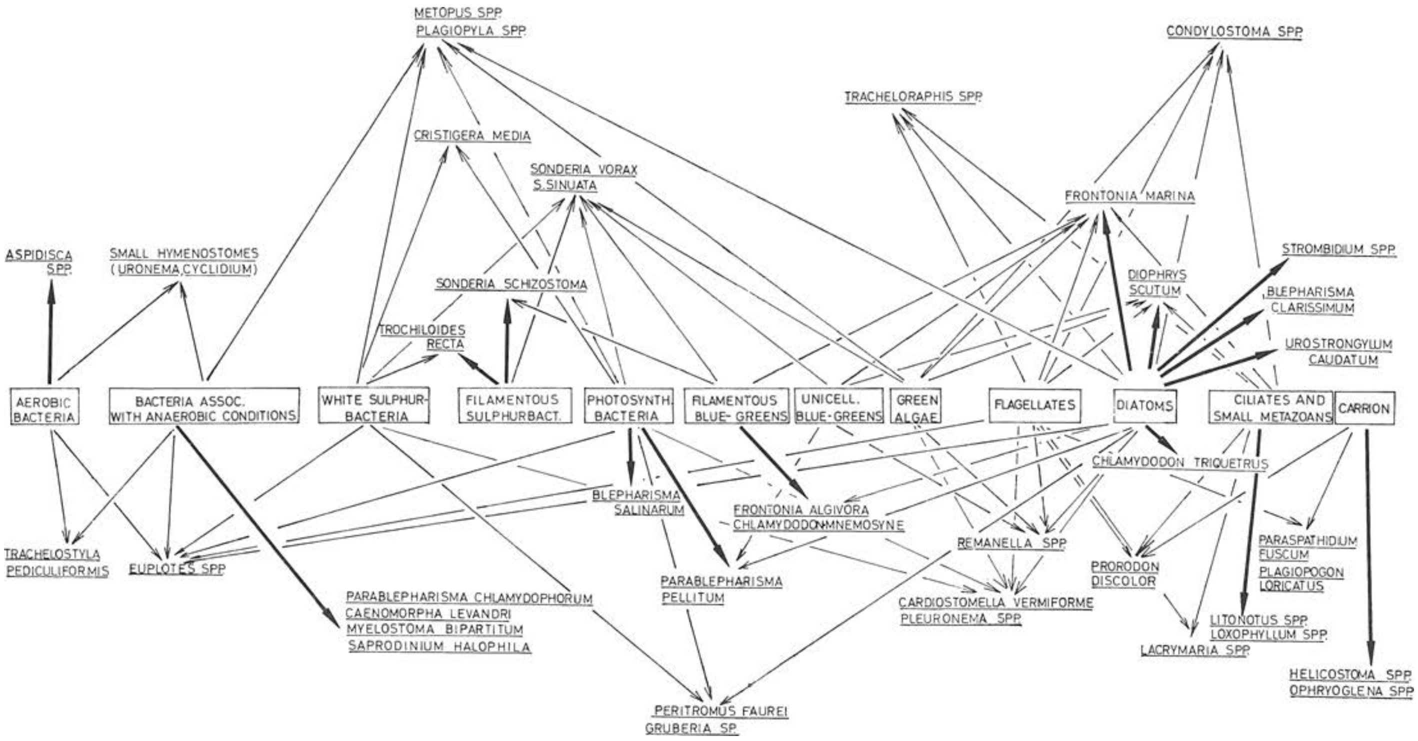

FIG. 57. The food relationships of the most common ciliates in "esturine" sediments and in sulphureta.

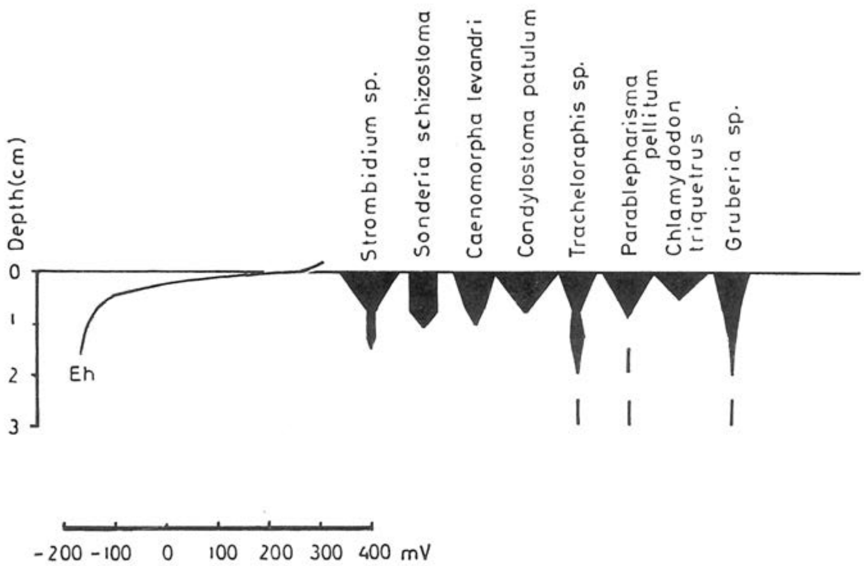

FIG. 58. The redox-profile and the vertical distribution of some common forms in the inner part of the Nivå Bay. 7 August 1968, 14^{30}.

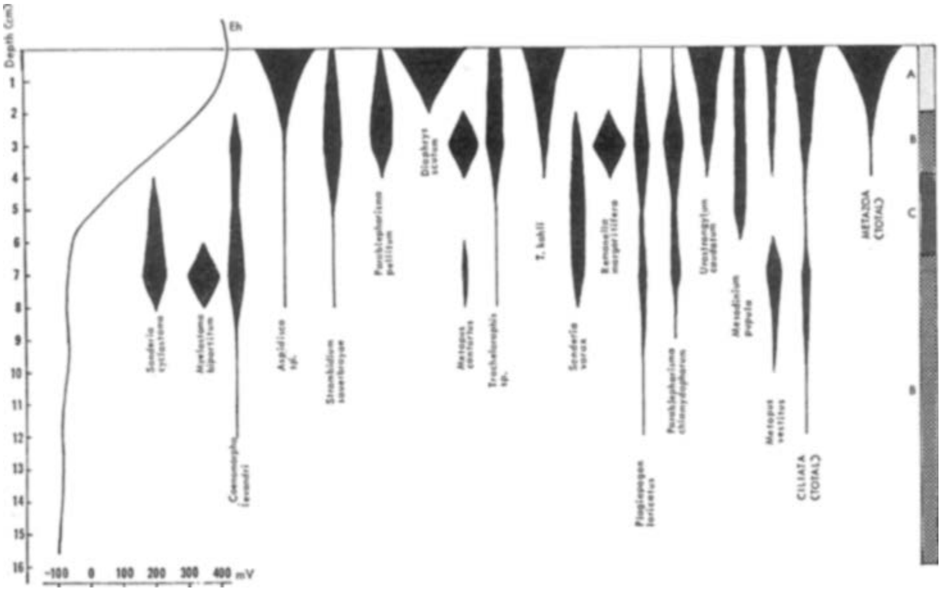

FIG. 59. The vertical distribution of some common forms and the redox-profile in the Nivå Bay, 6 April 1967. A, oxidized, yellow sand; B, blackish-greyish sand; C, decomposing *Zostera*, D, blackish sand.

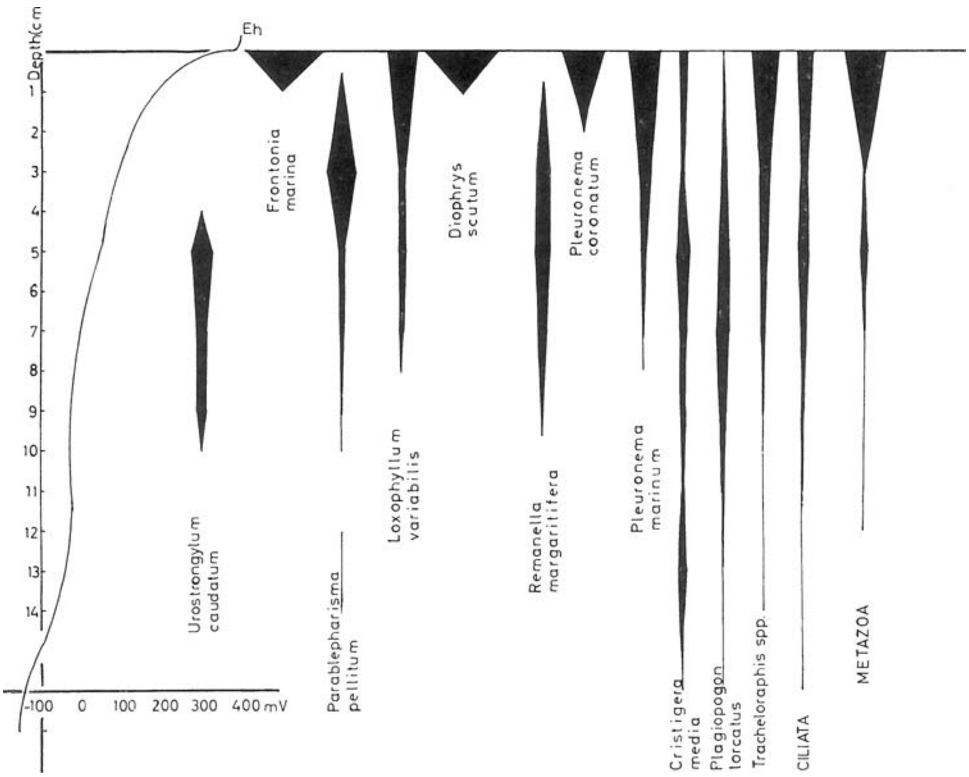

FIG. 60. The vertical distribution of some common forms and the redox-profile in the Nivå Bay, 27 July 1967.

addition, typical "sulphide forms" such as *Caenomorpha levandri, Saprodinium halophila, Myelostoma bipartitum, Parablepharisma chlamydopherum*, and *Mesodinium* sp. make their appearance. Very interesting is *Frontonia microstoma*, a ciliate exclusively feeding on large diatoms but restricted to H_2S containing sediments and thus nearly always living in the aphotic zone several cm below the surface.

Still deeper in the sediment the fauna becomes poorer in individuals and species, the forms extending deepest usually being the *Metopus* spp, *Caenomorpha levandri, Saprodinium halophila, Myelostoma bipartitum, Plagiopogon loricatus, Cristigera media*, and *Mesodinium* sp.

Nematodes and a few rotifers (*Colurella* sp., *Lecane cornuta*) may occur in the sulphide zone but no metazoans seem to tolerate so high concentrations of H_2S as do the ciliates (see Chapter 7). The euryoecious *Uronychia transfuga* and the *Euplotes* spp may occur in all strata of the sediment.

Fig. 57 shows schematically the food relations of the more important ciliates in the estuarine sediments.

In the estuarine sediments the redox discontinuity layer is often so close to

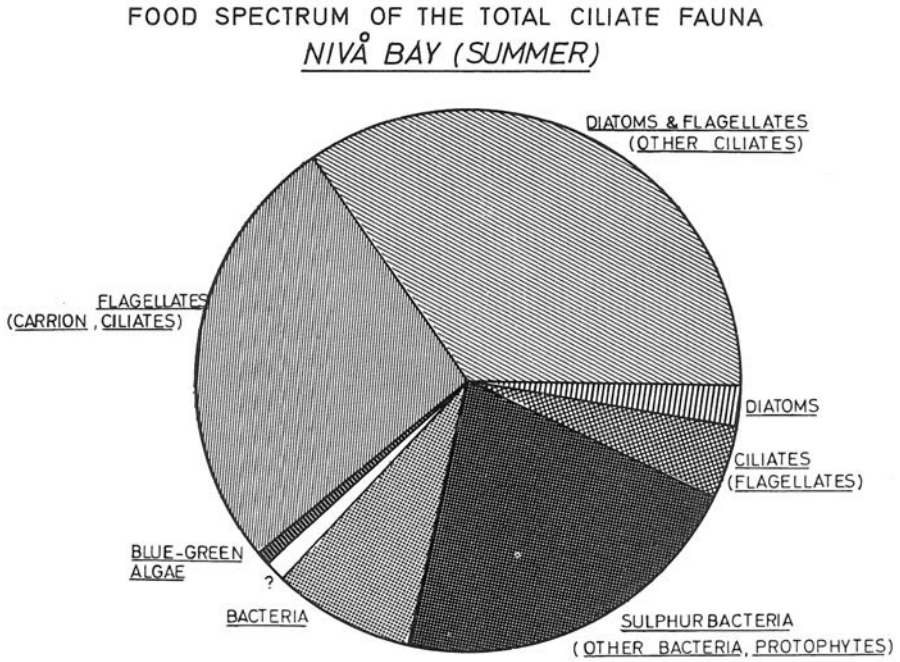

FIG. 61. The food spectrum of the ciliate fauna in the southernmost Nivå locality in summer.

the surface that it overlaps with the photic zone. It is therefore clear that the maximum number of ciliates is found at the surface or within the upper one or two cm.

NIVÅ. Of the two main localities investigated in the Nivå Bay the one situated southernmost (Fig. 5) is open towards the sea but sheltered by a sandbar some hundred metres from the shore. In most places the sediment is detritus-rich medium sand with patchy growths of *Zostera* and *Ruppia*. The oxidized layer is thickest seawards where sand is sometimes deposited by the waves. The examples shown in Figs 58-60 are based on samples taken from different places in the bay to show the variation in the redox-profile. The sampling locality represented by Fig. 61 is situated most landwards. At night reducing conditions prevail even in the surface (Chapter 5, pp. 65-66) and in daytime reducing conditions prevail already few mm below the surface and the locality could justifiably be called a sulphuretum. In the other, more seawards situated, localities the oxidized layer is thicker.

The composition and zonation of the fauna is in accordance with the above described idealized estuarine sand, as can be seen in Figs 59-60.

When the innermost sample is excluded, the number of ciliates varied between about 1000 and 2000 individuals per cm^2. The metazoan microfauna is rich. During summer harpacticoids are usually most numerous with up to 136/cm^2, while they are fewer during other seasons. Nematodes were found in numbers

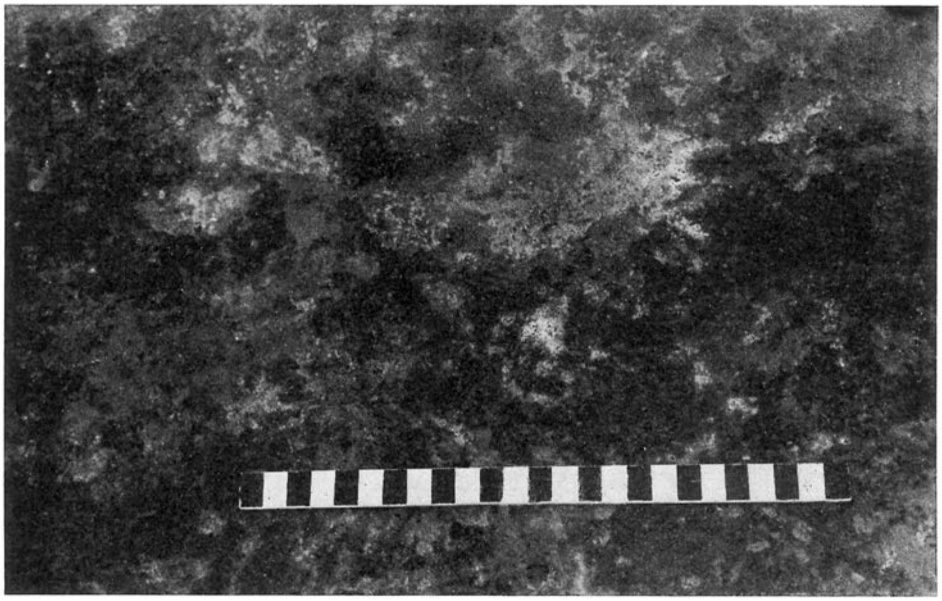

FIG. 62. Patches of *Beggiatoa* and other white sulphurbacteria in a cove in the northern part of the Nivå Bay. Scale: 25 cm.

varying from 17 to 56/cm². Rotifers, turbellarians, ostracods and oligochaetes were found in smaller numbers, usually fewer than 10/cm².

The relative importance of various food items for the ciliates (based on the samples from 29 June 1966 and 22 & 27 July 1967 from approximately the same place) is diagrammed on Fig. 61. The large importance of sulphurbacteria as food for the ciliates should especially be noted.

The sheltered cove in the northern part of the bay (Fig. 5) is a rather stable locality. The redox conditions were described in Chapter 5 (p. 66 and Fig. 26) where it was shown that the redox discontinuity layer, which lies close to the surface in autumn and winter, submerges during spring and summer. In summer the surface layers are mainly dominated by algae and purple sulphurbacteria, the remaining part of the year large patches of *Beggiatoa* dominate the surface together with *Thiothrix, Thiovolum, Macromonas,* purple bacteria like *Chromatium* and *Lamprocystis,* diatoms, blue-green algae, and flagellates (Figs 62-63).

The locality was sampled quantitatively at 7 occasions during the period October 1966 to August 1967. The number of ciliates varied between 356/cm² in late autumn to about 2200/cm² in late spring (Fig. 64). The micrometazoans found were nematodes, turbellarians, rotifers, harpacticoids, and oligochaetes. (A description of the rotifer fauna in this locality is given in Thane-Fenchel, 1968).

An example of the vertical distribution of some of the common forms and of the redox-profile is shown in Fig. 65, and Fig. 66 gives the relative importance of

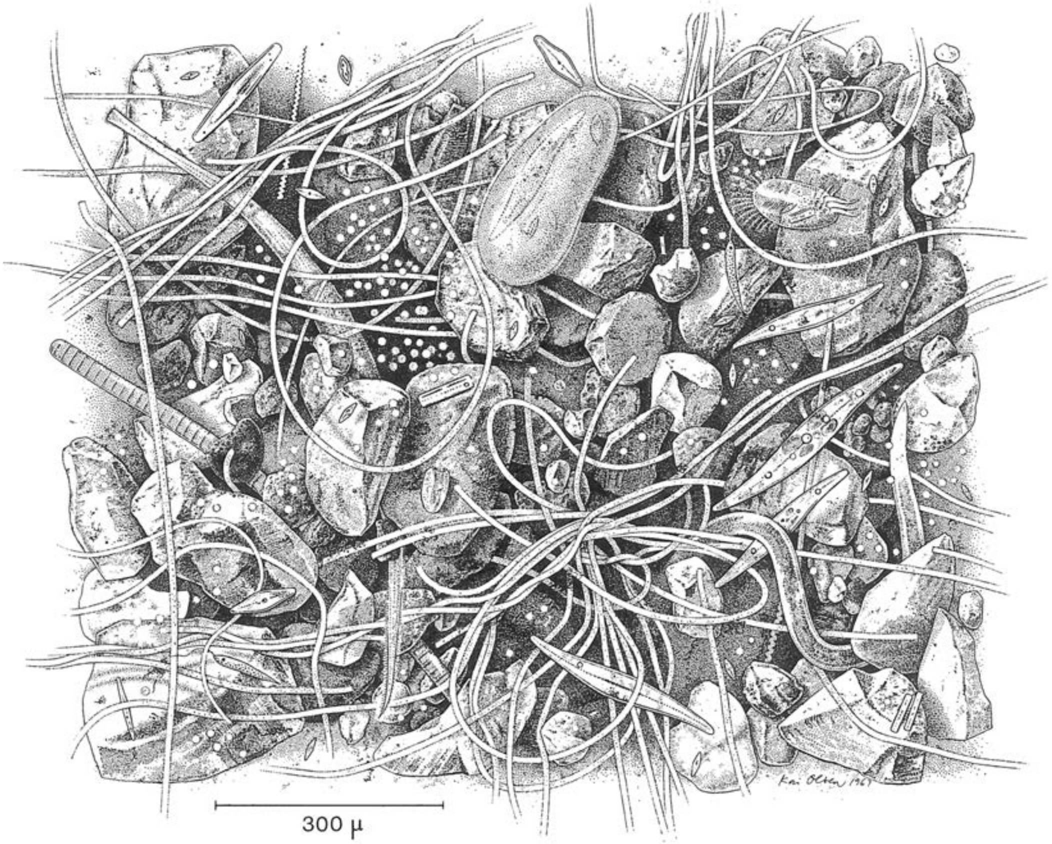

FIG. 63. The microflora and fauna in the surface of the *Beggiatoa* patches. (*Oscillatoria, Beggiatoa, Thiovolum,* diatoms, euglenoids, nematode, *Tracheloraphis* sp., *Frontonia marina, Diophrys scutum, Trochiloides recta*).

various food items for the ciliate populations in the 7 samples. As could be expected the relative and absolute importance of green plants (diatoms, blue-green algae, flagellates) as food for the ciliates is higher during summer, while the absolute numbers of bacteria-feeding forms remain rather constant throughout the year. The diagram should be interpreted with some caution. The class: "ciliates feeding on dinoflagellates" includes some forms which are also histophagous (*Plagiopogon loricatus, Paraspathidium fuscum* both numerous during summer). Also, it should be repeated that in the class: "ciliates eating sulphurbacteria" only the species exclusively or mainly eating purple sulphurbacteria or leucothiobacteriae are included.

Nevertheless, the graph does without doubt show the general trend and again demonstrates the trophic significance of chemoautotrophic and photoautotrophic bacteria, especially of those belonging to the sulphur cycle, for the ciliates.

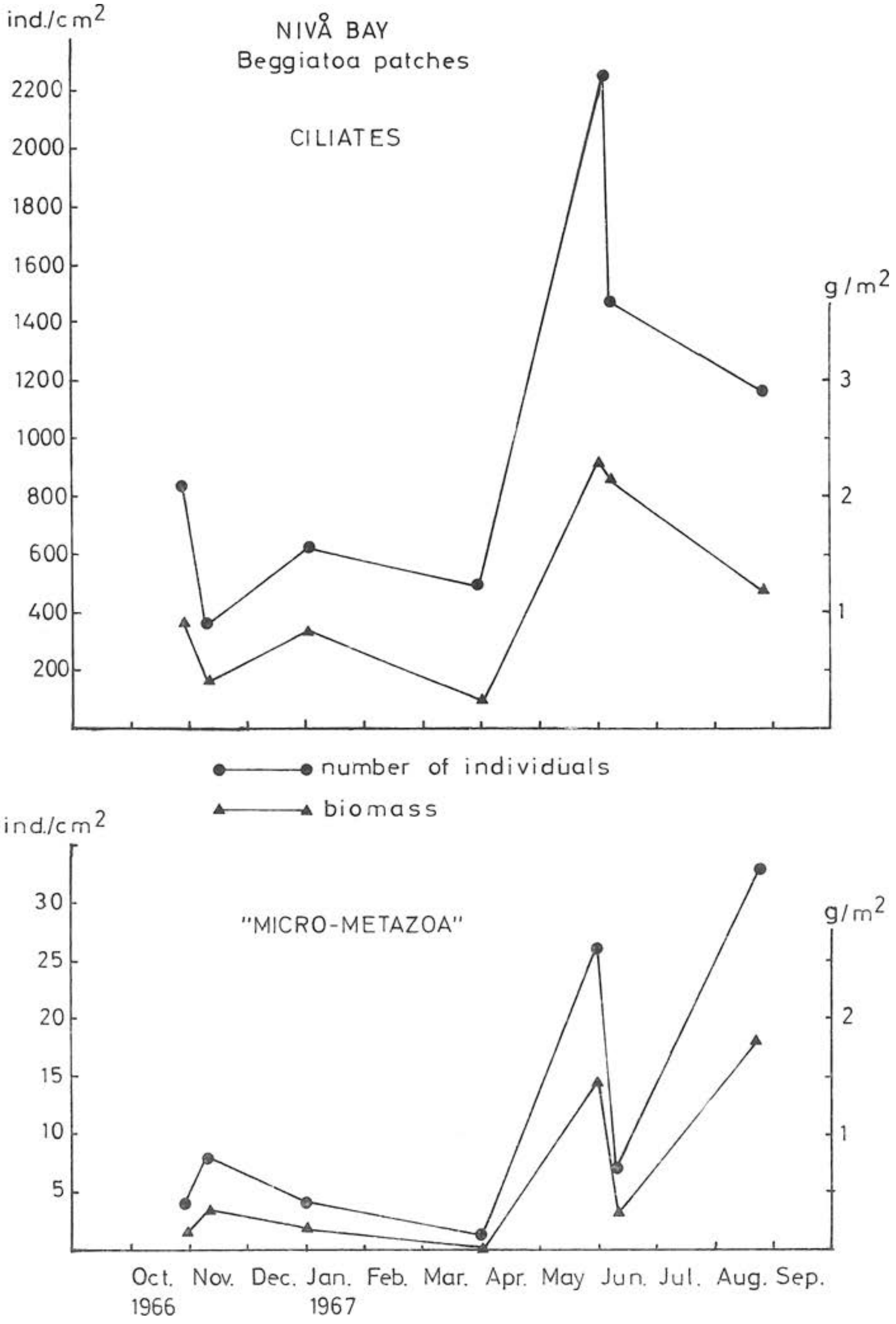

FIG. 64. The quantitative occurrences of ciliates and of micro metazoa in the "*Beggiatoa* patch locality."

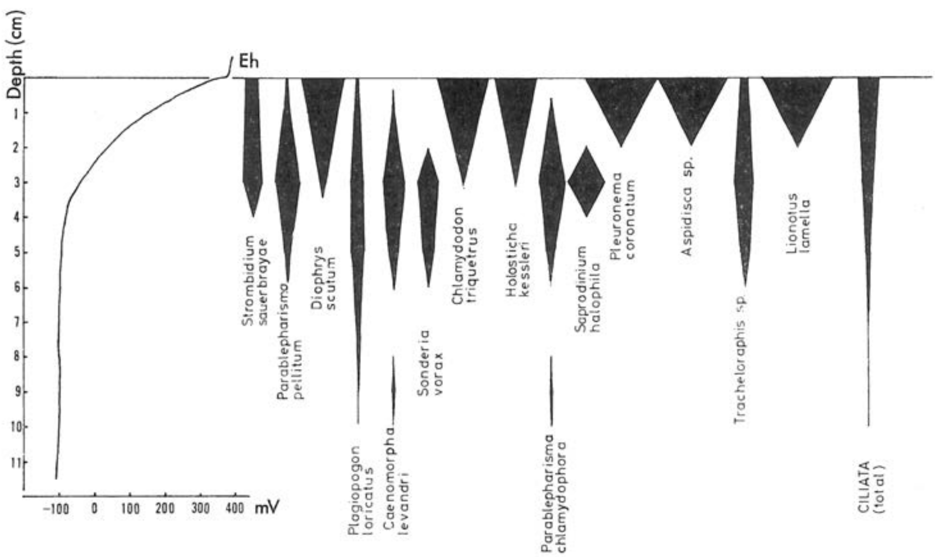

FIG. 65. The vertical distribution of some common forms and the redox-profile in the "*Beggiatoa* patch locality", Nivå Bay, 30 March 1967.

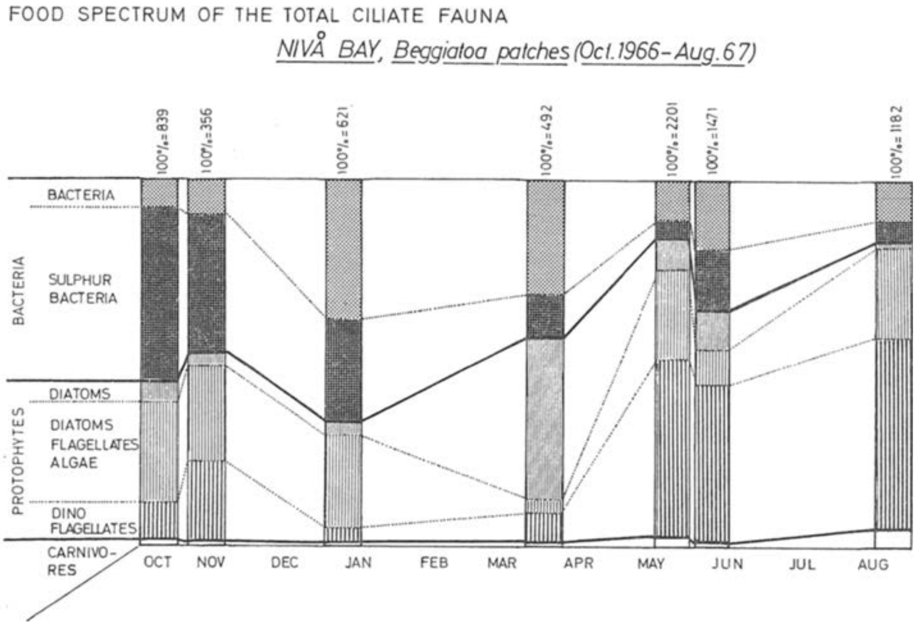

FIG. 66. The food spectrum of the ciliate fauna in the "*Beggiatoa* patch locality" from October 1966 to August 1967.

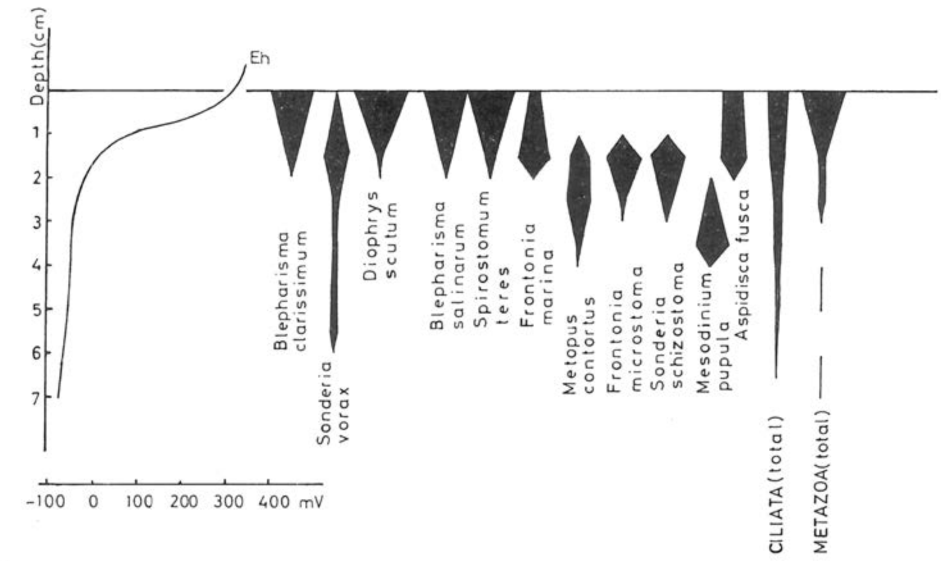

FIG. 67. The vertical distribution of some common forms and the redox-profile in the Askö Harbour, 7 August 1966.

Probably, purple sulphurbacteria are of greater importance than white ones during the summer, but this could not be proved since many species eat both kinds of sulphurbacteria.

Two quantitative samples were taken in the outlet of the cove. The locality resembles other Nivå localities except that under certain weather conditions it is rather exposed and clean sand is sometimes deposited on the surface, thus explaining the relatively high values of Eh measured on 2 March 1967.

ASKÖ. In the Askö Harbour 9 quantitative samples were taken in sand during the periods July-August 1966 and June 1967. A paper based on the 1966 samples has previously been published (Fenchel & Jansson, 1966) in which the vertical distribution of the fauna and its relation to the redox-profile was discussed.

The locality resembles the Nivå localities in several respects, but the sediment is coarser and poorly sorted. The redox-profile varies rather much from place to place but in most places reducing conditions prevail a few cm below the surface (Figs 67-68).

The surface is mainly dominated by diatoms and small patches of sulphurbacteria are found sporadically.

Composition and vertical zonation of the ciliate fauna also resemble the conditions found in the Nivå Bay. However, the ciliate populations (98-454/cm^2) are smaller than those found in the Nivå Bay while the metazoan microfauna is rich in the Askö locality probably due to the coarser sediment. The fauna of rotifers is rich both in species and individuals because of the constant, low salinity (6‰).

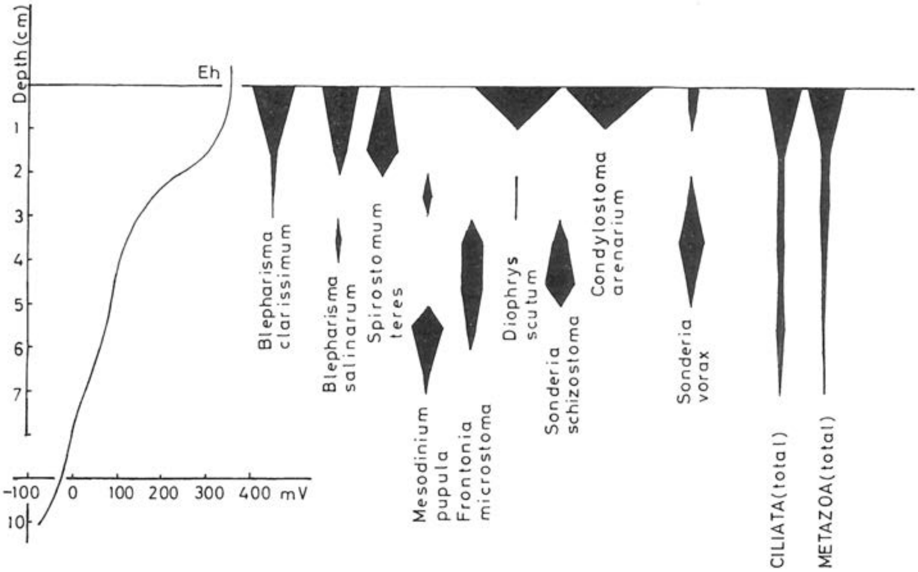

FIG. 68. The vertical distribution of some common forms and the redox-profile in the Askö Harbour, 21 July 1966.

(Thane-Fenchel, 1968, has described the rotifer fauna of this locality.) Also oligochaetes and chironomid larvae are common in this locality.

The occurrence of the ciliate *Spirostomum teres* is without doubt due to the salinity conditions. The species occurs close to the sediment surface and also in the upper part of the reduced zone but never in the presence of H_2S. It feeds on bacteria and on small diatoms and desmidiaceans. The presence of *Climacostomum virens* is also due to the low salinity (the species was not found in the quantitative samples).

Fig. 69 shows the relative importance of different food items for the ciliates. Noteworthy is the relatively large number of histophages (only the obligatory histophages *Ophryoglena* sp. and *Helicostoma notatum* are included). This is probably due to the fact that the metazoan fauna is relatively rich.

VELLERUP. In the Vellerup Vig in the Isefjord (Fig. 6) 3 quantitative samples were taken in April 1967. Two of these (Fig. 70) were from among dense growth of *Zostera* and the third from another area where only sparse growth of this plant occurs. In the latter locality the sediments consist of medium sand (Mdφ: 400 μ) in the two former samples the sand was fine (Mdφ: 125 μ). In both cases the sand was poorly sorted and rich in silt and detritus. The contents of clay increase with depth and at a certain depth hard glacial clay is found (at 4-6 cm depth in the fine sand locality and about 8 cm in the medium sand locality). As seen in Fig. 70 this influences the vertical distribution of the fauna strongly. The Eh falls to low values close to the surface but only in one of the samples negative values were

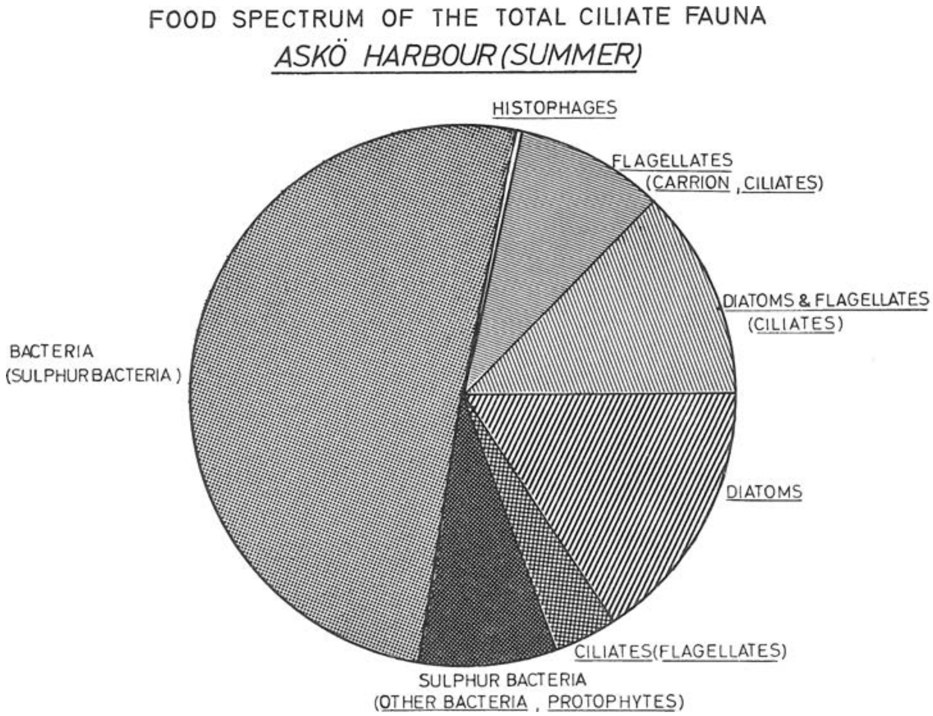

FIG. 69. The food spectrum of the ciliate fauna in the Askö Harbour in summer. Based on the 9 quantitative samples.

found and the presence of H_2S evident. Undoubtedly the preceding stormy winter and especially a stormy period in April 1967, only about 10 days prior to the sampling, had mixed the sediment resulting in higher values of Eh than typical of the locality. The presence of *Caenomorpha levandri, Parablepharisma pellitum, P. chlamydopherum, Sonderia sinuata, Peritromus* sp., *Metopus vestitus,* and *Plagiopyla frontata* indicates that the sediments are usually reducing. The faunal composition may therefore not be quite typical of the locality.

Between 597 and 751 ciliates per cm² were found. Diatom feeders constituted a dominating part of the ciliate fauna: *Tracheloraphis* spp (mainly *T. kahli*), *Strombidium sauerbrayae, Urostrongylum caudatum, Chlamydodon triquetrus,* and *Holosticha kessleri*. The dominating bacterivorous ciliates were *Trachelostyla pediculiformis* and *Aspidisca* sp. Other ciliates commonly found were *Pleuronema coronatum, P. marinum, Kentrophorus fasciolatum, Geleia fossata, Lacrymaria* spp, and *Loxophyllum* spp.

The species related to the sulphur cycle (i.e. those mainly feeding on sulphurbacteria or confined to H_2S containing environments) have already been mentioned.

The metazoan microfauna was rich and, as could be expected when the fine sediments rich in clay and silt are considered, the nematodes were dominating

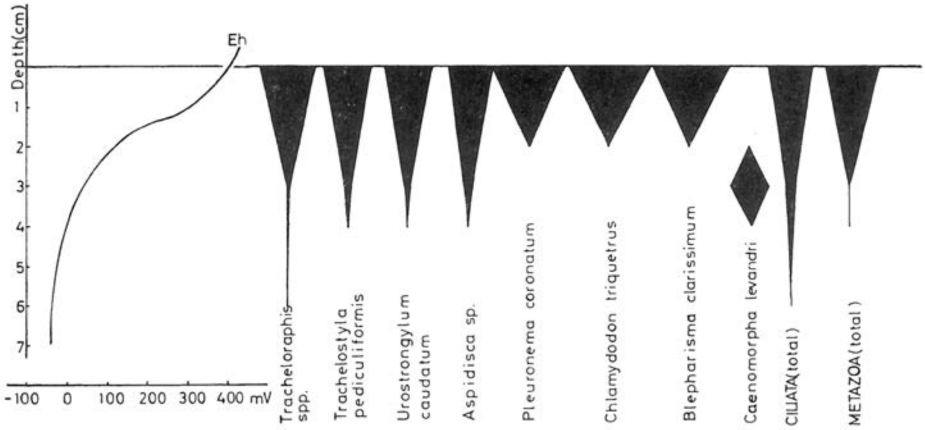

FIG. 70. The vertical distribution of some common forms and the redox-profile in the sediments of Vellerup Vig. 25 April 1967.

(20-70 individuals per cm^2). Also harpacticoids and turbellarians were common, of the latter group a diatom eating *Mecynostomum* was the most abundant.

A quantitative sample taken close to an outlet of a freshwater stream in the Vellerup Vig showed, that in spite of a continuous water current above a coarse sediment, reducing conditions may prevail few cm below the surface.

The sulphuretum

As mentioned on p. 79 the term "sulphuretum" describes an ecosystem totally dominated by the sulphur cycle. Sulphureta may develop due to supply of H_2S of volcanic origin in some parts of the world but in all sulphureta studied during the present investigation (and indeed in all sulphureta in Scandinavia) the H_2S source is the anaerobic decomposition of organic material, to a great extent through sulphate reduction carried out by *Desulphovibrio*. (The general features of the sulphur cycle in sediments and the organisms transforming sulphur compounds were discussed in Chapter 5). Obviously many other biological processes than those involving sulphur compounds take place in the sulphuretum (photosynthesis, heterotrophic, aerobic and anaerobic decomposition, chemosynthesis and photoreduction not involving sulphur compounds), but the large amounts of sulphurbacteria, which even constitute a great part of the substratum and the high concentrations of H_2S and elemental sulphur, are such conspicuous features that the term sulphuretum is justified.

Sulphureta are very common along the shores of the many fjords, lagoons, bays and coves of the inner Danish waters where drift-weed accumulates and conditions are lentic. The classical studies on sulphurbacteria by Warming (1875) were carried out in such localities not least along the Øresund coast.

Sulphureta occur, however, also in deeper water where drift-weed accumulates. J. Fabricius, B. Sc., has, during SCUBA diving, observed areas of the sea bottom of the Øresund down to 40 m depth quite covered with white sulphurbacteria, and in the same way. Dr. B.-O. Jansson observed patches of both purple and white sulphurbacteria on the sea bottom off Askö down to 10 m depth (personal communications).

While the bacterial flora of sulphureta has been studied by several authors (see Chapter 5 for references) the fauna of this biotope has been rather neglected. The works of Kirby (1934) and Kahl (1928, 1931) describe the ciliate fauna of sulphureta in inland salines.

The general lack of interest for the fauna of the sulphuretum is remarkable in view of the rather diversified microfauna present in such biotopes, and probably in no other biotope are so dense populations of living organisms found.

The distinction between the estuarine sediment and the sulphuretum is arbitrary and as previously mentioned the "*Beggiatoa* patch locality" described on p. 112 and the innermost part of the Nivå Bay described on p. 111 could have been described as a sulphureta.

A vertical zonation of the fauna is found in the sulphureta as well as in other sediments with a redox gradient but it is often difficult to study due to this steep gradient. In artificial laboratory sulphureta the vertical zonation was easy to study (Chapters 7 and 9).

All kinds of sulphurbacteria are present, but often either white or purple forms dominate. In the sulphureta studied during the present investigation purples dominated in summer and whites in winter. This can probably in part be explained by the light factor, but even in the laboratory under constant light conditions white sulphurbacteria precede the occurrence of purple bacteria in the succession (see Chapter 9).

NIVÅ. The sulphuretum studied in most detail is situated at the shore of Nivå Bay in a small cove measuring about 10 × 25 metres. It is about 1 m deep in the middle and bordered with sand dunes. The bottom consists of hard sand. Due to local hydrographical conditions the cove is filled with a mixture consisting of drift-weed and sand during winter storms so that at high water there is only about 20 cm free water above the sediment and at low tide only small pools remain.

Each year (the locality was observed regularly in the period from summer 1964 to summer 1968) the locality shows a number of characteristic changes through the seasons. In winter the sediment is regularly mixed and new supplies of drift-weed and sand are deposited, and the surface remains black from the decomposing leaves of *Zostera*.

As soon as calm periods prevail, white sulphurbacteria (*Beggiatoa, Thiovolum,* etc.) grow and cover the surface with white patches while purple bacteria remain few and are mainly found along the edges of the cove.

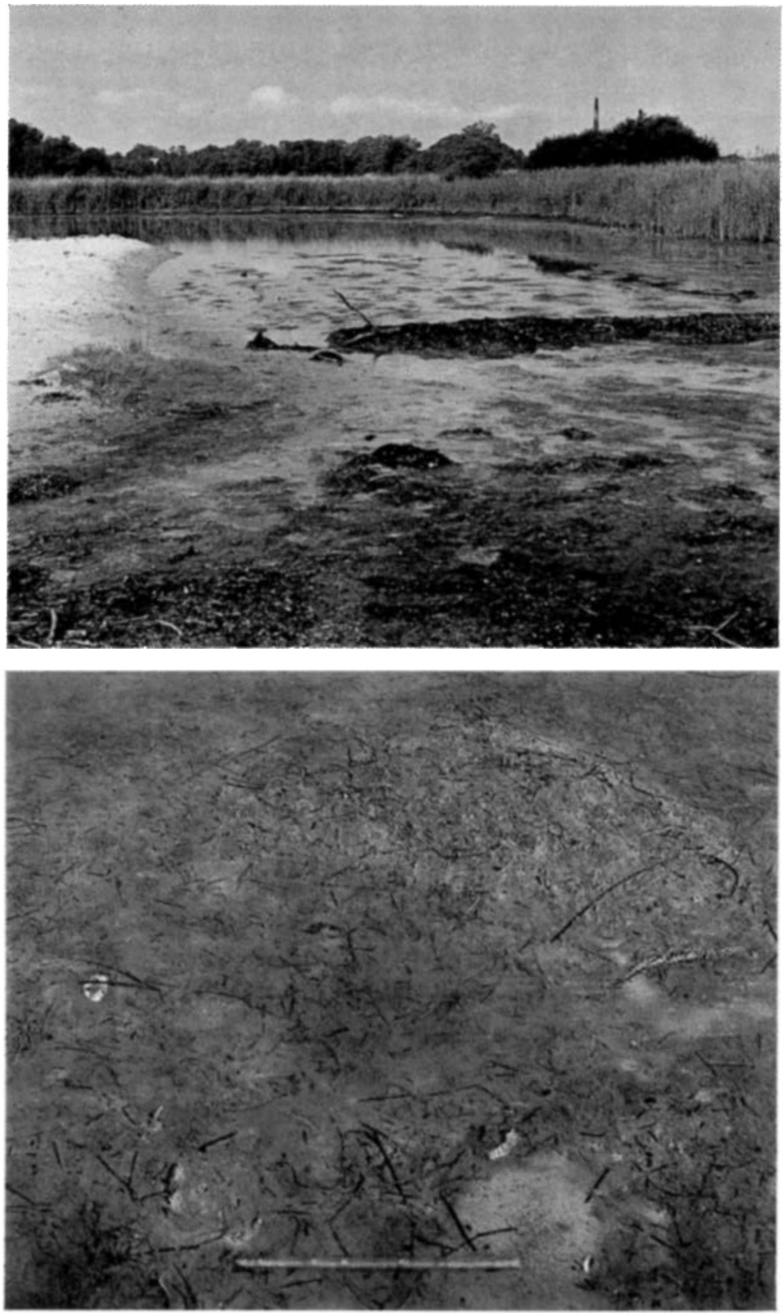

FIG. 71. The sulphuretum in Nivå Bay.

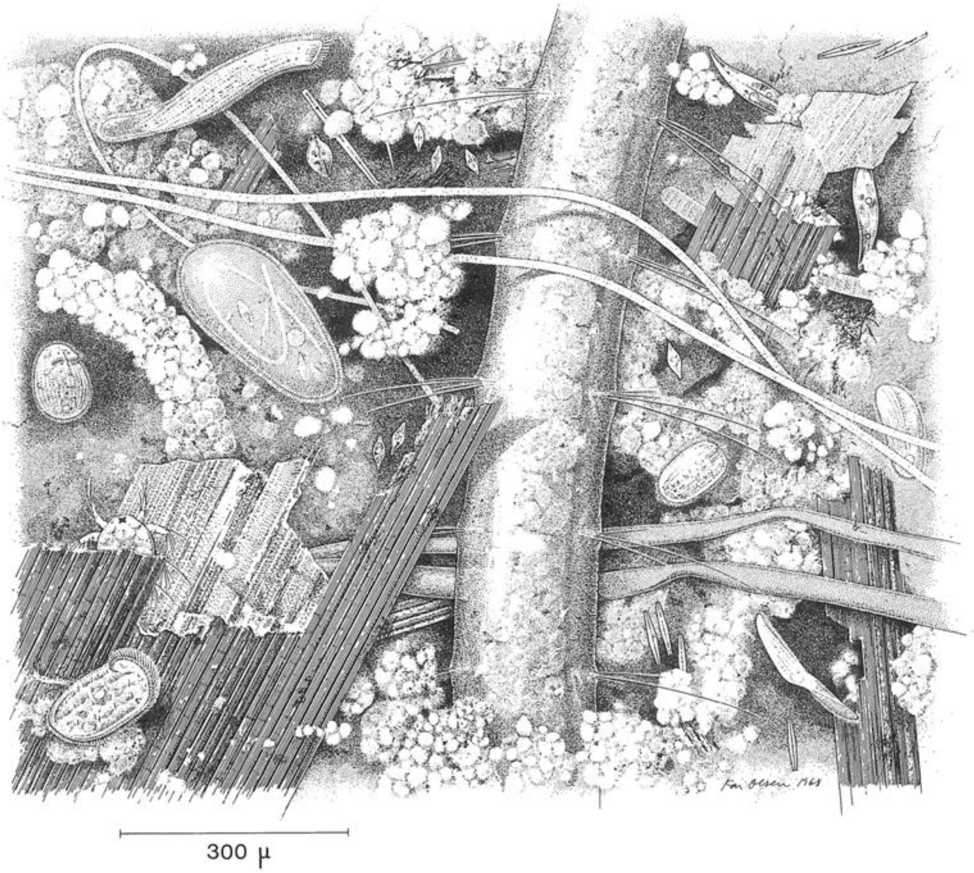

FIG. 72. The microflora and fauna in the surface layers of the Nivå sulphuretum (in summer). (*Plagiopyla frontata, Peritromus faurei, Paraspathidium fuscum, Frontonia marina*, harpacticoid nauplii, *Nais elinguis, Prorodon discolor, Blepharisma salinarum, Paramecium calkinsi*, diatoms, *Beggiatoa, Thiothrix, Oscillatoria*, and, making up a large part of the substrate, the purple *Lamprocystis* of which some colonies have passed the intestine of oligochaetes).

This was the stage of development attained by the locality in the end of March 1968. The fauna was then already rather well developed. Of the 45 species of ciliates found during summer (see below) 18 were found in a qualitative sample in addition to harpacticoids, nematodes, turbellarians and oligochaetes.

In the beginning of April purples became abundant but the surface was later mixed by another storm combined with a high water level and on 17 April the conditions resembled those found in March. The water above the sediment was oxidized (+329 mV) and consequently contained no H_2S. Below the surface the interstitial water contained 26 mg H_2S/l. Nineteen species of ciliates were found in qualitative samples and the above mentioned groups of metazoa were represented in addition to specimens of the rotifer *Encentrum marinum*.

On 27 May the sulphuretum was fully developed (see Fig. 71). The surface of the sediment was quite red from dense growth of *Lamprocystis* and *Chromatium* which made up most of the substratum of the surface. In addition to the purple sulphurbacteria large amounts of *Oscillatoria* and other blue-green algae, *Beggiatoa* and *Thiothrix*, numerous diatoms, dinoflagellates, and euglenoids were found (see Fig. 72). Blue-green algae dominated along the edges of the cove.

In a qualitative sample 26 species of ciliates, 4 species of turbellarians and 3 species of rotifers were found in addition to harpacticoids, ostracodes, nematodes and oligochaetes. The surface sediment contained 19 mg H_2S/l (sampled in the morning) and about 10 cm below the surface the interstitial water contained 211 mg H_2S/l.

Through the summer the concentration of H_2S in the free water above the surface varied from 0 to about 20 mg/l and the redox potential from +329 to —190 mV. The content of H_2S was generally higher during the night, as could be expected, but H_2S was only present at low tide when the water of the cove is not in connection with the sea. At more or less regular intervals, usually several times per day, the water was rising and H_2S removed from the free water of the sulphuretum. The contents of H_2S of the interstitial water increased during the spring and in June 740 mg/l was measured.

In late autumn, when the first storms occur, the system is destroyed leaving a layer of black decomposing plant residue.

During the summer 1968 the following 45 species of ciliates were found among the sulphurbacteria: *Prorodon discolor, P. morgani, Chaena* sp., *Plagiopogon loricatus, Lacrymaria* sp., *Mesodinium* sp., *Paraspathidium fuscum, Tracheloraphis* sp., *T. kahli, Litonotus lamella, Loxophyllum* sp., *L. variabilis, Chlamydodon triquetrus, Plagiopyla* sp(p), *Sonderia vorax, S. cyclostoma, Sonderia* sp., *Cardiostomella vermiforme, Pleuronema marinum, P. coronatum, Cyclidium citrullus, Ophryoglena* sp., *Paramecium calkinsi, Frontonia marina, Blepharisma* sp., *B. salinarum, Parablepharisma pellitum, P. chlamydopherum, Gruberia* sp., *Condylostoma patulum, Peritromus faurei, Metopus contortus, M. vestitus, Caenomorpha levandri, Myelostoma bipartitum, Saprodinium halophila, Strombidium* sp., *Strobilidium* sp., *Holosticha kessleri, Urostrongylum caudatum, Trachelostyla pediculiformis, Euplotes elegans, Diophrys scutum, Uronychia transfuga, Aspidisca* sp.; in addition a number of unidentified species, mainly small forms, were observed.

The following rotifers were found: *Encentrum villosum, E. marinum, Lindia tecusa, Brachionus plicatilis, Colurella colurus*, and *Notholca striata*. Turbellarians observed were *Monocelis lineata, Mecynostomum auritum, Provortex karlingi*, and *P. tubiferus*. Two oligochaetes, *Nais elinguis* and *Paranais littoralis* were identified but probably more species were present. The numerous harpacticoids, nematodes and ostracods were not identified.

Also a few representatives of the macrofauna were found in the sulphuretum, i.e. the gastropod *Hydrobia*, the polychaete *Nereis diversicolor* and some gam-

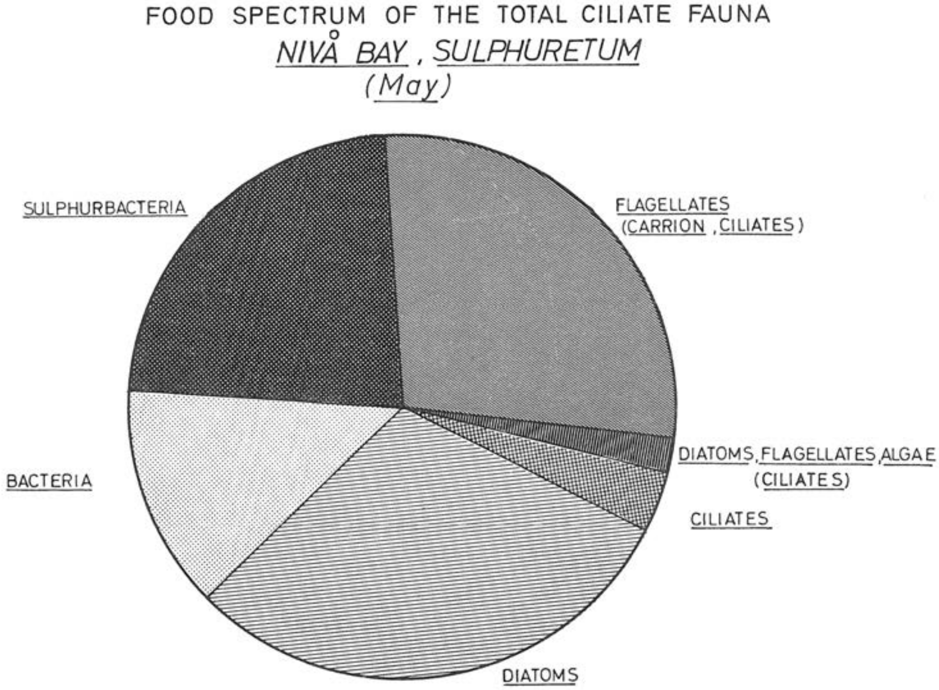

FIG. 73. The food spectrum of the ciliates in the Nivå sulphuretum.

marids. In day time at high tide fishes belonging to the genus *Gobius* were seen browsing on the carpet of sulphurbacteria. In another similar locality in Nivå Bay young eels were seen.

A vertical zonation of the fauna was present but difficult to study since nearly all animals were concentrated in the upper 0.5 to 1 cm of the sediment. In most places the sediment below the layer of sulphurbacteria is clogged by the large amounts of organic detritus. Core samples of the upper cm (nearly exclusively consisting of sulphurbacteria) could roughly be divided into an upper and a lower part. The lower part mainly harboured *Caenomorpha levandri, Myelostoma bipartitum, Saprodinium halophila,* the *Parablepharisma* spp, the *Sonderia* spp, the *Metopus* spp, and a few others, while the remaining species were concentrated in the upper part of the sample.

When H_2S was present in the free water of the stagnant pools many species, normally living in the sediments, were found swimming at the surface film of the water. This applies to the typical benthic forms *Frontonia marina, Paraspathidium fuscum, Prorodon discolor, Diophrys scutum,* rotifers, and harpacticoids. This effect was also studied in laboratory experiments (see Chapter 7).

Also the macrofauna leaves the sediment under such conditions. The vagile fauna (fishes, crustaceans) leaves the cove when H_2S appears. *Nereis diversicolor* leaves its burrow and the worms are found wriggling at the water surface. In longer, calm periods mass death of this species was observed.

In May 1968 a sample covering 1 cm^2 was taken to a depth of 1 cm. This cubic centimetre contained 1220 ciliates, 6000 dinoflagellates (mainly *Gymnodinium*) and 492 small metazoans. This corresponds to about 0.65 g wet weight ciliates per m^2 and 33 g micrometazoa per m^2 indicating the enormous biological activity of the sulphuretum. Fig. 73, which is based on the same sample, shows the relative importance of food items for the ciliates. Sulphurbacteria, here mainly purples, constitute about 25 % of the food eaten by the ciliates.

ISEFJORD. The other sulphuretum studied quantitatively is situated along the shore at Munkholm Bridge in the Isefjord (Fig. 6). It was studied in May 1968. The water is shallow and sheltered and large amounts of plant residue are accumulated along the shore. Patches dominated by purple sulphurbacteria alternate with patches covered by *Vaucheria* and blue-green algae.

Interstitial water from some cm below the surface, obtained by pressing a test tube down into the loose and soft sediment, contained 23 mg H_2S/l and had a redox potential of —176 mV. In the water specimens of *Caenomorpha levandri, Metopus contortus,* nematodes, and a few *Paramecium calkinsi* were present. The presence of the latter species may indicate some contamination with surface water and thus a too low value of H_2S contents. The water above the sediment probably never contains H_2S in any significant amounts since it is always in open connection with the sea.

A surface area of 1 cm^2 collected to a depth of 1 cm contained 1262 ciliates and 246 micrometazoa. The fauna resembled that found in the Nivå sulphuretum but was poorer in species. In total 23 species of ciliates (in addition to a few unidentified ones) have been found in a number of samples from this locality.

FREDERIKSSUND. Some pools found in the marshes along the shore at Frederikssund (Roskildefjord) quite dominated by purple sulphurbacteria were studied in May 1968. The salinity was extremely low (around 1‰) and this explains the small number of species found. The euglenoid flagellate *Eutreptia* and the ciliates *Paramecium calkinsi, Prorodon discolor* and *Cyclidium citrullus* occurred in enourmous amounts in addition to a *Holosticha* sp., an unidentified cyrtophorine ciliate and an unidentified, free swimming peritrich ciliate. The paramecia were feeding on sulphurbacteria, *Prorodon* on flagellates and *Cyclidium* on small bacteria. Thus, this is a very simple community compared with other sulphureta studied.

VELLERUP VIG. Finally, the fauna in 10 to 50 cm thick layers of decomposing *Zostera* leaves found in a locality along the shore of Vellerup Vig was studied. The layer of algae is constantly covered by 20-40 cm water. At the surface of the decomposing algae white patches of *Beggiatoa* and other white sulphurbacteria were seen, and under the microscope numerous, small colonies of *Lamprocystis* and diatoms could be seen. All these organisms also occurred below the surface in smaller numbers.

Water samples from 10-30 cm below the surface contained amounts of H_2S varying from 2.6 to 443 mg/l (Eh: —156 to —196 mV). The fauna of these water samples mainly consisted of *Metopus contortus, Caenomorpha levandri, Saprodinium halophila, Myelostoma bipartitum, Sonderia vorax, Trochiloides recta, Plagiopyla* sp., *Uronema* sp., *Cardiostomella vermiforme, Parablepharisma pellitum, Mesodinium pupula, Mesodinium* sp., *Trachelostyla pediculiformis, Euplotes* sp., nematodes, the rotifer *Colurella colurus,* oligochaetes and rarely some of the species of ciliates occurring at the surface.

At the surface of the layer of decomposing plants H_2S was not found in measurable amounts. Here large numbers of *Tracheloraphis* sp., *Pleuronema coronatum, Frontonia marina, Uronychia transfuga, Trochiloides recta, Prorodon discolor, Diophrys scutum, Aspidisca* sp., *Litonotus lamella,* and other forms of ciliates in addition to nematodes, turbellarians, harpacticoids and oligochaetes were found.

The splash zone of beaches

This biotope was only studied to a small extent during the present investigation. Remane (1933) called this zone of the beach the "*Otoplana* biocenosis" due to the presence of these turbellarians.

The sediment consists of coarse sand. Each wave causes movements of the interstitial water deep into the sediment since the water drains through the sand (see Jansson, 1968 b) and the sediment is therefore oxidized throughout.

A sample from the splash zone of an exposed beach on Askö, showed a constant redox potential through the whole sample. The fauna of the splash zone is poor with 68 ciliates per cm^2 and 9 metazoans per cm^2 of which the majority were specimens of the rotifer *Colurella colurus.* As could be expected from the redox-profile there is no clear vertical zonation of the fauna. The presence of a few specimens of *Mesodinium pupula* is remarkable; normally it only occurs in reduced sediments. The remaining species are all characteristic of oxidized sediments. The percentage of carnivorous ciliates is high (more than 25 %). This has also been observed at other occasions in the same locality and in the splash zone of the Helsingør Beach. Also histophagous ciliates are often common. In the quantitative Askö sample, however, only one specimen of *Coleps pulcher* occurred but in this beach as well as in the Helsingør Beach, specimens of *Ophryoglena* sp. were often observed. In the latter locality the ciliates were at several occasions seen feeding on planktonic copepods which were filtered from the waves by the sand. *Uronychia transfuga* is often common in the splash zone.

The observations on *Ophryoglena* and the large numbers of predators make it natural to suggest that the animal community of the splash zone largely depend on stranded plankton organisms. This may also apply to the diatom feeders of the biocenosis.

There is, however, too little evidence to make categoric statements on the food chains of this community but it may be concluded that —1. The splash zone constitutes an environment oxidized throughout and therefore shows no vertical zonation, and —2. The population sizes of both ciliates and metazoans are small.

Gravels

Two quantitative samples were taken in June 1966 off the Kronborg Castle at about 30 cm water depth. The water currents are very strong in this locality explaining the presence of an extremely coarse sediment (Mdφ: 3-4 mm).

A redox-profile was not measured but there was no sign of reducing conditions down to 8-9 cm depth, i.e. the length of the cores.

The fauna was poor in ciliates (31 and 62 individuals per cm^2, respectively). The large interstitia of the sediment, however, allowed the presence of large invertebrates (a syllid polychaete, oligochaetes) and some very long, unidentified turbellarians. In accordance with the mechanical properties of the sediment only few nematodes were found.

Vaucheria-carpets

Along the shores of estuaries and lagoons carpets of the alga *Vaucheria* sometimes occur, often in the vicinity of sulphureta. In these carpets there is often a rich growth of blue-green algae, diatoms and sulphurbacteria. The fauna is restricted to live in or on the carpet, below which reducing muds are found.

Only a few qualitative samples from Nivå Bay were studied in summer and autumn 1967 and in summer 1968. Of metazoans especially rotifers (*Encentrum* spp, *Colurella* spp, etc.) and nematodes are frequent. The ciliate fauna is very rich especially in herbivorous forms. Thus species eating blue-green algae (*Frontonia algivora* and *Chlamydodon mnemosyne*) are numerous. Also the alga-eating *Chlamydodon obliquus* is common here and so are several of the diatom-feeding ciliates also found in sulphureta. *Paramecium calkinsi* and *Blepharisma salinarum* are the most common consumers of sulphurbacteria though other forms such as *Gruberia* sp. and the *Sonderia* spp are also often found. The large *Euplotes* spp (*E. harpa, E. vannus*) are also frequent in this biotope.

Detritus layers on muds and clays

As discussed in Chapter 3 sediments with interstitia below a certain size only contain burrowing animals. The lower limit for the presence of the smallest interstitial animals, i.e. the ciliates, is 90-100 μ in median grain size for well sorted sands, but coarser and less well sorted sand is also uninhabitable if the contents of clay and silt exceed 3-4 %. Such "non capillary" sediments are, however, often covered by a more or less thick layer of looser sediment often consisting of organic detritus and this layer harbours a microfauna.

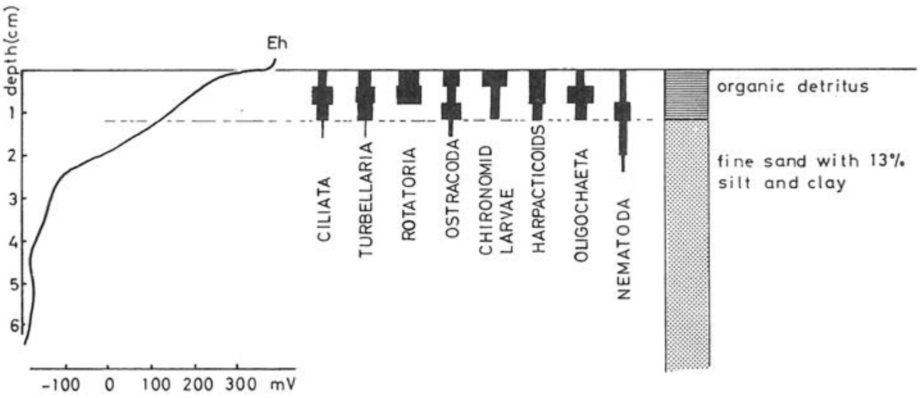

FIG. 74. The vertical distribution of the microfauna and the redox-profile in a detritus covered clayey sediment in Askö Harbour. 25 June 1967.

The mechanical properties of this biotope may favour larger and more robust animals for which pore size is of no importance but rather the ability to displace the detritus in order to move in the biotope. It could therefore be expected that ciliates play a modest role in this biotope in comparison with the metazoans.

Only a few quantitative samples were studied. They were collected in Askö Harbour in June 1967 between stalks of *Phragmites*. The sediment consisted of poorly sorted fine sand containing 13-14 % glacial clay and it could therefore not harbour an interstitial fauna. On the top of the sediment a 5 to 12 mm thick layer of organic detritus was found. In two of the samples 1 cm^2 of the detritus layer (which was 5-10 mm thick) was sorted in a petri dish with filtered water and the microfauna was enumerated. In a third sample (Fig. 74) in which the detritus layer was 12 mm thick the core was split vertically by removing 4 mm layers one by one and sorting them individually. Here also the upper 12 mm of the underlying clayey sand was sorted.

The two first samples (representing 1 cm^2 each) both contained 14 ciliates, but 100 and 39 small metazoans, respectively, of which 44 and 21 were nematodes, the remaining being rotifers, turbellarians, harpacticoids, oligochaetes, ostracods, and acarines. The ciliates found were *Strombidium* sp. and *Diophrys scutum* (diatom consumers), *Pleuronema coronatum* (eating diatoms and bacteria), an unidentified oxytrichid, the histophagous *Helicostoma notatum, Porodon discolor* (eating flagellates and carrion), and the omnivorous *Uronychia transfuga*.

In the third sample the detritus layer was, as mentioned, thicker. Fig. 74 shows that while all other groups were practically restricted to the detritus layer, the nematodes were able to burrow in the clayey sand where more than 33 % of them were found. Also here the ciliate fauna was very poor (91/cm^2) though somewhat richer than in the other two samples. The metazoan microfauna on the other hand was very rich (277/cm^2). Also here the nematodes dominated (154/cm^2) followed by the turbellarians (71/cm^2), but also relatively large numbers of rotifers, ostracods and harpacticoids were found. Characteristic was further the presence of large forms, i.e. chironomid larvae, acarines and oligochaetes.

The ciliate fauna consists, as in the above mentioned samples of diatom- and bacteria-feeding species in addition to the histophagous *Helicostoma notatum*. The finding of one specimen of each of the sulphurbacteria-eating species *Parablepharisma pellitum* and *Blepharisma salinarum* is not surprising considering the reducing properties of the underlying sediment.

Of course, not too much can be concluded from these samples taken in a single locality. However, also the samples from deeper water in the Øresund (i.e. Hornbæk, 22 m, and Ålsgårde, 20 m), likewise consisting of non capillary or nearly non-capillary sediments, indicate a modest role of the ciliates in the loose surface layers of clayey and muddy sediments.

7. INTERPRETATION OF THE CORRELATION BETWEEN REDOX-PROFILE AND VERTICAL ZONATION OF THE FAUNA. TOLERANCE TO ANAEROBIC CONDITIONS AND TO H_2S

As shown in Chapter 6 there is a clear correlation between the vertical zonation of the fauna and the redox-profile. The significance of the redox conditions for the distribution of the fauna can also be seen in Figs 75-79 where the numbers of animals per cm^3 sediment or water are plotted against the Eh of the sample. The graphs are based on field samples and on artificial sediments and mixed cultures of various kinds (see Chapter 9). No doubt, the graphs show somewhat too wide ranges for several species, since the animals have been extracted from sediment columns 1 to 3 cm high which may have a considerably higher Eh at the top than at the bottom. The Pt electrodes have a vertical extension of 0.4 to 0.8 cm and the Eh is therefore not sufficiently precisely measured when a steep redox gradient is present. This is especially the case in sediments with Eh falling to very low values within a cm or so below the surface.

Nevertheless, the graphs show that the distribution of the animals can be correlated with the measured electrode potentials.

Fig. 75 shows the "Eh-spectra" of four species of ciliates found commonly in the surface layers of sands. They are all in the main confined to oxidized environments. The apparent occurrence of a few speciments in reduced environments is probably due to the presence of some specimens in the upper part of a core segment, the middle and lower part of which was reduced.

Fig. 76 shows two species confined to moderately reduced environments; both have their main distributions in environments with Eh values between 0 and +200 mV, i.e. in the vicinity of the redox discontinuity layer. The slight tendency to bimodal distributions seen in these graphs is an artefact due to the main oc-

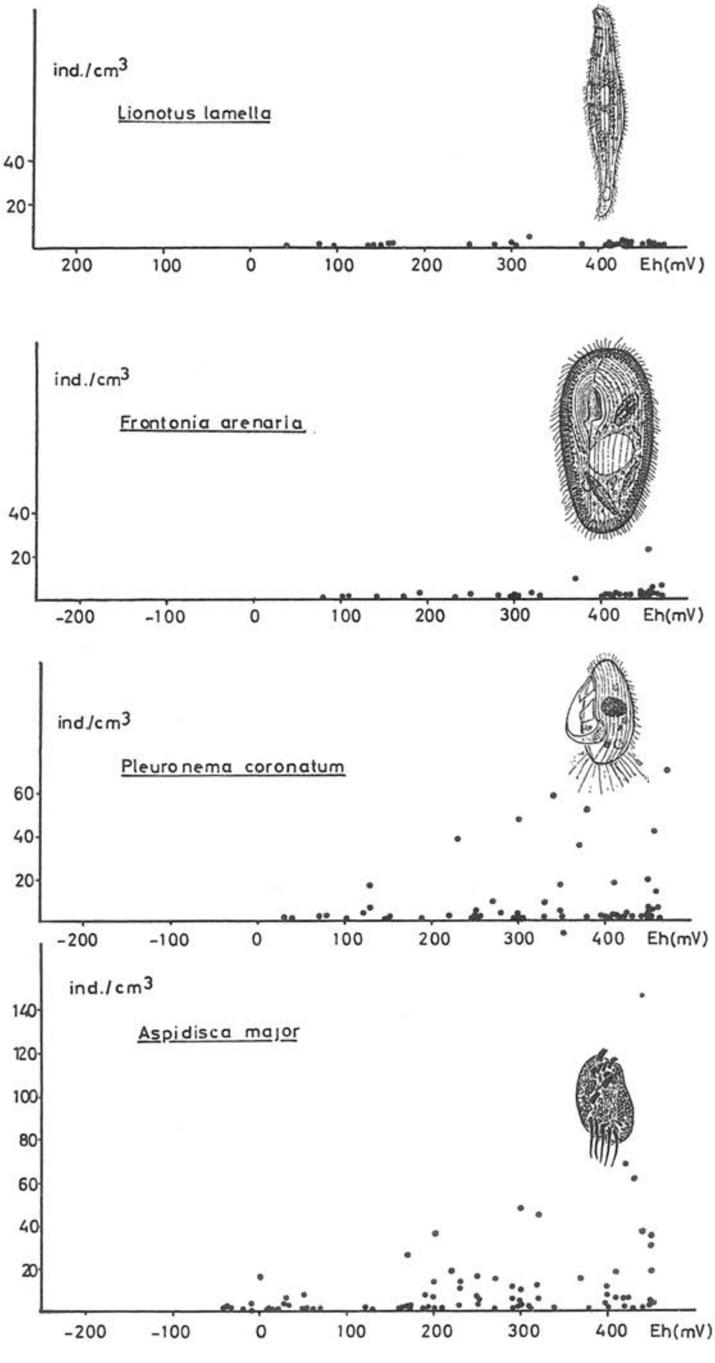

FIG. 75. The Eh spectra of four species of ciliates confined to the surface layers of sand.

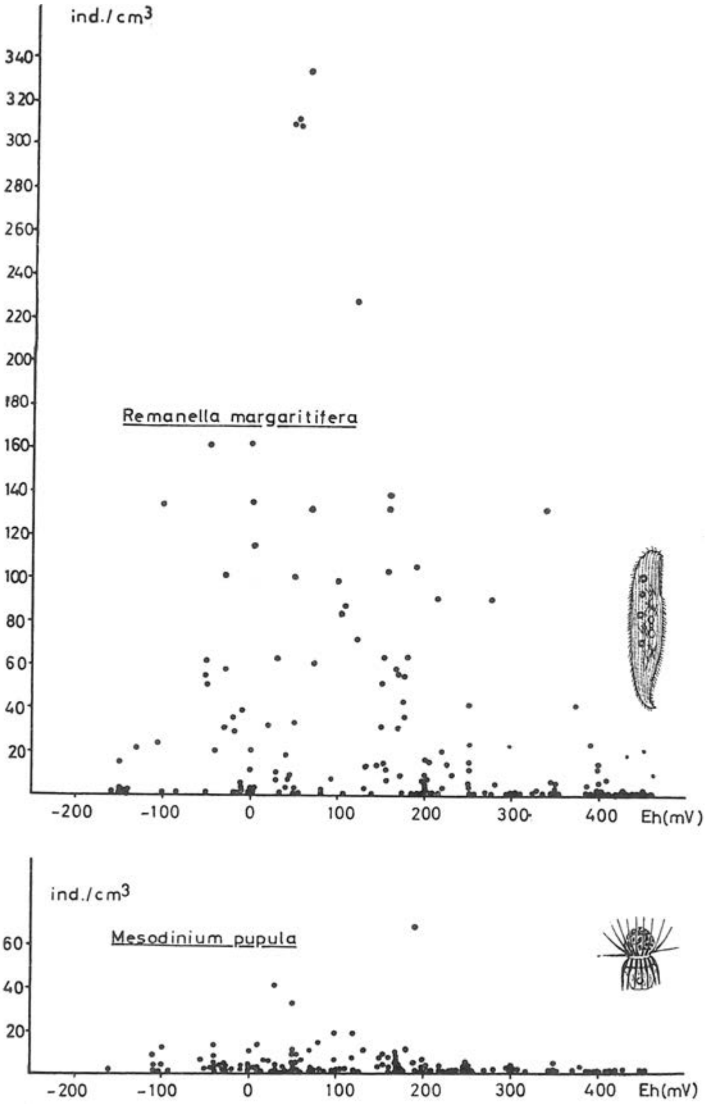

FIG. 76. The Eh spectra of two species of ciliates living in the vicinity of the redox discontinuity ayer.

currence around the discontinuity layer. Since the Eh was measured at fixed depth intervals one is often likely to measure above or below the discontinuity layer.

Similar Eh-spectra are characteristic of many other species, for example the other *Remanella* spp, the *Kentrophorus* spp and the *Geleia* spp.

Fig. 77 shows the Eh-spectra of 5 species living in or close to the sulphide zone of sediments. They have all been found at redox potentials as low as —250

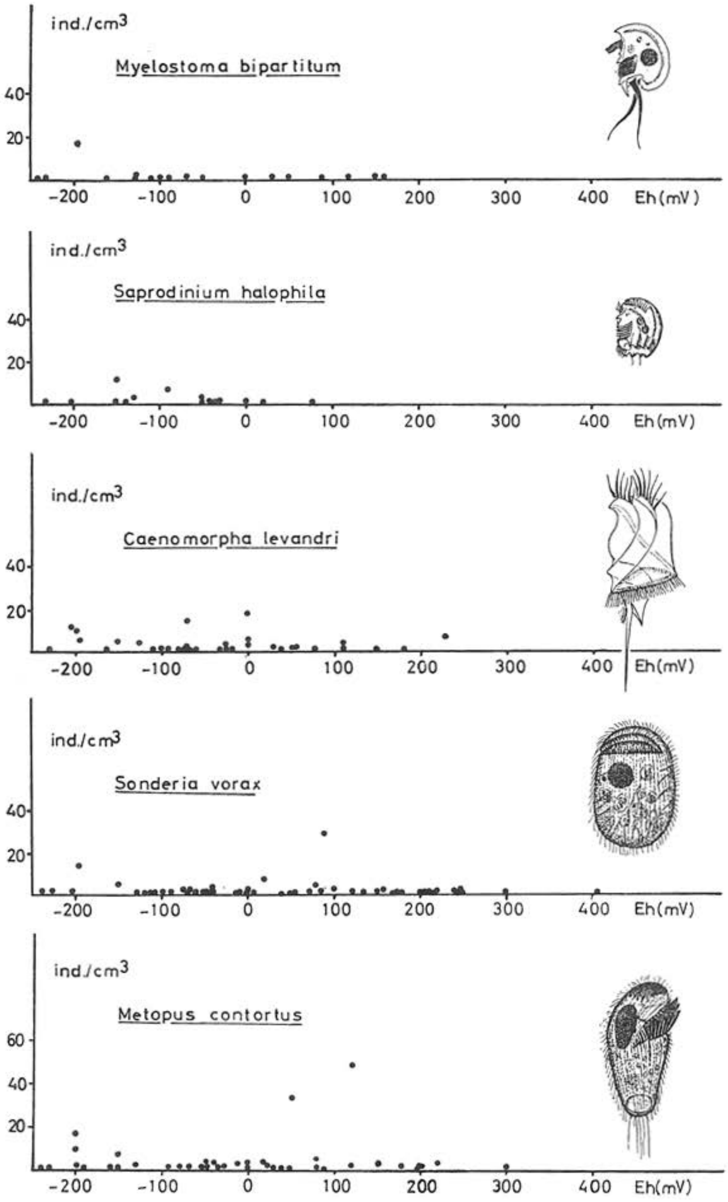

FIG. 77. The Eh spectra of five species of ciliates living in or close to the sulphide zone of sediments.

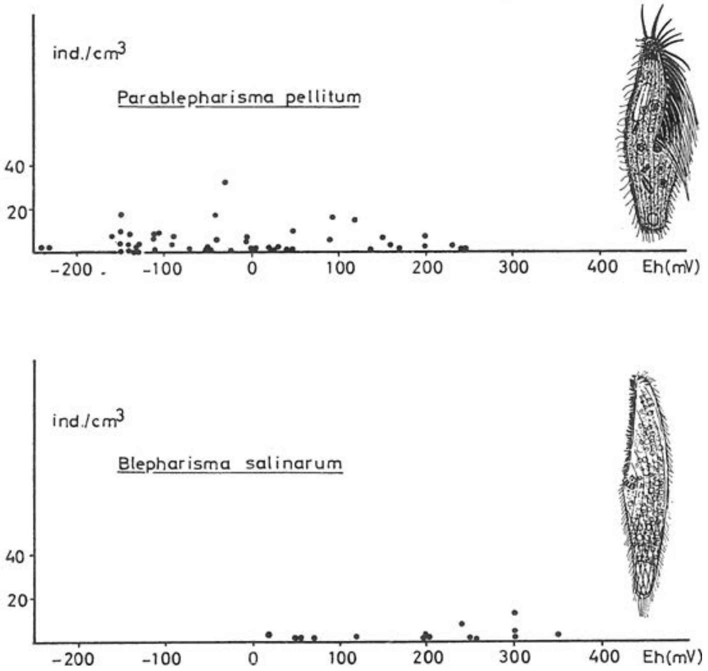

FIG. 78. The Eh spectra of two consumers of purple sulphurbacteria in estuarine sands.

mV and have their main distributions at negative potentials. The findings at high potentials may, as in the above mentioned cases, be due to an insufficiently precise localization of the fauna or/and the redox-profile. In laboratory cultures *Caenomorpha levandri, Myelostoma bipartitum* and *Saprodinium halophila* were never found in connection with redox potentials above 0 mV.

Fig. 78 shows the Eh-spectra of two ciliates which both predominantly feed on purple sulphurbacteria (Chapter 6); *Blepharisma salinarum* lives close to the surface while *Parablepharisma pellitum* is only found in the sulphide zone. As shown by Baas Becking & Wood (1955), purple sulphurbacteria may grow in reducing as well as oxidizing environments; in the latter case they utilize elemental sulphur and in the former case hydrogen sulphide as hydrogen donor. These bacteria are, however, consumed by different kinds of ciliates at the different environmental conditions.

Fig. 79 shows the Eh range for three diatom consumers which are common in and on estuarine sands.

In order to understand the relation between the distribution of the animals and the redox conditions three things should be considered for each species, viz the requirements of oxygen, the tolerance to reduced, toxic compounds (notably H_2S), and the food requirements.

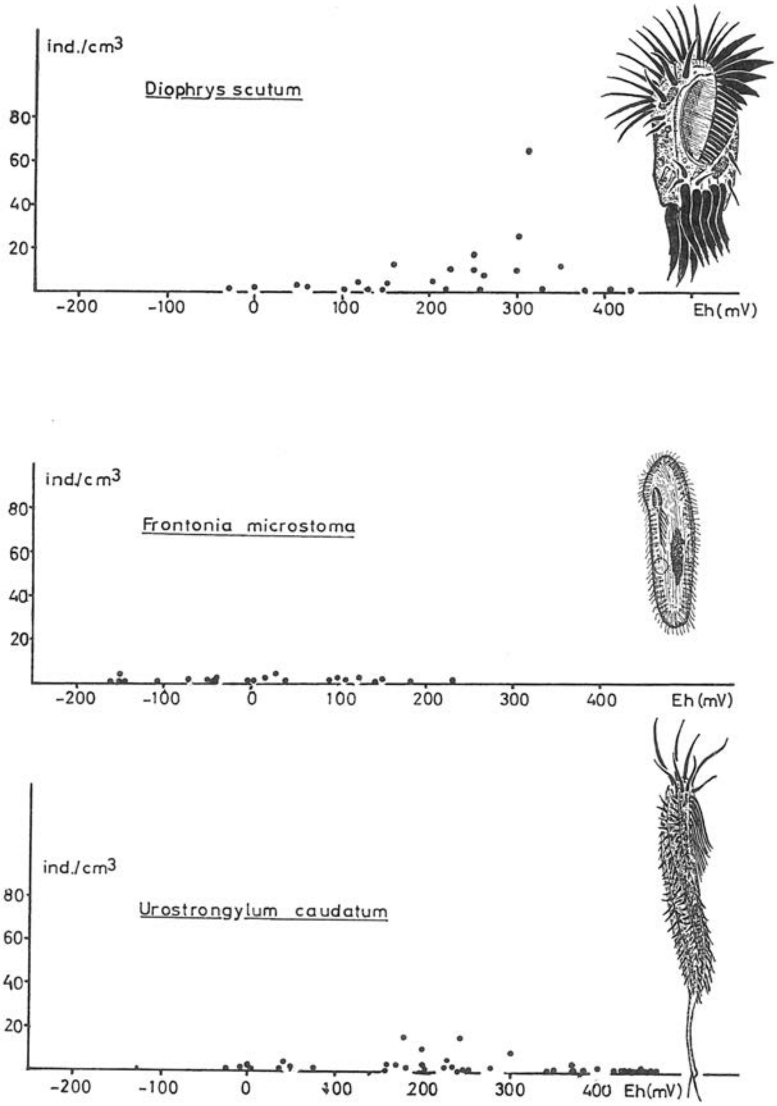

FIG. 79. The Eh spectra of three species of ciliates feeding on diatoms in estuarine sediments.

While there is no critical work on the effect of chemical factors (except salinity) on marine ciliates, much information can be found in the literature on freshwater ciliates.

In the earlier literature there are many reports stating that a large number of ciliates such as *Paramecium caudatum*, *Spirostomum* spp, *Coleps hirtus*, etc. can survive for long periods or even multiply under completely anaerobic conditions.

The results of these experiments are now believed to be inaccurate because it is difficult to obtain completely anaerobic conditions without adding reducing agents (commercial hydrogen and nitrogen do contain some oxygen). More recent investigations show that the great majority of freshwater ciliates can only survive completely anaerobic conditions for a short period, but many species for example the *Paramecium* and the *Spirostomum* spp can thrive well at very low oxygen tensions. This was demonstrated by Bick (1958, 1964) and others, as well in the field as in laboratory experiments. (For references to older and newer literature on the oxygen requirements of freshwater ciliates and for discussions see Brand, 1946, and Noland & Gojdics, 1967).

The existence of a genuine anaerobic freshwater fauna of ciliates living in sulphide-containing waters and sediments has, however, been demonstrated. It consists mainly of species of *Metopus, Caenomorpha, Luidio, Plagiopyla,* and the representatives of the order Odontostomatida (*Saprodinium,* etc.). For references see the above mentioned literature and Wetzel (1929).

It is certain that the species living in the sulphide zone of marine sediments, e.g. *Caenomorpha levandri, Metopus* spp, *Mesodinium* spp, *Saprodinium halophila, Myelostoma bipartitum,* and the *Parablepharisma* spp, can live in a completely anaerobic environment and that forms often occurring in this zone (*Sonderia* spp, *Plagiopyla* spp, *Cardiostomella vermiforme*) can at least thrive in anaerobic environments for long periods.

In an experiment, water and decomposing algae, initially containing 42 mg H_2S/l and with Eh value of —190 mV were kept in an airtight bottle in the dark for 79 days. After 17, 32 and 79 days, water samples, taken without introducing air, were analysed and the fauna studied. During the experiment the Eh decreased to – 210 mV and the contents of H_2S increased to 295 mg/l. The value of pH varied between 6.65 and 7.06. Oxygen was completely absent throughout the experiment.

After 17 days a rich fauna of *Plagiopyla* sp., *Mesodinium* sp., *Sonderia vorax, S. cyclostoma, Metopus contortus, Caenomorpha levandri, Saprodinium halophila, Myelostoma bipartitum,* some nematodes, and the rotifer *Colurella colurus* was found. After 32 days the metazoans and the two first mentioned ciliate species had disappeared while a population of an *Euplotes* sp. had become established. After 79 days only *Metopus contortus* and *Caenomorpha levandri* (and an unidentified small holotrich) were found. All the ciliate populations found, and probably also *Colurella,* were reproducing at one time or another and *Metopus contortus* showed an epidemic of conjugation at 32 days. Thus, there is no doubt that these species all can thrive in completely anaerobic environments.

In the experiment the ability to live anaerobically was not studied separately from the ability to endure the toxic compounds associated with anaerobiosis. Probably an additional number of species endure anaerobiosis but not H_2S in any significant amounts.

Bick (1958) studied the vertical distribution of ciliates in ponds containing large amounts of decomposing leaves. In the quite anaerobic and H_2S containing deeper layers a fauna mainly dominated by *Metopus* spp was found. Immediately above this layer a fauna tolerating very low oxygen tensions but only very low concentrations of hydrogen sulphide was found comprising *Spirostomum ambiguum, Halteria grandinella* and *Cyclidium citrullus*. It is reasonable to consider this fauna as analogous to that found in the vicinity of the redox discontinuity layer of marine sediments, i.e. where conditions are reducing but where H_2S is absent or occurs in very low concentrations.

Wetzel (1929) also demonstrated a vertical zonation of the ciliates in freshwater ponds, which could be correlated with the distribution of H_2S and O_2.

Very relevant to the discussion is the investigation by Rylow (1923) on *Loxodes rostrum*, a freshwater relative of *Remanella*. Rylow found that this ciliate avoids water containing H_2S and O_2 in larger amounts and thus populations of this ciliate are concentrated in the stratum between the oxygen and the H_2S zones.

It is probable that the ciliates confined to the oxidized surface layers of sediments are dependent on a relatively high oxygen tension but direct proof is lacking.

Bick (1964) studied the influence of ammonia on various freshwater ciliates. He found that many, but not all, forms tolerated this compound in higher concentrations at low values of pH indicating that NH_3 is more toxic than NH_4^+. Thus when pH was above 8.6 only 1 mg/l was tolerated while below pH 7.6 more than 20 mg/l was tolerated by *Cyclidium citrullus*.

Nikitinsky & Mudrezowa-Wyss (1930) investigated the effect of H_2S, H_2, CO_2, and CH_4 on a large number of freshwater organisms including many ciliates. They found that, in contrast to H_2S and CO_2, H_2 and CH_4 are not toxic. They also found, as could be expected, that H_2S is more toxic than CO_2. They tested their experimental organisms by placing them in water saturated with the gases which in all cases quickly killed the organisms. Thus all organisms with the exception of the sulphurbacteria *Beggiatoa*, which survived for about 40 min, died within few minutes or seconds when placed in saturated H_2S solutions. The authors generally found a correlation between a longer survival of some species in the CO_2 and H_2S solutions and their ability to live anaerobically, but the experiments, exposing all animals to lethal concentrations, give only restricted information of importance for the understanding of the field distributions.

Baas Becking & Wood (1955) mentions that some flagellates tolerate higher concentrations of H_2S at lower pH indicating that H_2S is less toxic than HS^- and/or S^{--}.

During the present investigation 41 samples of sediments or water from Nivå Bay, Helsingør Beach, Julebæk Beach, Vellerup Vig, Frederikssund, and from laboratory cultures were analysed for contents of total H_2S and the species present were noted. The contents of H_2S varied from 0 to more than 700 mg/l.

In Fig. 80 the occurrence of some species of ciliates at different H_2S concentrations is shown. All species found in more than 50 mg H_2S/l are shown while those only occurring at lower concentrations or not tolerating H_2S at all (the majority of species) are only represented by a few examples. At one occasion two samples from the Helsingør Beach with unusually high concentrations of H_2S (about 400 and 500 mg/l respectively) contained specimens of some ciliates (*Remanella* spp, *Mesodinium pupula, Pleuronema marinum, Cardiostomella vermiforme*) normally not occurring at such high concentrations. These and a few similar cases are indicated with crosses in the diagram.

Concerning some of the ciliates found in small concentrations of H_2S, the results only indicate that they may live in concentrations up to 10-20 mg/l for a limited time since these forms were mainly found in environments not containing H_2S. Also in some cases the microdistribution of H_2S and of the animals may not always have been homogeneous within the sample analysed. (Of the sediments, 0.5 to 4 cm high core segments were analysed, whereas samples from the free water, usually 5 or 10 ml, were taken by a pipette.)

It is noteworthy that some of the species belonging to the sulphide zone apparently occur only when free H_2S is present.

Among the metazoans, few forms endure such high concentrations of H_2S as do many ciliates. Only the nematodes and some rotifers are comparable. Living nematodes have been found in small numbers at concentrations up to 250 mg/l and more numerously in concentrations up to 100 mg H_2S/l. Wieser & Kanwisher (1961) found living specimens of a few species in sealed tubes filled with anaerobic muds with values of Eh as low as 160 mV. Among rotifers *Colurella colurus* has been found in water containing up to 360 mg H_2S/l and they may apparently reproduce in water containing more than 100 mg/l. In the Askö Harbour specimens of *Monostyla cornuta* were found in sediments with redox potentials between —200 and —100 mV indicating that it endures H_2S in considerable amounts and *Rotatoria citrina* was also found at negative potentials.

Other rotifers were never found under such conditions though a few findings of several other species were made in cultures and in sulphureta at concentrations below 10 mg/l.

Some oligochaetes are also resistant to H_2S; some have been found in water containing up to 50 mg/l.

Turbellarians were generally not observed in H_2S containing environments though a few specimens have occasionally been found in samples containing as much as 50 mg/l. Harpacticoids are never found in sulphide containing sediments but have been observed in water with up to 10 mg/l. The gastropod *Hydrobia* leaves the water or creeps along the water film when H_2S appears.

A glass container with water and sediment from the Nivå sulphuretum was kept for about 20 hours in the laboratory. The water column above the sediment was about 15 cm high and was coloured intensely red from sulphurbacteria. Close to the surface 8 mg H_2S/l and at the surface of the sediment 34 mg/l were

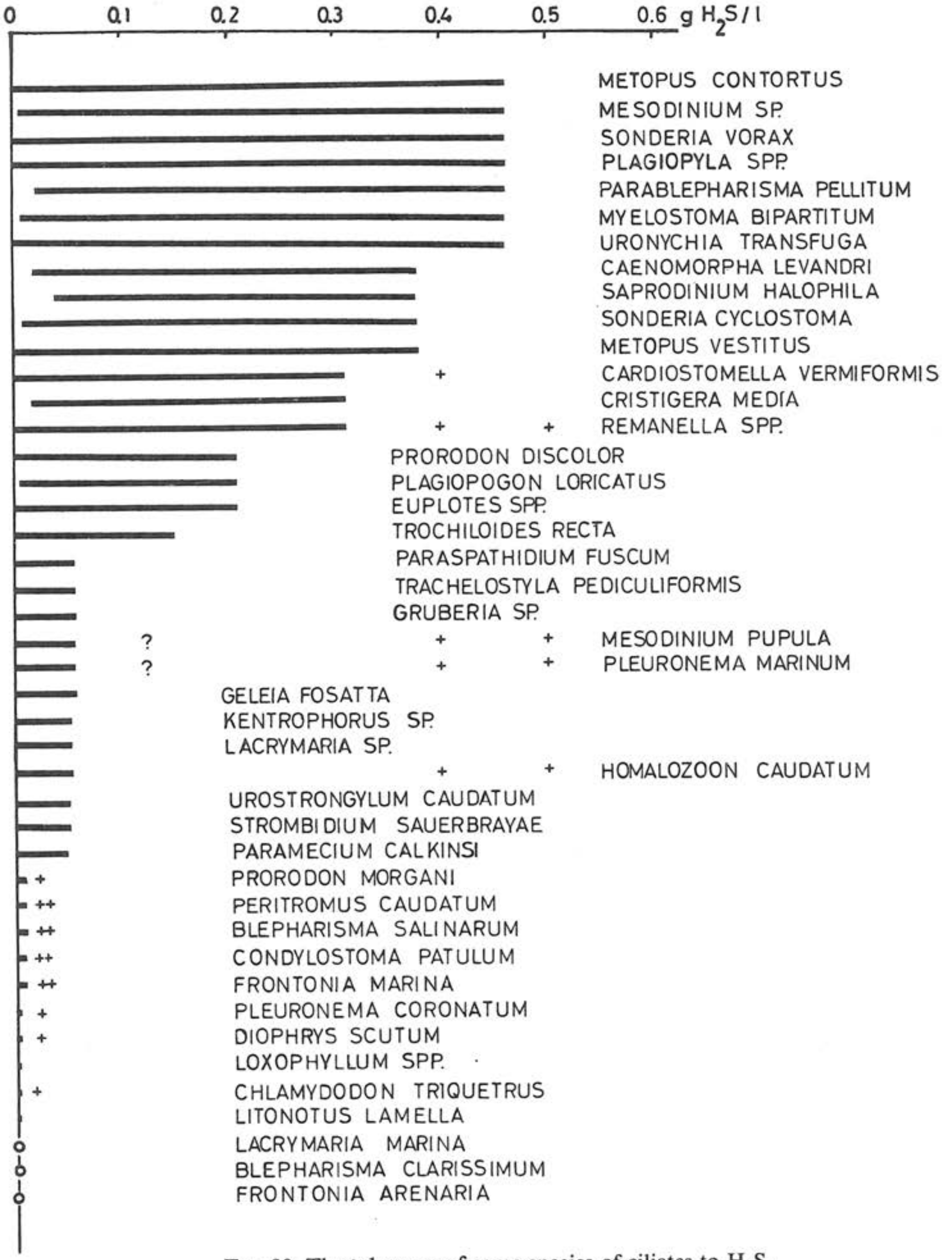

FIG. 80. The tolerance of some species of ciliates to H_2S.

found. Probably the concentrations of H_2S immediately below the water surface are lower, but this was not possible to demonstrate. Here large concentrations of harpacticoids, turbellarians and nematodes in addition to numerous specimens of the ciliates *Frontonia marina, Paraspathidium fuscum, Condylostoma patulum, Prorodon discolor, Gruberia* sp., *Blepharisma salinarum, Holosticha kessleri, Ophryoglena* sp., *Tracheloraphis* sp., *Paramecium calkinsi,* and *Peritromus faurei* congregated though all these forms (with the exception of *Paramecium calkinsi*) are benthic normally seeking downwards to the sediment. Apparently H_2S changes the geotaxis of these species.

At the sediment surface the fauna was dominated by *Metopus contortus, Plagiopyla* sp., *Sonderia vorax* and *Parablepharisma pellitum* which apparently show positive geotaxis even in the higher concentrations of H_2S. In addition to these forms a few specimens of *Frontonia marina, Tracheloraphis* sp., *Prorodon discolor,* and *Blepharisma salinarum* were also found at the bottom.

Oligochaetes were found along the glass wall close to the water surface and in the sediment. Some *Nereis diversicolor* in the sample were all dead next day in spite of their ability to swim at the surface.

From this it can be concluded that —1. The different species of ciliates in sediments show different ranges of tolerances to oxygen and H_2S, —2. The vertical distributions of the animals may be controlled by the distribution of these compounds which partly explains the correlation between vertical distribution and redox-profile, —3. The ciliates comprise many species which can endure or even do require high concentrations of H_2S, and —4. Several representatives of the microfauna normally living in the oxidized environments or at least environments free from H_2S may endure moderate concentrations of H_2S for some time. This seems not to be the case with the macrofauna which can migrate from areas where the toxic H_2S is present.

The vertical and horizontal distribution of the microflora (the food of the microfauna) and the relation to the redox potential was discussed in Chapter 5. Here only a few examples showing the significance for the microfauna will be mentioned.

The anaerobic decomposers will be found in the strongly reducing layers of the sediment and it seems probable that the majority of the inhabitants of the sulphide zone feed on these microbes. At least the majority of the ciliates living in this zone feeds on bacteria.

In the vicinity of the redox discontinuity layer, bacterial activity is great (see Chapter 5). That the animals eating chemoautotrophic and other types of bacteria are concentrated in this layer is not strange; numerous examples demonstrating this are mentioned in Chapter 6.

Photoreduction requires that reduced compounds reach the photic zone. When this is the case a number of ciliates feeding on photoautotrophic bacteria will be present. Among these ciliates some require strongly reducing conditions (e.g.

Parablepharisma pellitum), others (e.g. *Blepharisma salinarum*) require more oxidizing conditions resulting in a zonation within the layers where photoreduction may take place.

The distributions of many forms are more dependent on chemical factors than on food. Thus *Metopus contortus* may feed on white or purple sulphurbacteria, other bacteria, diatoms, and other protophytes, but is strictly confined to reducing environments. *Pleuronema coronatum* feeds on the same items, but is confined to the oxidized surface layers.

As shown in Chapter 6 a large number of forms, e.g. the *Remanella* spp, are found in reducing environments but feed mainly or exclusively on diatoms and other unicellular plants. As mentioned previously in this chapter the relative of *Remanella, Loxodes,* shows the same "redox preference" in chemically stratified water bodies and this ciliate is known to feed on sulphurbacteria, which are not eaten by *Remanella.*

8. TAXONOMIC COMPOSITION OF THE CILIATE FAUNAS OF DIFFERENT COMMUNITIES AND ADAPTATIONS FOUND IN BENTHIC CILIATES

In Fenchel (1968a) the fact, that several ciliate groups are conservative in their ecological demands, was discussed and exemplified by their preference of food. Also other ecological features are characteristic of lower or higher taxa. Several genera, families and even orders may be characteristic of or dominating in certain communities (Chapter 6).

In and on estuarine sediments and sulphureta a number of ciliate groups, rather sparsely or not at all represented in other biotopes, are numerous both in number of species and numbers of individuals. For example a number of representatives of the order Heterotrichida (species of the genera *Blepharisma, Peritromus, Gruberia, Condylostoma, Spirostomum* and *Fabrea*) live mainly in sulphureta and in sediments of estuarine character but only in the oxidized surface layers or the reduced layers not containing H_2S. Other heterotrichids living in the sulphide zone have undoubtedly evolved from some of these forms.

The morphological studies of Jankowski (1964) have shown that the genus *Blepharisma,* which is the most primitive group within the order, has given rise to the other genera within the Heterotrichida and thus to all the heterotrich inhabitants of the sulphide zone, i.e. *Parablepharisma, Metopus, Caenomorpha,* and also, as shown by Jankowski, the order Odontostomatida which only comprise "sulphide forms". The ciliate fauna characteristic of these environments has been called "sapropelic" by Lauterborn (1916), Wetzel (1929), Jankowski (1964) and other students of the freshwater representatives. The Greek word "sapros", meaning decaying, is, however, not very appropriate to designate this community since the characteristic feature of the environment in question is not the presence of decomposing organic material but the absence of oxygen and the presence of hydrogen sulphide. Aerobically decomposing organic material contains a different fauna, while the deeper strata of marine sands may contain significant amounts of H_2S but rather little organic material and still harbour a characteristic "sapropelic fauna". A large part of this fauna feeds on sulphurbacteria which are generally not dependent on the presence of organic material. In free waters where the supply of oxygen is high, large accumulations of organic material are necessary in order to create reducing conditions, in sediments where oxygen availability is poor, relatively little organic material is needed before anaerobiosis takes over.

The heterotrichids have, however, evolved many more species within most genera in similar freshwater biotopes than they have in the sea as is apparent from the work of Jankowski. This may partly be explained by the relatively larger role played by the Plagiopylidae in marine environments, but the representatives of this group are mostly macrophagous and probably do not replace the microphagous caenomorphids and odontostomatids which are numerous in freshwater.

Other ciliate groups than the heterotrichs also contribute to the sulphide fauna. Among them the representatives of the Plagiopylidae are of large importance in the sea but less so in freshwater.

The herbivorous, cyrtophorine gymnostomes are also well represented in the estuarine microbiocenosis (*Chlamydodon, Chilodontopsis, Cryptopharynx*) but within this group only one, the *Beggiatoa*-eating *Trochiloides recta,* can be considered to belong to the sulphide fauna and the species does not seem to tolerate very high concentrations of H_2S.

A number of species belonging to groups, predominantly comprising aerobic species, also occur in the sulphide fauna, e.g. *Frontonia microstoma, Cristigera media, Mesodinium* sp., *Plagiopogon loricatus.*

The representatives of the sulphide fauna, notably the heterotrichs, the odontostomatids and the plagiopylids, show a number of characteristic adaptations, the significance of which is not always clear. One is the occurrence of symbiontic bacteria on the body surface found in most *Metopus* spp, all *Parablepharisma* spp, *Caenomorpha* spp, *Sonderia* spp, odontostomatids, and on some *Euplotes* spp living in reduced sediments. In some cases the layer of bacteria is very dense and is orientated perpendicular to the surface of the ciliate (*Parablepharisma* spp). In other forms the bacteria lie parallel to the surface in what seems to be a layer of mucus covering the animal (*Sonderia* spp). In some forms, e.g. *Parablepharisma* and *Sonderia* spp, the whole surface is covered by the bacteria, in other cases, for example the caenomorphids, only certain parts of the body carry bacteria. Also the species of the genus *Kentrophorus,* which live in the reduced zone, though rarely at high concentrations of H_2S, carry a dense layer of sulphur bacteria on one side of the body and these bacteria contain grains of sulphur and pigments, (Fauré-Fremiet, 1950b); they are probably not, however, photosynthetic.

The significance of the symbiontic bacteria remains unknown. The present investigation has shown that they are associated with ciliates living in reducing environments. In all cases the association seems to be obligatory and one species of ciliate is always associated with a certain species of bacteria.

Within the Heterotrichida and the Odontostomatida the development of the species of the sulphide fauna has been associated with a torsion of the body (*Metopus, Parablepharisma* and even more extremely in *Caenomorpha* and the odontostomatids), development of rigid cell surfaces often carrying spines and crests, and of cirri in connection with a general reduction of the ciliature (caenomorphids, odontostomatids). A rigid cell surface is also found in the *Sonderia* spp.

The significance of these features is not clear. They may be adaptations to the locomotion in accumulations of organic debris, the rigid, twisted bodies enabling the ciliates to screw themselves through the viscous medium. Dogiel (1965) called attention to the resemblance of the odontostomatids to the ciliates living in the digestive system of ruminants. This is undoubtedly an example of parallel evolution. More interesting is the fact that some of the marine "sapropels", i. e. metopids and plagiopylids, have become obligatory commensals in the intestine of sea-urchins (Beers, 1954; Lucas, 1934) probably due to their ability to live anaerobically.

The systematic composition of the ciliates of sublittoral sands and their morphological adaptations have been treated thoroughly in the literature already (notably by Dragesco, 1960).

Especially the rhabdophorine gymnostomes are dominant in fine and medium sands and the number of known species of this group has increased drastically as a result of the studies on sand ciliates carried out by Dragesco, Fauré-Fremiet, Raikov, and others in recent years.

The rhabdophorine families confined to sands are Loxodidae and Geleiidae while several representatives of Enchelyidae, Sphathidiidae, Trachelocercidae, Amphileptidae, and Trachelidae are more or less confined to interstitial life.

As shown in Chapter 6 the Loxodidae and the Geleiidae are confined to the reduced layers of the sediment, the Amphileptidae and the Trachelidae live only in the oxidized surface layers and within the other families representatives are found in both layers of the sands.

The morphological adaptations of sand ciliates of which many are easily understandable (elongated, often ribbon shaped bodies, contractibility, thigmotactic behaviour, reduced ciliation on one side of the body) have alle been discussed by Dragesco (1960). He also found that many of these ciliates are characteristic by containing only small amounts of DNA. The works of Raikov (1958, 1959, 1963) have shown that these forms have macronuclei which are only diploid in contrast to the polyploid nuclei found in most other ciliates.

These diploid macronuclei cannot undergo division. Raikov considers these conditions to be primitive but the fact that they occur in groups only related ecologically but not very closely systematically and in some cases in forms which are not at all systematically primitive (viz. *Geleia*) indicate a convergent evolution, the ecological and physiological significance of which is unknown.

A feature characteristic of a great number of ciliates from all kinds of benthic communities is the presence of refringent bodies in the cytoplasm. These inclusions are probably always of calcareous nature. Usually they are rounded bodies concentrated in the anterior end of the body (*Tracheloraphis* spp, *Prorodon* spp, *Lacrymaria* spp, *Cardiostomella vermiforme*, *Metopus contortus*, and many other forms). In *Remanella* two kinds are present; the "corpuscles of Müller" which contain calcium but are not soluble in acid, and fine needles which consist of calcium carbonate. The latter are birefringent in contrast to other calcareous inclusions of ciliates. In *Ciliofaurea* other shapes are found (discs, wheels). André & Fauré-Fremiet (1967) studied the fine structure and morphogenesis of the calcareous inclusions of *Prorodon morgani* and Fauré-Fremiet & Gauchery (1957) and Dragesco (1960) studied the inclusions of *Remanella*.

It should be remembered that side by side with species specialized to different biotopes a number of ubiquitous forms "specialized in not specializing" are found. An example is *Uronychia transfuga* which occurs in the oxidized splash zone, in sands of all grain sizes, in algal mats, in anaerobic environments with high concentrations of H_2S and in any other concievable sea-water biotope. In the Helsingør Laboratory the species has been found in considerable numbers in bottles containing several months old sea-water, in cultures of invertebrate larvae and everywhere in the sea-water system. In the field it usually occurs in relatively small numbers. Also some *Euplotes* spp and some small hymenostomous ciliates are ubiquitous.

The ability to form cysts is an important factor in protozoan ecology and is found in a great number of freshwater ciliates. Few observations on encystation in marine benthic ciliates are reported in the literature, but probably encystation is rather widely distributed.

In obligatory histophages (*Helicostoma notatum*, *Ophryoglena* spp) encystation is an obligatory part of the life cycle (Fauré-Fremiet, 1935; Mazoué, 1935; Mugard, 1949). Webb (1956) observed that *Nassula citrea*, *Lacrymaria marina* and *Diophrys appendiculata* may form cysts.

During the present investigation formation of cysts was observed in *Diophrys scutum*, *Lacrymaria marina*, *Uronema marina* and *Aspidisca angulata*. In all cases the encystation was a result of depletion of food in cultures and new supplies of food resulted in excystation. (*Diophrys scutum* was fed on diatoms, *Lacrymaria marina* on *Uronema* and the two last mentioned forms on bacteria grown in peptone solutions, see Fenchel, 1968b). Addition of peptone to cycts of *Uronema* and *Aspidisca* results in excystation within 2 to 24 hours. Cysts of *Aspidisca angulata* kept at 10°C and at 5°C were able to excyst after nearly 2 years. In all species cysts were attached to the glass walls of the culture containers; in the field they probably attach to sand grains or other objects.

The number of cysts in proportion to that of active animals in the field is unknown, but cysts of ciliates were occasionally observed in field samples.

Neither slowly nor quickly desiccated cysts of *Aspidisca* were able to excyst when water was added. Sand samples from Nivå Bay which were dried at various temperatures and samples first drained for water and then dried did not contain living ciliates some time after water had been added again. Thus cysts of marine ciliates seem not to survive desiccation.

9. LABORATORY MODELS OF MICROBENTHIC ECOSYSTEMS

Ciliates and other microorganisms are of special value as objects for ecological research because they are relatively easy to keep in complete ecosystems in the laboratory. Micro ecosystems have been studied by several authors. In Chapter 5 references are given to works, in which the ecology of various microorganisms have been studied in mixed cultures. The work of Bick (1964) should also be mentioned as an example demonstrating the large amount of information which can be derived from such studies. In this work the succession of microorganisms, algae, protozoa and small metazoans in water enriched with cellulose was studied. Other examples are the works of Beyers (1965), Margalef (1967) and papers cited therein.

Much work, also with an ecological scope, is carried out on pure cultures and it is clear that this approach is indispensable. It is believed, however, that a deeper understanding of natural ecosystems can only be achieved by parallel studies on the behaviour of mixed cultures. Such experiments may provide information on population dynamics, element cycles, energetics, zonation, and other aspects of ecosystems.

I believe that during the present investigation the potentials of laboratory ecosystems were far from fully exploited. The experiments have, however, demonstrated some features and principles of the ecosystems in question. It is hoped that they will serve to demonstrate the potentials of such experiments for further research on the ecology of the microfauna.

As shown in Chapter 6 the oxidized sand layers of the Helsingør Beach locality are characterized by a dominance of bacterivorous forms and a rather poor fauna of herbivorores. This microfauna could be expected mainly to be based on the bacterial decomposition of bits of sea-weeds into the sediment. The dominating bacterivorous forms are *Aspidisca major* and other *Aspidisca* spp, *Holosticha* spp and *Trachelostyla pediculiformis*. The important carnivores are *Litonotus lamella, Loxophyllum* spp, *Hemiophrys filum, Uronychia transfuga*, and *Tracheloraphis* spp.

In order to study this community in the laboratory two plastic trays measuring

25 × 29 cm in area and about 5 cm high were both filled with 1.5 l rinsed and heated sand (to a heigth of about 2 cm). One g thallus of *Fucus serratus* was cut into small bits and mixed into the sand of each tray, and millipore filtered, aged sea-water was added to a height of 1 cm above the surface of the sand. Finally, 10 cm^3 surface sand from the Helsingør Beach were added to each tray. One of the trays was exposed to the light conditions of the laboratory, the other one was kept in the dark. The temperature was about 20°C throughout the experiment (42 days). Every third or fourth day Eh and pH were measured in the water and in the sediment and samples of 1 cm^2 taken to the bottom of the trays (i.e. 2 cm^3 sand + 1 cm^3 water) were collected and extracted for microfauna. The oxygen contents of the water and in the sediment were occasionally measured.

The results are shown in Fig. 81. Since the dark experiment nearly down to every detail resembled the light experiment with respect to population sizes and sequence of each species, Eh, pH, and oxygen, only the latter is shown. (The ciliate *Euplotes*, however, occurred in significantly larger numbers in the dark experiment.) All species, with the exception of a few which occurred very sporadically and in very small numbers, are shown. Also a few unidentified forms (never found with more than 11/cm^2) have been omitted.

The water remained oxidized throughout the experiment. In the sediment the Eh decreased during the first 6 days to values somewhat below +200 mV as a result of the activity of heterotrophic bacteria. After a week it increased again. The oxygen content remained rather constant throughout the experiment and was all the time lower in the sediment, but it never fell to 0. The value of pH also remained rather constant, indicating, together with the constantly low oxygen contents and the fact that the light and the dark experiment progressed identically, that photosynthesis was of no significance. The dinoflagellates (*Amphidinium* sp.) were colourless and occurred in the same numbers in the dark and the light experiment. Diatoms and other algae were practically absent.

All successions on organic material are initiated by a growth of heterotrophic bacteria quickly followed by a growth of bacterivorous ciliates among which *Uronema marina* is always the first to appear. As shown in Fenchel (1968a, b) this is due to the fact that this ciliate has specialized on bacteria which decompose proteins and also that this small ciliate has a high reproductive potential with a generation time of 2-3 hours at 20°C under optimal conditions.

In this experiment the growth of *Uronema* was slower, probably due to slower bacterial growth. This was again due to a low concentration of organic matter and also to slow decomposition of the bits of *Fucus* by the bacteria.

Uronema marina is rarely abundant in the field except around decomposing corpses of animals. It probably occurs in all successions based on organic material, but when its food, bacteria, becomes scarce *Uronema* encysts and rarely reappears later in the succession. In my experiment, however, it showed another small maximum after about 4 weeks.

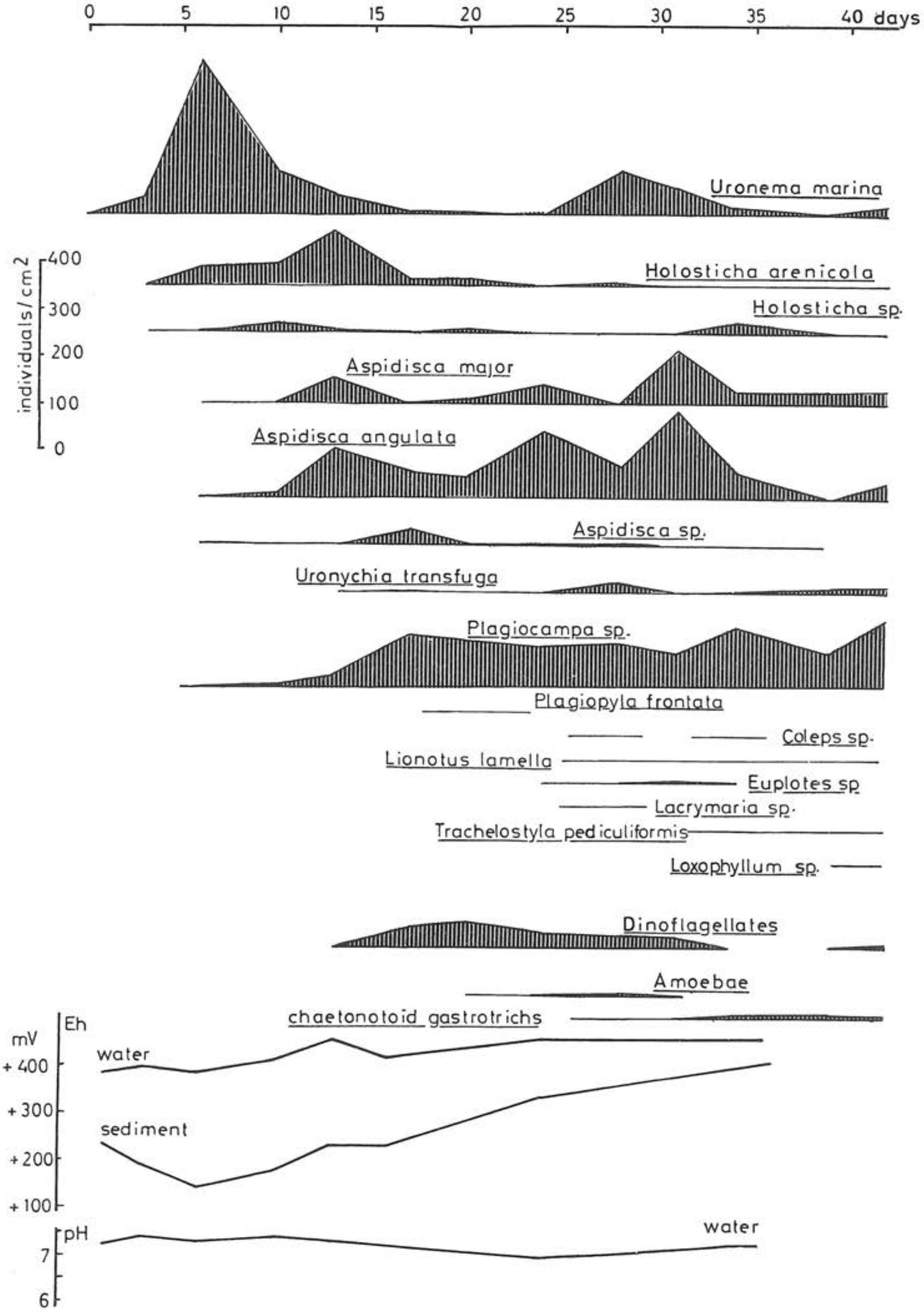

FIG. 81. The succession of species and the Eh and pH conditions in a succession experiment based on clean sand enriched with bits of *Fucus*.

After 5-10 days other bacterivorous ciliates take over, i.e. the somewhat larger *Holosticha* spp, which in addition to bacteria also eat small zooflagellates (*Bodo, Rhynchomonas*), the *Aspidisca* spp and, less numerously represented, *Trachelostyla pediculiformis* and *Euplotes* sp. The amoebae and the chaetonotoid gastrotrichs are also feeding on bacteria.

After 15 days the first carnivorous ciliates made their appearance; *Uronychia transfuga* followed by *Lacrymaria* sp., *Litonotus lamella* and *Loxophyllum* sp. and a few specimens of the histophagous *Coleps*. The role played by *Plagiocampa* sp. is uncertain; it may be a histophage or a carnivore feeding on ciliates and flagellates. Its cytoplasm is filled with clear refringent feeding vacuoles suggesting that its food is of animal origin. Like other forms occuring in the experiment it is a normal representative of the fauna of the surface sands of Helsingør Beach.

Only one species, *Plagiopyla frontata,* does not fit into the scheme being, like its relatives, normally confined to localities where sulphurbacteria are growing, i.e. where a reduction layer is developed. However, the species was sometimes encountered in small numbers in the Helsingør Beach during periods when the sediment was oxidized throughout (Fig. 48). During the succession experiment the sediments became somewhat reduced in the beginning, but far too little to induce sulphate reduction, and sulphurbacteria were quite absent. The presence of *Plagiopyla* cannot be explained as "accidental" as the species also occurred in the dark experiment in the same period. From its field distribution and its occurrence in this experiment it must be concluded that it is not so strictly dependent on sulphurbacteria and/or reducing conditions as are other plagiopylids.

The experiment shows that it is possible to reproduce the oxidized surface layers of sublittoral sands. The energy contained in the added bits of algae was only utilized through bacteria and possibly through small zooflagellates which may, however, also feed on the bacteria. The bacterial growth is quickly followed by growth of ciliate populations. First late in the succession other animals appear. The poor growth of metazoans (i.e. some chaetonotoids and a few nematodes not shown in Fig. 81) may be due to an insufficient introduction of appropriate species in the beginning of the experiment. The herbivorous forms, macrodasyoids, some turbellarians and nematodes, could of course not have been expected to grow in the experiment. Also time may have been too short to form populations of significant size at least for the larger micrometazoans, the reproductive potential of which is much lower than that of the ciliates (see Fenchel, 1968b).

The absence of diatoms and other protophytes was striking. It seems as if the presence of a reduction layer is necessary for the growth of protophytes (see also Chapter 5) and this may, together with the mechanical mixing of the surface layers, explain the poor growth of these plants in clean beaches and their occurrence at greater depths in the sediment.

Models involving reducing conditions and chemosynthesis were studied in more detail. The simplest model of this kind is made by placing a small crystal

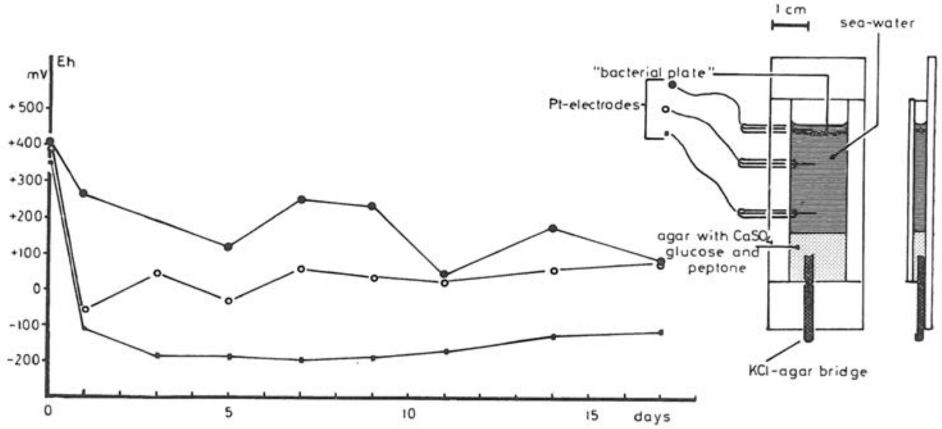

FIG. 82. A micro aquarium fitted with electrodes and the redox conditions through 17 days.

of Na_2S in a glass tube filled with sea-water. The tube is sealed at one end and closed at the other by a cotton plug or a piece of Jena filter and placed in a petri dish with filtered sea-water and inoculated with a few drops of water from a natural sample of estuarine sand. Sometimes the supply of sulphide from the tube was too high, killing everything in the dish but often growth of thiobacilli and of filamentous sulphurbacteria took place at some distance from the end of the tube followed by growth of populations of ciliates, mainly *Cyclidium citrullus* and *Plagiopyla* spp, feeding on the sulphurbacteria. These cultures rarely lasted longer than about a week and their development was difficult to predict. They are, however, of interest as they are an example of an ecosystem based solely on the energy of sulphide (i.e. H_2S → *Thiobacillus* → ciliates).

More interesting and more stable cultures were made by using a medium in which anaerobic decomposers and especially *Desulphovibrio* thrive. The medium used was a sea-water version of one used by Baas Becking (1925). To 1 litre of sea-water, 10 g $CaSO_4$, 1 g glucose and 1 g peptone were added, the solution autoclaved and stored at 5°C. For experiments the solution was turned into a solid medium by boiling with some agar. Still hot, the solution was poured into test tubes to a depth of about 2 cm or introduced into one end of a "micro aquarium" with a pipette. The micro aquaria measured 1.5×5 cm and were about 3 mm high; they were made of microscopic slides and coverglasses held together with vaseline. These micro aquaria could easily be studied under the compound microscope. Platinum-electrodes and a KCl-agar bridge could also be introduced to measure Eh at various levels in the aquaria (Fig. 82).

After the agar medium had congealed, filtered sea-water or water from a field sample was added. In the first case the cultures were inoculated with drops of water from natural samples (mainly from Nivå Bay). In the experiments, which were all made at room temperature, the water surface (3×15 mm in the case of the micro aquaria) was in free contact with the air.

These experiments offered a fine opportunity to study the zonation of the microflora and fauna. The cultures could be kept in the dark or in the light.

The fauna which developed in the cultures varied somewhat from experiment to experiment though they were kept under identical conditions. This is due to the fact that it is somewhat accidental which species are introduced when the inoculate consists of only few drops of water. However, several species were common to most experiments. In the test tube experiments the inoculates were larger and the fauna consequently somewhat more diverse.

Fig. 82 shows the development of the redox conditions in a typical dark experiment. Within the first 24 hours the Eh of the lower part (i.e. towards the agar) of the micro aquarium falls drastically due to bacterial activity. During the following days the Eh decreases further to values around —200 mV. Sulphate reduction follows as a result of the initial reduction of the medium and seems to dominate after 2-5 days. As seen on the graph, the Eh of this region begins to increase slowly after 7-10 days due to a slow oxidation of the system as the reduced compounds are used up at the upper end of the aquarium. The redox discontinuity layer moves downwards in the course of the experiment; in the shown example it passed the middle electrode at 5-7 days. The irregular measurements of the Eh by the upper electrode are due to small (1-2 mm) changes in water level. In spite of the fact that the micro aquaria were kept in a moist chamber between observations, some evaporation took place and small amounts of distilled water were added once in a while. The distance of the electrode to the air water interface was therefore not quite constant throughout the experiment.

The fauna and flora were particularly easy to study in the micro aquaria, but supplementary observations were made in the test tube cultures.

The cultures kept in the dark were most simple and will be described first. In the nutrient agar dense populations of decomposing bacteria soon flourish. Above the agar and through a considerable length of the micro aquaria only few bacteria are found. At the level of the redox discontinuity layer (around 0 mV) dense masses of various kinds of bacteria occur. Most conspicuous are masses of filamentous sulphurbacteria, mainly *Beggiatoa* (Figs. 83-84), which appear after 2-5 days and persist as long as H_2S is formed (some months in the test tube experiments, 14-30 days in the micro aquaria). Other white sulphurbacteria, and bacteria not belonging to the sulphur cycle, also occur. Different kinds of bacteria develop at different levels in the "bacterial plate". Above the redox discontinuity layer the number of bacteria decreases again.

Some species of diatoms were nearly always present in a few specimens, mainly above the bacterial plate. Colourless dinoflagellates were often found in a dense, well defined layer immediately below the layer of *Beggiatoa*. Amoebae were often very common in the bacterial plate.

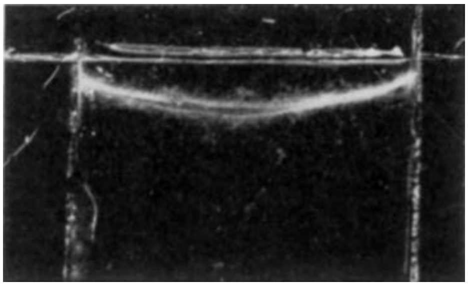

FIG. 83. A micro aquarium (5 days old) showing a bacterial plate (mainly *Beggiatoa*).

FIG. 84. Drawing made by tracing a micrograph of the bacterial plate in a micro aquarium (same experiment as shown on Fig. 83). Most conspicuous are the filaments of *Beggiatoa* and the ciliates *Cyclidium citrullus*, *Euplotes elegans* and *Holosticha* sp. Below the *Oscillatoria* filament (lower left) a *Plagiopogon loricatus* is seen. Bacteria (except *Beggiatoa*) are not shown.

The dominating ciliates in these experiments were *Euplotes elegans*, *Cyclidium citrullus* and a *Holosticha* sp. (Fig. 84) all confined to the bacterial plate. In some of the experiments, *Pleuronema coronatum* was found in the upper part of the containers in large numbers and sometimes also the *Beggiatoa*-eating *Sonderia schizostoma* and *Plagiopyla* sp. occurred in the bacterial plate and below it. Below the discontinuity layer *Metopus contortus*, *Plagiopogon loricatus* and *Cardiostomella vermiforme* occurred regularly in small numbers.

In these dark experiments the fauna and flora and its zonation remained rather unchanged until the energy of the medium was exhausted whereafter the zonation broke down and the flora and fauna died out. However, as shown above, the redox discontinuity layer moves downwards during the experiment and the zonation of microflora and fauna consequently follows it. The zonation was in accordance with that found in the field and the experiments show in a generalized way the priciple of the bacterial production based on the end products of anaerobic decomposition and their significance for the fauna. While a few diatoms were found throughout the dark experiments, photosynthetic bacteria were absent. Also blue-green algae did not grow though specimens introduced accidentally at the inocculation of the cultures lived for long periods. Some colourless forms, however, were often found in numbers among the *Beggiatoa* filaments.

If freshly inoculated cultures are exposed to light they develop like the dark experiments during the first 3-4 days; i.e. a well defined bacterial plate consisting of *Beggiatoa* and other colourless bacteria is formed and the same associated fauna develops. In the following days, however, green and purple sulphurbacteria and other photosynthetic bacteria become increasingly abundant, first at the redox discontinuity layer and later expanding downwards. The green bacteria always occur below the purple ones. Among the first occurring purple sulphurbacteria is a *Chromatium* sp. Diatoms occur in large numbers first above the bacterial plate where they may cover the glass of the micro aquaria completely and after some time they are found everywhere in the culture. All these photosynthetic organisms become more and more abundant to the expence of the colourless bacteria. After about 15 days the blue-green alga *Oscillatoria* and related forms become rather common and after about one month they are quite dominating.

Since the photoautotrophic organisms, in contrast to the chemoautotrophs, can remove reduced compounds under anaerobic conditions their activity destroys the original zonation of the chemical factors and consequently the zonation of the flora and fauna.

After some weeks both photoautotrophic bacteria and algae invade the agar. During the last stages of development mainly diatoms, blue-green algae (which may become rarer again), a colonial purple sulphurbacterium and the white sulphurbacterium *Achromatium* are found. The latter two probably utilized the deposits of elemental sulphur formed on the glass of the culture vessels, both in the light and the dark experiments. At this stage the solid nutrient medium had quite disappeared. Of course, theoretically the light experiments may continue infinitely as autotrophic systems after the energy originally added to the system in the form of the nutrient agar has been utilized. This was achieved after 1 to 2 months when the cultures were dominated by algae but the fauna was then rather poor.

The ciliate species found in the dark experiments also occurred in the light experiments and they showed a similar zonation as long as the chemical and bacterial zonations were intact. Some other species were characteristic of the light experiments. Large populations of *Diophrys scutum* often occurred above the redox discontinuity layer feeding on the diatoms. In one experiment *Peritromus* *faurei,* which mainly feeds on sulphurbacteria, was numerous. Its absence from the dark experiments may have been accidental. In one light experiment a small population of *Urostrongylum caudatum* was also found; like *Diophrys,* it feeds on small diatoms.

In another series of experiments the succession and zonation in sandy sediments were studied in the laboratory. These experiments were of two kinds. In one, clean sand, with organic material, was inoculated with field samples; in the other, the organisms in smaller or larger amounts of natural sands were followed in the laboratory under various conditions.

The following experiment is of the first type. In a plastic tray measuring 42 × 52 cm a 0.5 cm thick layer of 300 g *Zostera* leaves was covered with clean sand to 3 cm height which again was covered with a 5 cm thick layer of filtered sea-water. The tray received light, ca. 3000 Lux, from a daylight mercury lamp suspended about 0.5 m above it. The water was slowly bubbled with air during the experiment and the temperature varied between 19 and 23°C. The artificial sediment was inoculated with 10 cm^3 sand from Nivå Bay. Every second or third day measurements were made of Eh of the water and the sediment and the pH and the oxygen content of the water. On the same occasions samples of the water and sediment corresponding to 1 cm^2 surface area were extracted for microfauna. From time to time small samples of water and sediment were observed under the compound microscope.

Results are shown in Figs 85-86. As could be expected the Eh of the sediment decreased markedly within the first days of the experiment to reach values around —150 mV after 10 to 15 days. In experiments of this sort there is always an immediately lowering of the Eh if fresh plant leaves are used. This is due to the release of respiratory enzymes of the plant leaves. Thus the Eh of the water was somewhat below +200 mV immediately after adding them. This effect was of too short duration to be seen in Fig. 85.

After the first 15 to 20 days, the Eh of the sediment slowly increased during the rest of the experiment to values between —100 and 0 mV. The system was therefore far from completely oxidized after about 4 months when the experiment was terminated.

In the water Eh also decreased somewhat during the first days, then increased again. Fifteen to twenty days after the beginning of the experiments H_2S was liberated into the water and the Eh decreased to —80 mV in spite of continuous aeration. The presence of H_2S in the water resulted in a rich growth of the sulphurbacterium *Macromonas* (Fig. 86) followed by other white sulphurbacteria (mainly *Beggiatoa*) and cyanophyceans. The filaments of *Beggiatoa* and of blue-green algae formed a dense carpet on the surface of the sediment. This carpet formed a well defined layer between the water, which remained oxidized during the rest of the experiment, and the reduced sediment.

The oxygen content of the water decreased strongly during the first days of

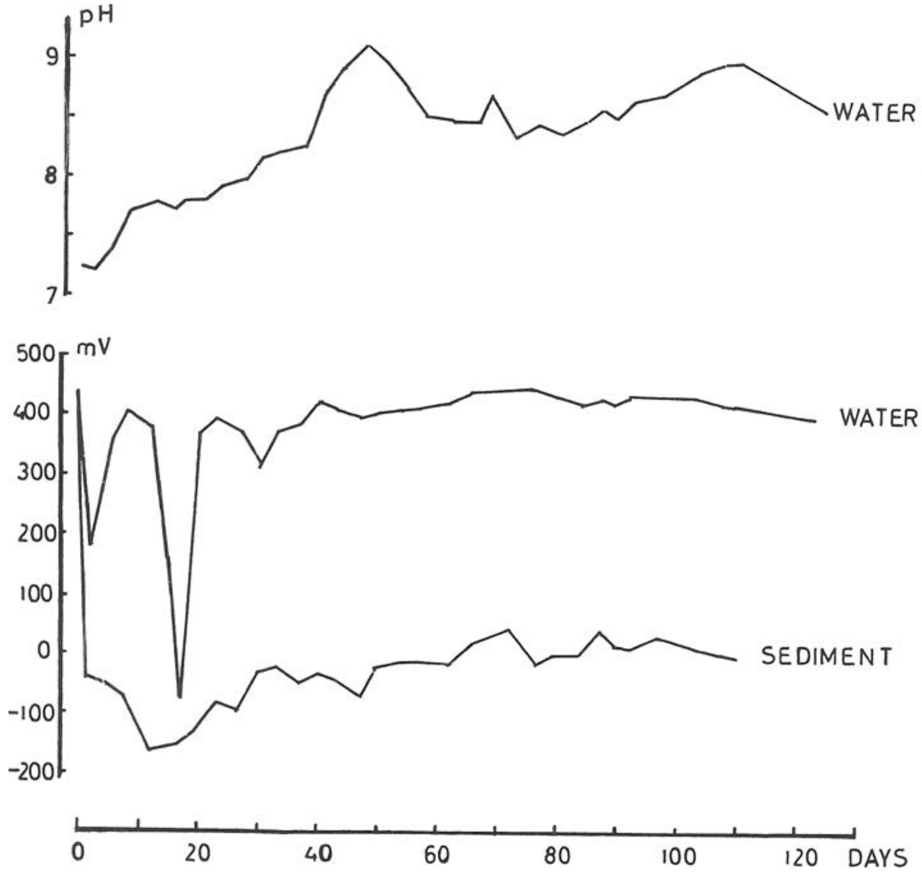

FIG. 85. The development of Eh and pH in the experiment based on sand and decomposing *Zostera.*

the experiment to values around 30 % saturation during the period when the water was reduced. This relatively high value in the presence of free H_2S was due to the continuous aeration. After the formation of the bacterial and algal carpet the oxygen content rose to values of 70 to 90 % sat. and remained so during the rest of the experiment. The oxygen content of the sediment was not measured; it seems, however, safe to suppose completely anaerobic conditions in the sediment during the experiment.

The pH of the water increased steadily during the first 60 days from initial values around 7.2 to values around 9 after 50 days reflecting the increasing numbers of cyanophyceans and later of diatoms. After about 50 days these two groups of algae dominated the sediment surface and the value of pH remained between 8.5 and 9. *Beggiatoa* and some other white sulphurbacteria remained common in the surface layers throughout the experiment. Purple sulphurbacteria and other

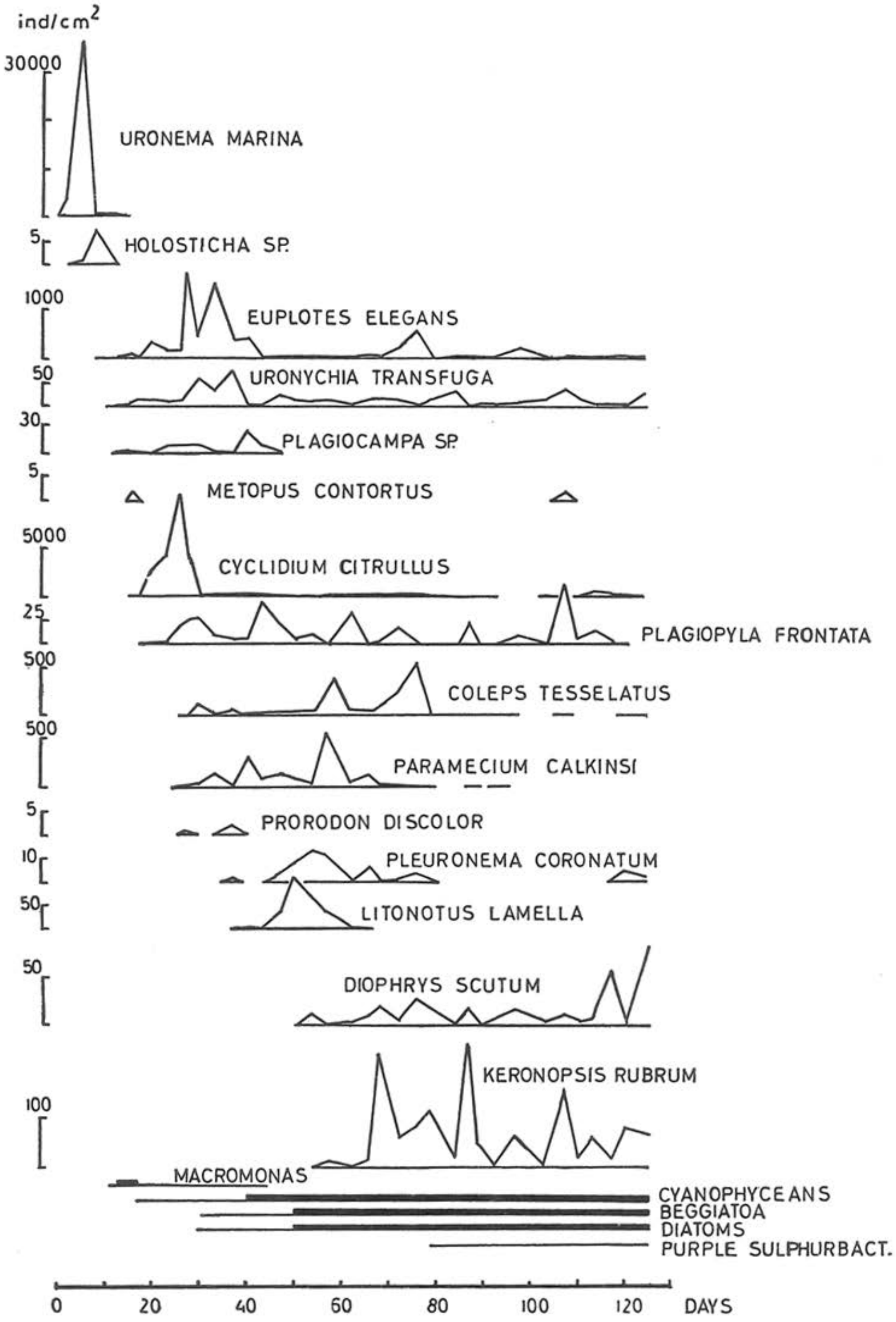

FIG. 86. Faunal and floral succession in the experiment referred to in Fig. 85.

photoautotrophic bacteria, though present after about 20 days and common during the last part of the experiment, never dominated. Possibly this was due to the colour of the artificial light used which may not have been appropriate for these organisms.

Amoebae became abundant after 30-40 days and remained common through the rest of the experiment; up to 443/cm^2 were counted on one occasion. Dinoflagellates and other flagellates were present most of the time though always in small numbers. Metazoans were rare, probably due to an incomplete inoculation of appropriate forms. One to three turbellarians (*Provortex* sp.) per cm^3 were found a couple of times after 70 days and a few nematodes and ostracods were likewise found occasionally.

The occurrences of the ciliates are shown in Fig. 86. Only forms which occurred in very few specimens and a few unidentified animals have been omitted. As will be discussed later in this chapter the diversity of species was relatively low and the succession of species therefore relatively easy to interprete.

As in the experiments with oxidized sands (pp. 146-148) a large population of *Uronema marina* appeared within the first few days (36,000/cm^2 after 5 days). This population soon declined, and it disappeared completely after 13 days. It was followed by a *Holosticha* sp. which, however, only occurred in very small numbers. The populations of these two species were based on the growth of decomposing bacteria.

The anaerobic and reducing conditions in the sediment brought about by heterotrophic bacteria resulted in a rich growth of chemoautotrophic bacteria (mainly sulphurbacteria) at the sediment surface and ciliates feeding on these bacteria dominated the fauna for a long period; *Euplotes elegans,* which for a short period occurred in numbers of about 1500/cm^2 and which mainly fed on *Macromonas,* and *Cyclidium citrullus* (up to 10,000/cm^2) which fed on small unidentified bacteria. The experiments described on p. 149 indicate that *C. citrullus* may thrive on *Thiobacillus*. Other forms which occurred during this period of the experiment and which mainly or partly feed on sulphurbacteria were *Plagiopyla frontata, Metopus contortus, Pleuronema coronatum,* and *Paramecium calkinsi.* The last mentioned species is not a sediment dweller. It shows, in contrast to the other forms, a pronounced negative geotaxis and was always found in the water above the sediment.

A number of carnivorous ciliates also made their appearance: *Litonotus lamella* and *Uronychia transfuga. Coleps tesselatus* is a carnivore as well as a histophage. The role played by *Plagiocampa* sp. is uncertain as discussed on p. 148. A few specimens of *Prorodon discolor* appeared after about 40 days; it feeds on corpses and on dinoflagellates.

In connection with the appearance and bloom of diatoms after about 50 days, the diatom consuming ciliates *Diophrys scutum* and *Keronopsis rubrum* appeared and they remained numerous through the last part of the experiment.

The diversity of the species in this experiment was rather small when compared to natural sediments and the experiments described below. Thus forms feeding on cyanophyceans, filamentous sulphurbacteria and large diatoms were lacking in spite of a good supply with these food items, and forms specialized on photoautotrophic bacteria were also absent. A characteristic sulphide fauna did not develop.

Nevertheless, the experiment shows the characteristic succession based on organic material in sediments, the sequence of the dominating processes being aerobic decomposition quickly followed by anaerobic decomposition, chemosynthesis and photoautotrophy, and this sequence is reflected in occurrence of the different ciliate species. This experiment will be discussed further in connection with the experiments described below.

A number of experiments were made in which containers with natural sediments were kept in the laboratory. One of these experiments will be described in detail.

In two glass dishes reduced sulphide-containing sand from Nivå Bay was placed to a depth of 1.5 cm and covered with a 2 cm layer of filtered sea-water of 19‰ S. One of the containers was close to a window facing west; the other one was kept constantly in the dark. Measurements of pH and Eh, quantitative samplings of the microfauna and qualitative observations on the microflora were carried out as in the experiments described above.

In the light experiment (Fig. 87) the water became oxidized within a few weeks due to the activity of white sulphurbacteria like *Macromonas* and *Thiovolum*, which formed white clouds in the water, and later by the development of a carpet of *Beggiatoa* on the sediment surface. The white sulphurbacterium *Achromatium* is the latest one to occur. The *Beggiatoa* carpet became slowly invaded by cyanophyceans, diatoms and purple sulphurbacteria. After about 50 days the Eh of the sediment had increased to values around +300 mV. The pH of the water increased from initial values around 7 to values around 8 in later third of the experiment due to photosynthetic activity.

The ciliate fauna may be classified into several ecological groups. One is constituted by the species confined to reduced sulphide containing environments (*Caenomorpha, Saprodinium, Myelostoma, Sonderia, Parablepharisma, Cristigera, Metopus, Plagiopyla*) of which many feed on sulphurbacteria. This fauna declines and disappears as the sediment becomes oxidized. Other forms, which also feed mainly or partly on sulphurbacteria, are not confined to reduced conditions, i.e. *Euplotes elegans* and *Pleuronema coronatum*. *Blepharisma salinarum* which dominantly feeds on purple bacteria is confined to oxidizing environments (Chapter 7, Fig. 78).

The important diatom consumers were *Strombidium sauerbrayae, Frontonia marina* and *Diophrys scutum*, but also *Euplotes elegans* and *Pleuronema coronatum* eat small diatoms. Forms eating flagellates and to some extent other ciliates are

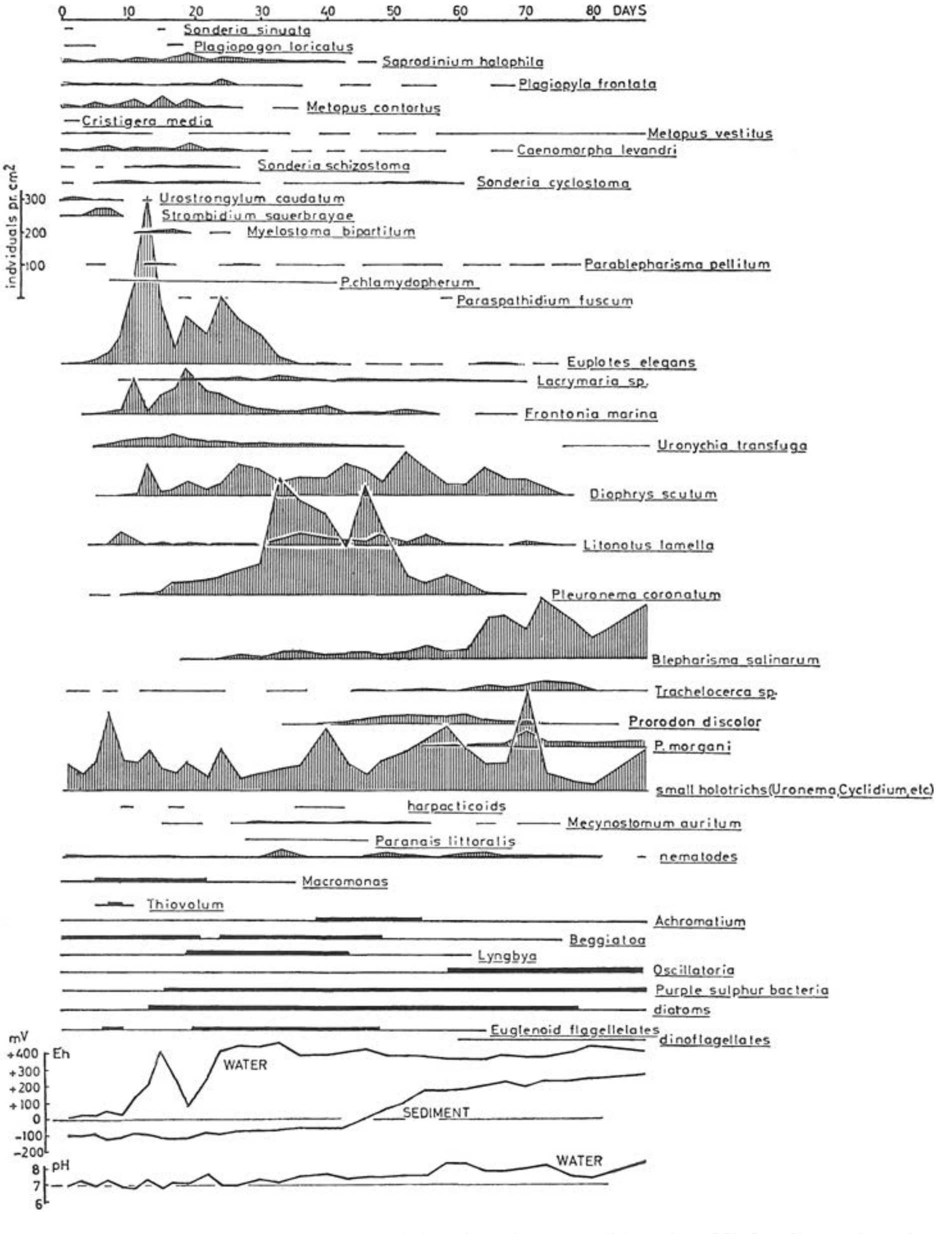

FIG. 87. Succession of fauna and flora and the Eh and pH conditions in a Nivå sediment kept in the laboratory. Light experiment.

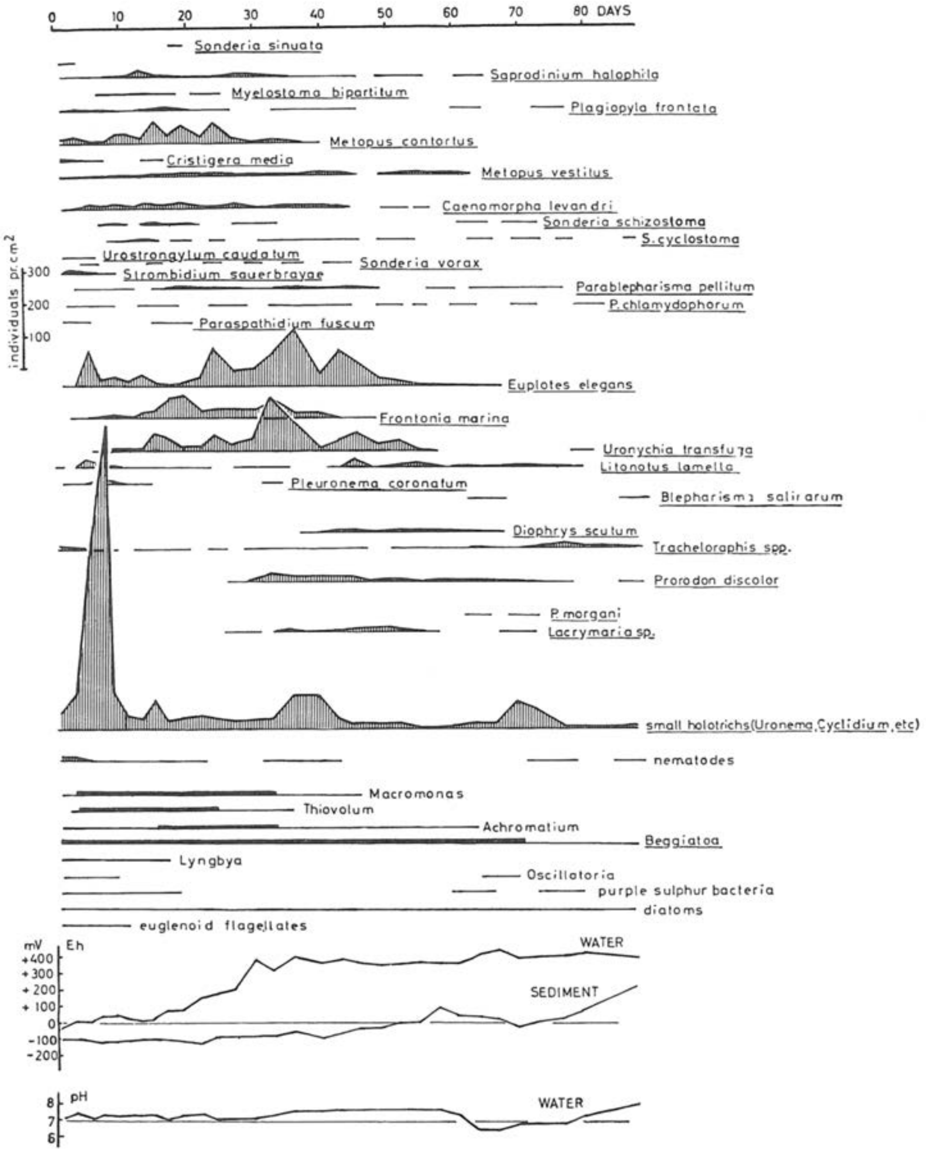

FIG. 88. Same as Fig. 87. Dark experiment.

the *Prorodon* spp and *Tracheloraphis* sp. which all occurred late in the succession. The ciliate predators were *Litonotus lamella, Lacrymaria* sp. and *Uronychia transfuga*.

The group "small holotrichs" in Figs 87-88 represent mainly bacteria feeders.

Fig. 89 shows the total number of ciliates during the experiment and the relative importance of various food items for the ciliates. It can be seen that the largest numbers of ciliates occurred while the sediment was still reducing whereafter the numbers declined.

The sequence of the most important food items is the same as mentioned previously for other experiments; heterotrophic bacteria, chemoautotrophic bacteria and photoautotrophic organisms. It is remarkable that the diatoms declined in numbers after the sediments had became oxidized, and this was reflected in the decline of numbers of diatom-consuming ciliates. The abundance of purple sulphurbacteria remained constant until the end of the experiment. This must be due to an ability of these bacteria to utilize elemental sulphur since H_2S was not present after about 60 days (cf. the Eh of the sediments).

The experiment was not followed in detail after the 88th day, but during the following months filamentous green algae slowly took over. The ciliate fauna became poor while some metazoans (harpacticoids, rotifers) became more numerous.

The interpretation of the experiment is complicated by the fact that in reality two systems are present; the supernatant water which oxidized rather early and the sediment which oxidized late in the experiment. Nevertheless, it can be seen that the succession of microbial processes and of the species shows a resemblance to the vertical zonation of sediments. Early in the experiment as well as at greater depths in the sediment, conditions are reducing and a special sulphide fauna is found. Later in the experiment or closer to the surface of the sediment around the redox discontinuity layer, mainly species eating chemoautotrophic bacteria are found and latest or closest to the surface photosynthetic organisms and animals feeding on them are dominating. This principle can also be exemplified by studying single species. Thus among the species eating purple bacteria the *Sonderia* spp and *Parablepharisma pellitum*, which require reducing conditions, appear early in the succession or deeper in the sediment, while *Blepharisma salinarum*, which requires more oxidizing conditions, appears later in the succession and closer to the sediment surface.

Finally it should be mentioned that even though the fauna of this experiment is much more diverse than that described previously (also metazoans were much better represented), the sequences of the important species common to the two experiments (*Euplotes elegans, Plagiopyla frontata, Uronychia transfuga, Pleuronema coronatum, Diophrys scutum*) are identical.

The results of the dark experiment are shown in Figs 88 and 90. At the beginning this experiment resembled the light experiment in most respects as to

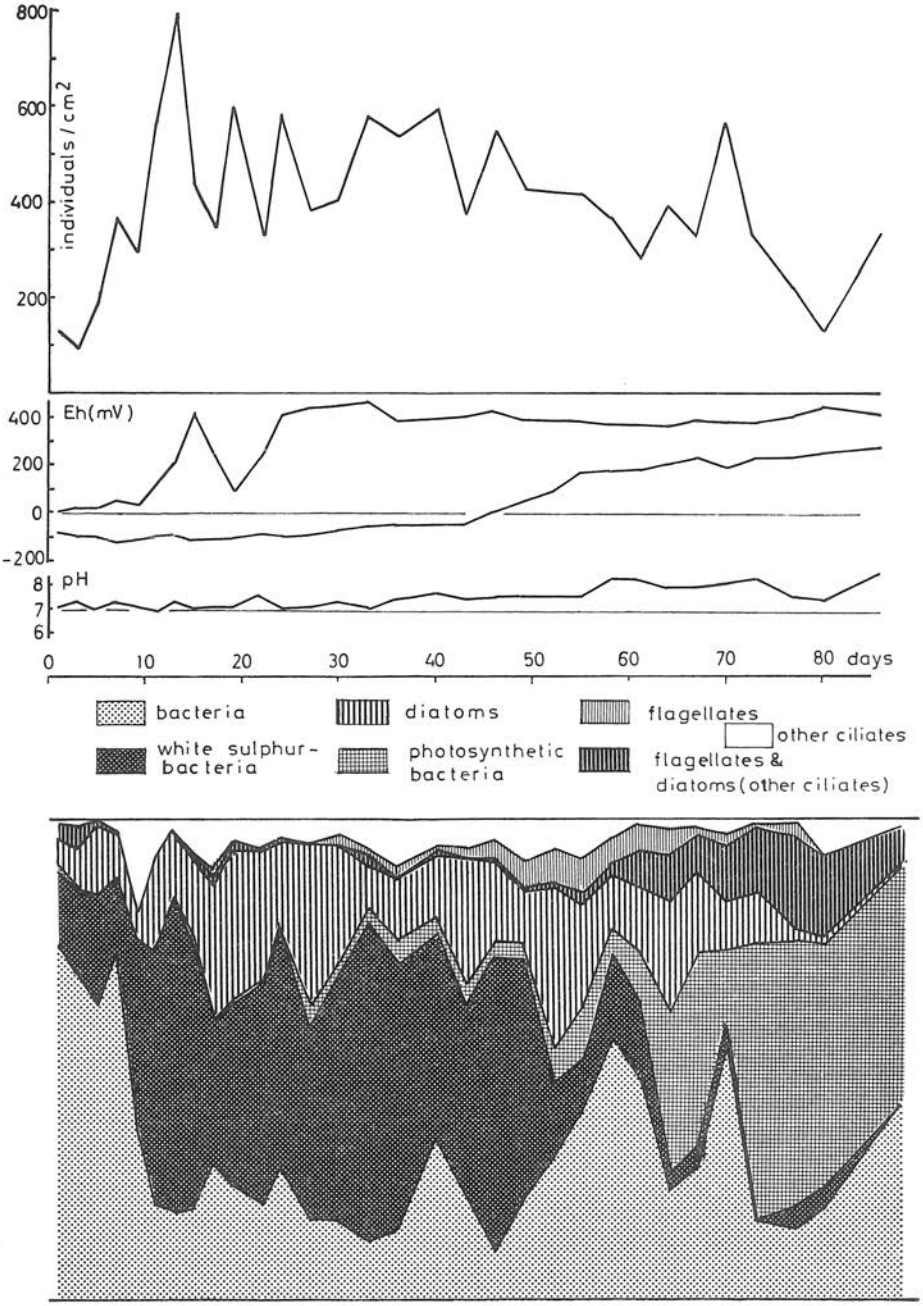

FIG. 89. The total numbers of ciliates and their food spectrum in the experiment referred to in Fig. 87 (light experiment).

fauna and redox conditions. However, the lack of photoautotrophic activity results in —1. Slower oxidation of both water and sediment, which took about twice as long as in the light experiment, and —2. Absence or poor development of herbivorous ciliates and ciliates eating photoautotrophic bacteria. However, small diatom populations were found throughout the experimental period and populations of some diatom eating forms, e.g. *Frontonia marina, Diophrys scutum,* occurred, though in much smaller numbers than in the light experiment. Also other, normally photosynthetic organisms (cyanophyceans, purple sulphurbacteria), occurred in very small numbers and consequently a small number of ciliates eating purple sulphurbacteria also occurred in the dark experiment. It is interesting to note that the populations of ciliate species living on photosynthetic organisms occurred in the same sequence in the dark and in the light experiment.

Fig. 90 shows that the total numbers of ciliates decrease strongly as the sediment becomes oxidized. The reducing properties of the sediment are thus a qualitative expression of the presence of potential energy which can be utilized by the system. The graph showing the relative importance of various food items throughout the experiment demonstrates that protophytes and photoautotrophic bacteria play a small role. The relatively high numbers of predators remain unexplained. It should be mentioned that the percentages which were calculated from the quantitative samples are not very reliable after 50-60 days in the dark experiment since they are based on small numbers of animals only.

Before concluding the description of these experiments it should be mentioned that the vertical zonation of the microfauna and the redox-profile was studied in a number of artificial and natural sediments kept in the laboratory for shorter and longer periods. In all cases the vertical zonation resembled that found in field samples and could be correlated with the redox-profile.

The dynamics of ciliate populations in succession experiments were studied in detail by Bick (1964, 1966). Essentially, my findings agree with those of Bick and this aspect will therefore only be treated briefly here.

As found by Bick the growth of many of the ciliate populations is initiated by an exponential growth phase as is the case in pure cultures (Fenchel, 1968b). After this phase the population either remains rather constant for some time or oscillates. Finally the population declines and dies out. Examples can be seen in Figs 81 and 86-88.

In Fenchel (1968b) the growth rates of ciliate populations in pure cultures were studied. Attempts were made to obtain maximum growth rates so that the reproductive potential of different species could be compared.

It was found that under optimal conditions the "intrinsic rate of natural increase" is dependent on the body size of the species in question so that the smallest species had the shortest generation time (*Uronema marina,* 2.5 hours at 20°C) and the largest species had the longest generation time (*Condylostoma patulum,* 46 hours at 20°C). In general it was found that $T = 0.0065 \times V^{0.44}$ (at 20°C),

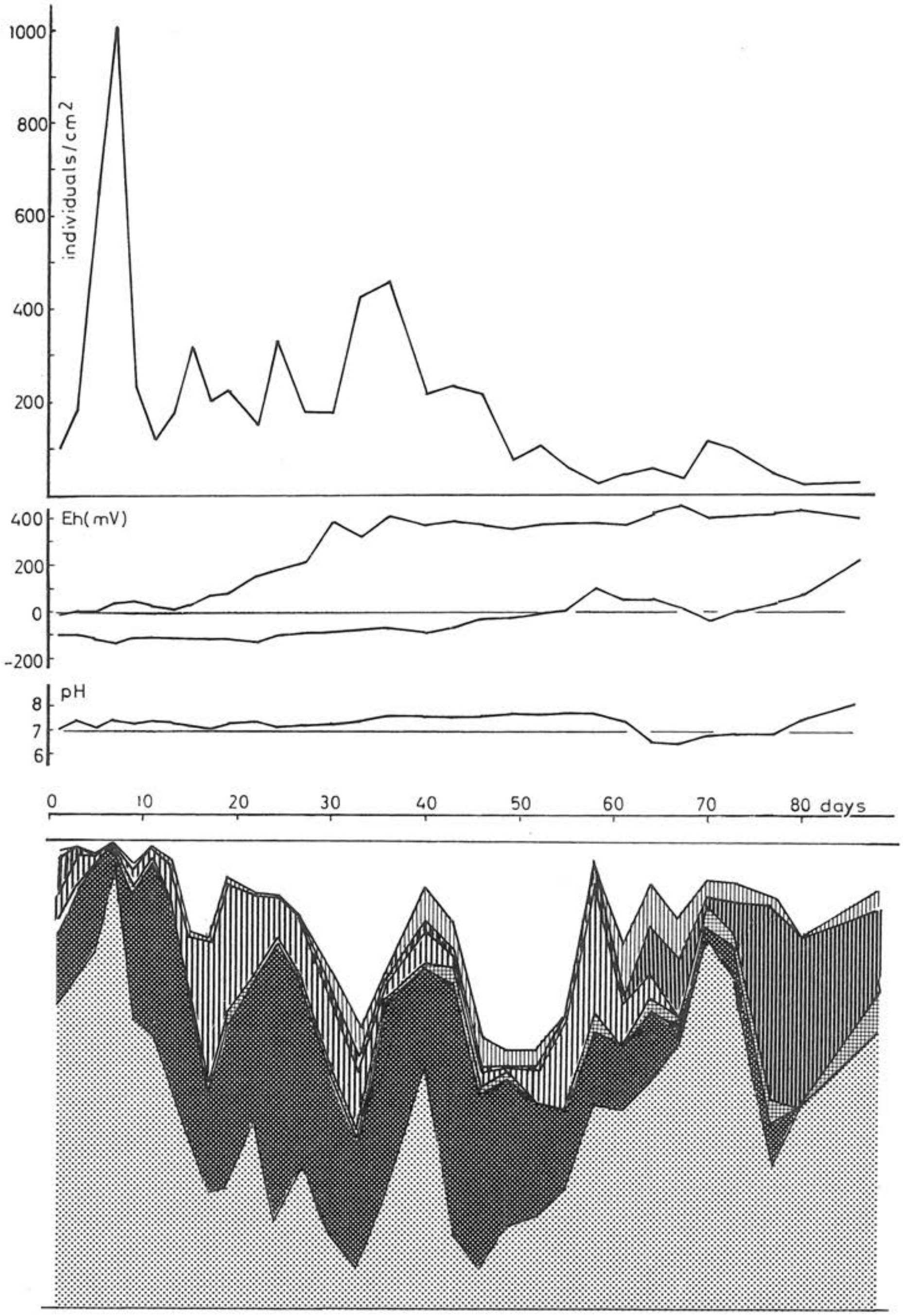

FIG. 90. The total numbers of ciliates and their food spectrum in the experiment referred to in Fig. 88 (dark experiment).

when T is the generation time in hours and V the cell volume in μ^3. Q_{10} was found to vary between 2.3 and 3.5 in the different species.

It would be of interest to see, to what extent this growth potential was reached in the populations of the succession experiments since these are more comparable to field conditions than are pure cultures. Plotting the exponential growth phases on semilog paper showed that the growth rates in mixed cultures rarely attained the potential value.

Two populations of *Diophrys scutum* showed a generation time of 25 and 35 hours, respectively, at 15° in a mixed culture. The former value corresponds rather closely to the rate found in pure cultures. In most cases, however, generation time was considerably longer than could be expected from the findings in pure cultures. Thus, populations of *Euplotes elegans* had generation times of 19, 38, 42, and 48 hours, respectively, during the exponential growth phase in mixed cultures at 17-20°C. The related *E. vannus,* which is of the same size as *E. elegans* and which was studied in pure cultures, has an optimum generation time of less than 10 hours at the same temperature.

Uronema marina, which under optimum conditions divides every 2.5 hours at 20°C had generation times of 12 to 16 hours in the succession experiments. Also many other examples from succession experiments show that in most cases the growth rates of the ciliate populations were lower than the maximum rate.

In Fenchel (1968b) it was shown that populations of *Aspidisca angulata* kept in cultures with different concentrations of food all showed exponential growth but at different rates. That the rate of the exponential growth in mixed cultures is lower than the potential rate is probably due to suboptimal food conditions.

Bick (1966) demonstrated that conjugation epidemics of ciliates often occur at the end of an exponential growth phase. This was also observed during the present investigation for populations of *Frontonia marina, Euplotes elegans, E. vannus,* and *Metopus contortus.*

The difference in faunal diversity between experiments based on natural sediments and those based on artificial sediments inoculated with small field samples may have two explanations. Possibly the organic material added to the artificial sediment was devoid of some micronutrient necessary for certain microorganisms and consequently for some representatives of the fauna. The other explanation is based on the role of large carnivores. It is certain that many more species were contained in the inoculate than those actually found in the succession experiments with artificial sediments. It is, however, possible that a few larger species, for example some metazoans, may have been absent in the inoculate. As shown by Paine (1966) in the field and by Slobodkin (1964) in laboratory experiments, predators feeding on more than one prey species tend to increase the diversity of a community by "invalidating the theorem of Gause".

More experiments and more detailed analysis are necessary to clarify the reason for the different diversities in the different types of experiments.

The following conclusions can be drawn from the experiments described in this chapter:

—1. A characteristic succession of microflora and fauna occurs as a result of decomposition of organic material in sediments. The species occurring in the succession and their sequence are dependent on whether anaerobiosis develops and on the presence or absence of light.

—2. When anaerobiosis develops the sequence of species resembles the vertical zonation of species in the sediments, the species occurring early in the succession being those occurring deepest in the sediments.

—3. The ciliates play a predominant role among the animals as consumers of bacteria and protophytes in such successions, probably due to their high reproductive potential.

—4. Chemosynthesis may play a predominant trophic role.

—5. The growth rates of the populations are lower than their potential growth rates in pure cultures.

10. QUANTITATIVE AND QUALITATIVE SIGNIFICANCE OF THE MICROFAUNA AND ESPECIALLY OF THE CILIATES IN BENTHIC ECOSYSTEMS

Since the classical works on microfauna by Krogh & Spärck (1936) and by Mare (1942) several authors have tried to compare the quantitative importance of the benthic micro- and macrofauna. Parts of this literature, which has recently been reviewed (Thorson, 1966), are difficult to evaluate. As discussed in the introduction to this paper, terms like macro-, meio- and microfauna have no precise biological meaning except with reference to certain sediments. Many workers have, following Mare (op. cit.), defined the meiofauna as the animals passing through a sieve with an arbitrarily chosen mesh size. Different authors have used different mesh sizes, and it is often rather a question of shape and behaviour than of size whether an animal will pass the sieve or not. Many authors have not defined the lower size limit of the meiofauna. Protozoa have, with the possible exception of foraminifera, always been disregarded, probably also rotifers, chaetonotoids and smaller turbellarians, and most of the comparisons are thus without meaning except for confirming the fact that when standard methods for quantitative work on marine benthos are used at least 90 % of the animals are lost.

However, several mainly recent investigations (e.g. Muus, 1967; Renaud-Debyser & Salvat, 1963; Wieser, 1960) have, due to the use of adequate methods and sufficient taxonomic knowledge, given a reliable picture of the quantitative composition and importance of the metazoan microfauna. Further improvements of our understanding of the role played by the microfauna have resulted from measurements of total bottom respiration and of the respiration of single members of the microfauna (Wieser & Kanwisher, 1961; Carey, 1967).

Only the papers of Fenchel (1967) and Fenchel & Jansson (1966), which both are based on part of the material treated in the present paper, give reliable estimates of the quantitative importance of other protozoa than foraminifera.

Before discussing the relative importance of different animal groups it should be mentioned that the total fauna, comprising all sizes of animals, has probably only a modest share in the metabolic activity of the sea bottom when compared to the bacteria. As discussed in Chapters 5 and 9 the utilization of dead organic matter by animals is dependent on the production of bacteria.

Quantitative evaluations of bacteria are uncertain; generally they are too low since only a restricted number of physiological types will grow on the various culture media used for quantitative evaluation. For example the anaerobes and the chemoautotrophs are very rarely considered in quantitative evaluations of bacteria. Mare (1942) found 3.5×10^{11} bacteria per m^2 corresponding to 0.4 g/m^2 (wet weight) in mud. ZoBell (1963b) states that numbers of bacteria vary between 10 and 10^9 per g sediment in various types of sediment, according to the literature. Most recently Westheide (1968) found between 1×10^6 and 1.4×10^7 bacteria per cm^3 sediment in a sandy wadden.

Under optimal conditions bacteria have several generations per hour but the division rate in nature is poorly known and apparently varies considerably (ZoBell, 1963b). Wieser & Kanwisher (1961) and Carey (1967) agree that bacteria are probably responsible for the greater part of the community respiration.

In the present paper animals weighing more than 10^{-4} g are, in accordance with Muus (1967), considered as macrofauna which thus includes forms such as oligochaetes which are mostly lost by sieving through 1 or 2 mm sieves. Metazoans weighing less than 10^{-4} g will be called metazoan microfauna and comprise nematodes, turbellarians, harpacticoids, ostracods, gastrotrichs, tardigrades, rotifers, archiannelids and juvenile stages of larger animals. As a third group ciliates are considered. Other protozoan groups were only incompletely quantified. Amoebae play a modest role; more than $10^5/m^2$ were rarely found. Foraminifera were not studied as this would have required another technique. Hansen (1965) found between 5 and 50 individuals/cm^2 in the Hornbæk Bay in the area sampled during the present investigation. Phytoflagellates were discussed in Chapters 5 and 6. Large dinoflagellates occur in numbers up to 6×10^7 per m^2; 0.5-1 $\times 10^7$ are often found. Euglenoids are very numerous in sulphureta, but have not been counted. In other biotopes they play a quantitatively smaller role. Neither were smaller flagellates, including the very small zooflagellates, enumerated. When the results given below are evaluated it should be kept in mind that the extraction method employed is not equally efficient for all animal groups (Chapter 2, p. 21).

In fine sublittoral sands of median grain sizes between 125 and 250 μ, ciliates are found in numbers varying between 5×10^6 and $3 \times 10^7/m^2$ corresponding to

0.3 to 1.5 g wet weight/m^2. Numbers around 10^7/m^2 predominate. Micrometazoa are usually found in numbers varying between 10^5 and 10^6 per m^2 corresponding to 0.5 to 6.5 g/m^2. Nematodes are most frequent, usually constituting about 50 % of the meiofauna in these sediments though turbellarians and gastrotrichs may also be abundant (see Figs 11-13). As shown in Chapter 3 the numbers of interstitial metazoa (excluding the nematodes) and of ciliates decrease with decreasing grain size and in sands finer than about 100 μ nematodes are quite dominating the microfauna.

In non-capillary sediments, practically only nematodes represent the microfauna. In the detritus layers covering such sediments a rich and varied microfauna may be present, in which the ciliates, however, play a very modest role even from a numerically point of view (see Chapter 6, pp. 128-130).

In coarser sands with median grain sizes larger than 250 μ, the ciliates play a decreasing and the micrometazoans an increasing role with increasing grain sizes. In medium and coarse sands ciliates occur in numbers between 5×10^5 and 2×10^6/m^2. Here nematodes play a modest role in contrast to most other metazoan groups.

In "estuarine" sands ciliates generally occur in numbers between 5×10^6 and 2×10^7 corresponding to weights between 0.2 and 2.3 g/m^2. The higher maximum weight of ciliates in this type of sediment as compared to "fine sublittoral sands" is due to a greater percentage of large ciliates. Micrometazoans are often very numerous in estuarine sands; 5×10^5-2×10^6/m^2 corresponding to 1.5-8 g/m^2 being common though smaller numbers are often found in winter. Nematodes and harpacticoids are mostly dominating. As in sublittoral sands, finer grain sizes are correlated with a greater relative and absolute importance of ciliates.

Sulphureta have the highest concentrations of animals (i.e. more than 10^7 ciliates per m^2 and up to 5×10^6 micrometazoans per m^2).

The relative quantitative importance of macrofauna, metazoan microfauna and ciliates were studied in more detail in three sandy localities.

One of them is the fine sand on 10 m depth off Ålsgårde in the Øresund. Two 0.1 m^2 samples taken in May 1968 with a Smith-McIntyre bottom grab were sieved through 0.5 mm meshes and the macrofauna sorted out and counted. The meiofauna was extracted from four 3.5 cm^2 core samples by the alcohol method of Jansson (see Chapter 2). The enumeration of ciliates was based on a 1 cm^2 sample from May 1967. The individuals of each macrofauna species were counted and the animals weighed on an analytical balance. The weight estimates of the micro metazoans are based on the literature and the biomass of the ciliates was calculated for each species (see Chapter 2).

In Fig. 91 all major taxonomic groups are arranged according to number of individuals and biomass per m^2. Fig. 92 shows the quantitative importance of different weight categories. It is remarkable that the size category 10^{-5}-10^{-4} g seems to be lacking in the sediment. Since the animals belonging to the category

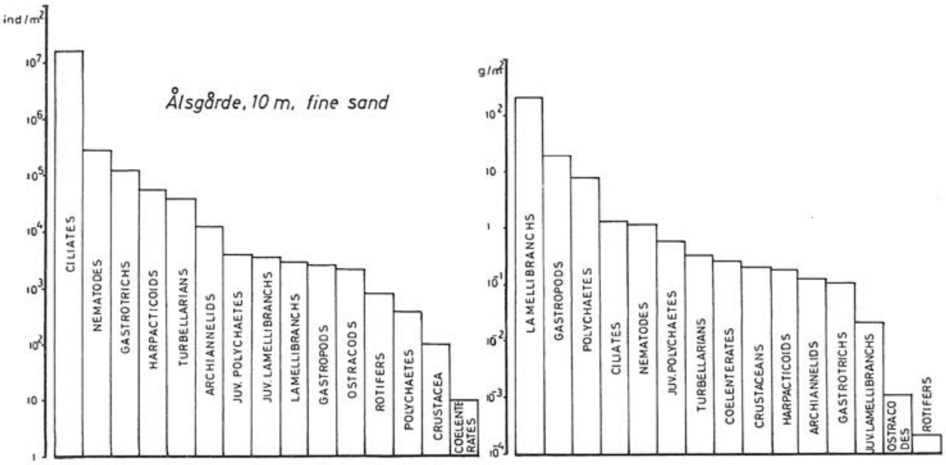

FIG. 91. The quantitative occurrence of the different animal groups on 10 m depth off Ålsgårde in summer.

10^{-4}-10^{-3} g were sufficiently well represented in the meiofauna samples it cannot be assumed that the animals belonging to the weight category 10^{-5}-10^{-4} were lost due to a hiatus between the two extraction methods used for the macro- and the metazoan microfauna, respectively.

The lack of the size group may therefore be real. The explanation is probably that there is a hiatus between the macrofauna which is large enough to burrow in the sand and the microfauna which is small enough to live interstitially the median grain size of this locality being around 175 μ.

The macrofauna is extremely rich in this locality especially due to a great

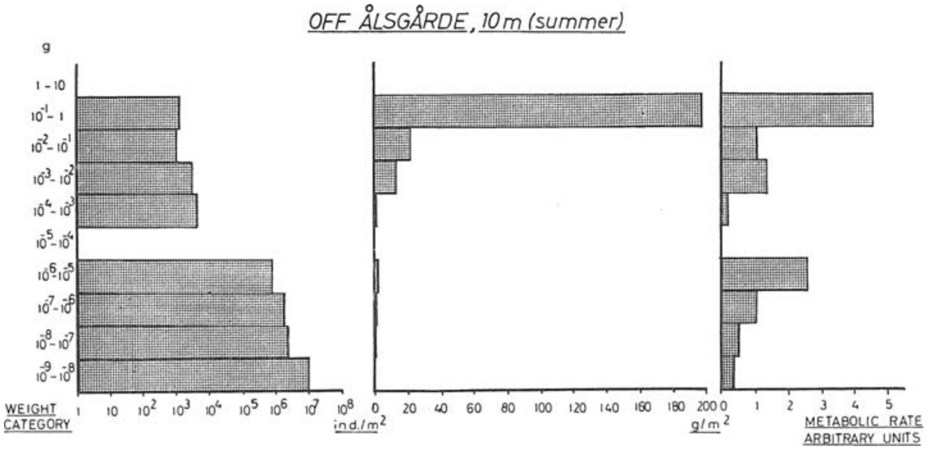

FIG. 92. The numbers, biomasses and relative metabolic rates of different size groups of animals in the 10 m locality of Ålsgårde.

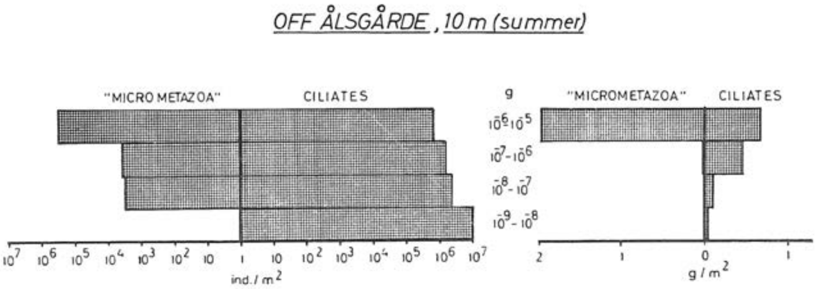

FIG. 93. The numbers and biomasses of different size groups of micro metazoa and of ciliates (10 m, off Ålsgårde).

number of molluscs (*Hydrobia, Macoma balthica, Mya arenaria, Mysella bidentata, Cardium edule*) and large numbers of small polychaetes, mainly spionids, so that a total of about 10^4 individuals or 230 g wet weight is found per m^2. The micro metazoans are represented by about 4×10^5 individuals per m^2 corresponding to about 2 g/m^2. As seen in Fig. 93 the great majority of the micro metazoans belong to the size group 10^{-6}-10^{-5} g. The ciliates were found in numbers of 1.5×10^7/m^2 representing about 1.4 g/m^2. The weight of the individual ciliates vary between 10^{-9} and 10^{-5} g.

It is well known that the metabolic rate per unit weight increases with decreasing body weight when animals of different sizes are compared. The relation between body weight and metabolic rate is known to be closely approximated by the formula; $\text{cal/h} = K\sqrt[3]{W^2}$ or $\text{cal/W}\cdot\text{h} = K\sqrt[3]{W^{-1}}$ when W is body weight (Zeuthen, 1947). Thus, a decrease in body weight by a factor 10^3 means an increase in metabolic rate per unit weight by a factor 10. The relation does not, however, hold strictly throughout the animal kingdom.

Nevertheless, by applying it we may get a crude picture of the relative metabo-

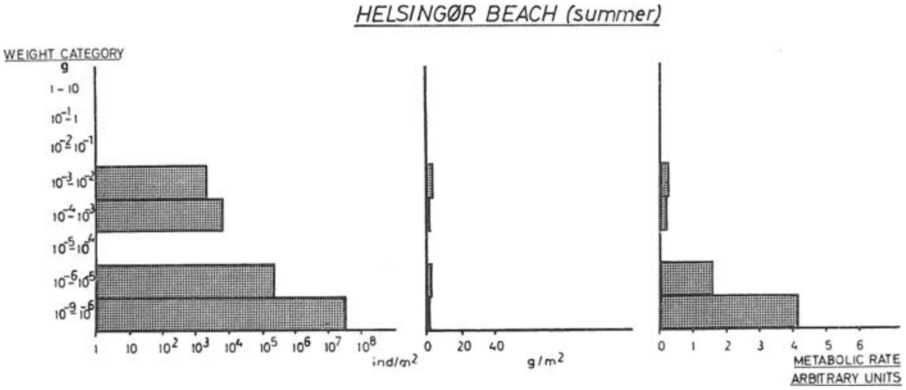

FIG. 94. The numbers, biomasses and relative metabolic rates of different size groups of animals in the locality off the Helsingør Beach.

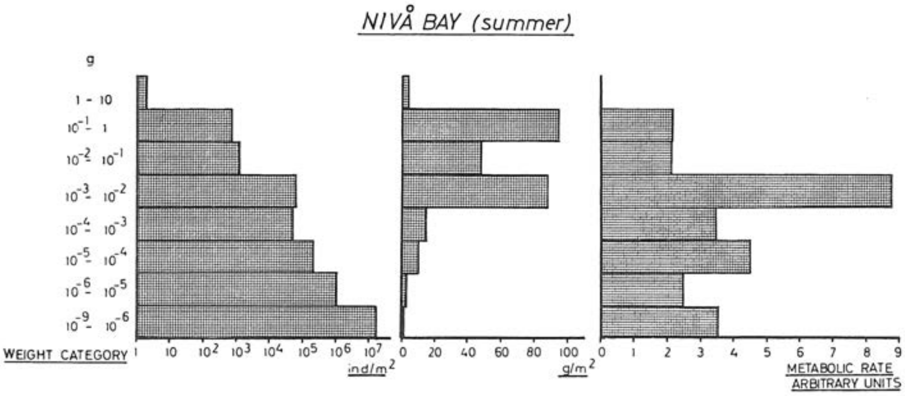

FIG. 95. The numbers, biomasses and relative metabolic rates of different size groups of animals in the Nivå Bay.

lic importance of the different weight categories of animals. This is shown in Fig. 92.

If we extract the ciliates from the weight categories 10^{-9}-10^{-5} we find that the relative importance of macro metazoa: micro metazoa: ciliates is approximately 1: 40: 1500 when numbers of individuals are considered, 190: 1.5: 1 when weights are considered, and 3.7: 1: 1.4 when relative metabolic rate is considered; i.e. the microfauna is responsible for about 40 % of the animal respiration and the ciliates for about 25 %.

In the locality off the Helsingør Beach large invertebrates are extremely rare, nearly only haustoriid amphipods and oligochaetes represent the macrofauna. The relative importance of different weight categories are shown in Fig. 94. The weight categories between 10^{-9} and 10^{-6} have been grouped together on the graph; they comprise almost exclusively ciliates. The relative metabolic rate was calculated for each size group and then added.

Also here a gap between animals larger than 10^{-4} and those smaller than 10^{-5} g is apparant (the median grain size of the sediment is about 200 μ).

The proportion macro metazoa: micro metazoa: ciliates is 1:28 :3000 when numbers are considered, 3.9:1.6:1 when weights are considered, and 1:3:8 when the metabolic rates are considered; i.e. in this locality ciliates play a predominant role.

The third example is the Nivå Bay (Fig. 95). Data on the macrofauna are taken from Muus (1967) who worked in the same locality studied in the present paper. Muus also enumerated the metazoan microfauna and his results are in accordance with mine. Here there is apparently no hiatus between the macro and the microfauna. This is in accordance with the fact that a rich meiofauna lives not interstitially, but on the surface of the sediments among algae and detritus, a biotope practically absent in sublittoral sands. As in Fig. 94, animals weighing between

10^{-9} and 10^{-6} (predominantly ciliates) have been grouped together in one weight category in Fig. 99.

Due to the huge numbers of oligochaetes, small polychaetes and specimens of the gastropod *Hydrobia*, the macrofauna is extremely rich in individuals (order of magnitude: $10^5/m^2$. *Hydrobia* (weight category: 10^{-3}-10^{-2} g) alone has a biomass of 50-90 g/m² thus making up 25-50 % of the total animal biomass of the locality.

The proportion macrofauna: meiofauna: ciliates is 1:10:50 (individuals), 170:10:1 (weights) and 4:2:1 (metabolic rates), i.e. the ciliates are responsible for about 14 % and the total microfauna more than 40 % of the animal respiration in and on the sediments of this locality.

It should be stressed that the graphs shown in Figs 92-95 are not "ecological pyramids" in the sense of Elton, i.e. the groupings are not carried out according to any trophic relationships but only according to size. That the graphs are not ecological pyramids can be seen at once if we accept that the calculated metabolic rates in any way reflect reality.

There is also no reason to believe that a classification according to size should reflect trophic relationships directly. The works of Muus (1967), Newell (1965), Perkins (1958) a.o. show that while it is true that some representatives of the macrofauna feed on representatives of the microfauna, many macro invertebrates, among which many are quantitatively important (e.g. oligochaetes, *Hydrobia*) feed directly on protophytes and bacteria; thus they occupy to a large extent the same trophic level as a great part of the microfauna. Also several benthic macro invertebrates are filter feeders deriving their energy from the pelagic ecosystem. Besides much of the benthos, not least the microfauna serves as food for the vagile organisms (fishes, prawns) not included in the present compilation of quantitative data.

Thus, in spite of a successful quantification of the various inhabitants of the benthos, we are still far from being able to quantify the trophic relationships due to the complexity of food webs in marine environments (see Fig. 57 which shows the food relationships of only one animal group, the ciliates, in estuarine sediments). Also exact knowledge on the food of several groups is still lacking.

In order to evaluate the role played by different animal groups in an ecosystem it is also possible to compare reproductive rates. Fenchel (1968b) studied the maximum reproductive rates of various ciliates at different temperatures. It was found that at 20° the generation time varies between 2.5 and 46 hours for different species of ciliates according to body size (see also discussion Chapter 9, pp. 162-164). Also the reproductive rates of various micro metazoa were estimated on the basis of data found in the literature. If we consider the factor by which a population increases in 24 hours (λ) at 20° we find that a population of ciliates under optimal conditions may increase by a factor 1.4 to 1000 in 24 hours according to the species. Nematode populations may increase by a factor 1.1-1.2 per day and harpacticoid populations by a factor 1.2-1.5.

If the animal populations grew at maximum rate in the field it would be easy to estimate the production of animals, but we have no reason to believe that this is so (see discussion in Chapter 9, p. 164). The data in ZoBell (1963b) indicate that bacteria do usually not reproduce at optimum rates in the field.

The quantitative methods, the methods for estimating age groups and the knowledge on life cycles are still not sufficiently advanced in order to evaluate the reproductive rates in the field.

Nevertheless, values for reproductive rates under optimal conditions of different animals will probably reflect the relative importance of the animals to some extent.

It should at last be mentioned that metabolic and reproductive rates may not be sufficient measures for evaluating the effect of a species on its environment. A significant experiment is described by Edwards & Heath (1963). The experiment was carried out in a terrestrial environment but is also relevant when discussing marine ecosystems. Knowing that the chemical decomposition of dead leaves is for a greater part due to the activity of microorganisms, the authors placed leaves in nylon bags with different mesh sizes which were buried in soil. It was found that the decomposition of leaves proceeded slowly in the bags with fine meshes; thus mechanical breakdown by the macrofauna is necessary in order to make the microbial decomposition efficient.

When comparing Figs 51, 53, 55, 61, 66, 69, and 73, which all show the composition of the food for the ciliate fauna in different localities the following may be concluded. Carnivores play a modest role; usually about 10 % of the ciliates are predators; this is intelligible since the most important prey for carnivorous ciliates are other ciliates. Histophages play a similar or even smaller role, but are difficult to evaluate exactly since many ciliates which feed on corpses or mechanically damaged animals are rather omnivorous preying also on ciliates or flagellates.

Zooflagellates are eaten by several ciliates, often by forms which also consume bacteria or phytoflagellates.

The two most important food items for the ciliates are bacteria and protophytes. Among the latter, diatoms are most important followed by flagellates and other unicellular algae. Filamentous algae are eaten by a few specialized forms which rarely play a large quantitative role.

The proportions between bacterivorous and herbivorous ciliates vary according to the biotope and to the season of the year. In lentic shallow water regions herbivores may often constitute up to 70-80 % of the ciliates in summer (Julebæk Beach, Nivå localities), during other periods of the year bacterivores are predominant. In deeper water bacteria seem to predominate as food for the ciliate fauna throughout the year.

As discussed in Chapters 5 and 9, many of the flagellates and probably some other algae (diatoms) living in sediments may be partly or obligatory heterotrophic.

The great importance of chemoautotrophic and photoautotrophic bacteria as food for the ciliates is striking; the sulphurbacteria alone may constitute 25-50 % of the food of the ciliates in certain localities. This indicates a large trophic role of reduced low-molecular endproducts of anaerobic decomposition. It is possible that this applies more to the ciliates than to other animals since many species of ciliates tolerate or require reducing environments.

Perkins (1958) observed that some nematodes feed on sulphurbacteria and I have observed naidid oligochaetes which apparantly mainly fed on these bacteria. Thane-Fenchel (1968) observed that several rotifers feed on sulphurbacteria.

The feeding habits, the quantitative importance and the reproductive potential of ciliates all indicate that these animals play a great role by diminishing the numbers of bacteria and protophytes in sediments. This seems especially to be the case under decomposition of larger amounts of organic matter as shown experimentally in Chapter 9.

The activity of bacterivorous ciliates may increase the rate of bacterial decomposition since constant removal of bacteria will keep their populations in a constant growth phase. Johannes (1965) showed that the presence of ciliates in bacterial cultures increases the rate of regeneration of phosphate from organic material.

It remains to discuss the role played by the ciliates as food for other animals. Unfortunately there is very little evidence at hand.

It is certain that many representatives of the macrofauna (e.g. *Arenicola,* many lamellibranchs, etc.) which swallow the sediment or siphon the surface must also benefit from the presence of the ciliates. It seems likely that the sand-licking haustoriid amphipods may eat ciliates, but evidence is lacking.

There is but little evidence showing that micro metazoans prey on ciliates. This is probably to a great extent due to the fact that ciliates leave no recognizable remains in the intestine of a predator.

Thane-Fenchel (1968) showed that some rotifers eat ciliates. I have observed that an unidentified turbellarian swallowed a *Tracheloraphis* sp. Remane (1927) mentions that the gastrotrich *Turbanella* may eat ciliates. Work in progress carried out by Birthe Jessie Jensen B. Sc. (Helsingør Laboratory) on the ecology of turbellarians has shown that several species representing different systematic groups feed on ciliates in the field.

This poor and scattered information does not signify very much concerning the role of ciliates as food for metazoans. Altogether, not very much is known on the feeding and food of many micro metazoan groups; future investigations, which must include direct observation on the feeding, will clarify the importance of ciliates in this respect.

It seems still premature to consider the ciliates as a "short circuit" of the food chains of marine benthos.

11. FUTURE RESEARCH ON MICROBENTHOS

The present paper has described the role of the ciliated protozoa in the sediment ecosystem and has outlined a number of general principles for an understanding of this system. At the same time it leaves a number of problems unsolved. This chapter will briefly propose a few projects for future research which seem promising.

It has been demonstrated that the redox conditions of sediments influence the distribution of the microflora and fauna and some probable reasons for this are discussed. Further research along these lines will imply a better chemical understanding of the sediment and especially a more complete chemical interpretation of the measured electrode potentials. Such investigations will require quantification of a larger number of ions and compounds of the interstitial water and this will to some extent require a development of new methods. It is already some time ago that the only electrochemical tool known to the ecologist was the pH-meter. In recent years this field has undergone a great development and a large number of ions and compounds can now be measured quantitatively with minute electrodes. This has been utilized to some extent in the present investigation, but no doubt this approach for investigating the microdistribution of chemical factors may be developed further. Improved measurements of oxygen contents in interstitial water are highly desirable, and reliable methods for measuring oxygen in reducing environments are still to be developed.

The microbiology of sediments is a promising subject and increased knowledge in this field is necessary in order to improve our understanding of the marine ecosystem. An obvious task is to enumerate all types of bacteria in a sediment. Direct counts of bacteria should be made simultaneously with dilution culture methods. The quantitative importance and the microdistribution of various physiological types should be investigated, not least such forms as anaerobes and chemoautotrophs which have hitherto been rather neglected.

While several plankton algae have been the object of physiological and biochemical research, the protophytes in sediments offer a rather unexplored field, for example concerning the exact demands of nutrients and light of these forms.

Many of the ciliates of the sediment constitute interesting objects for students of cellular metabolism. Thus the ciliates living constantly in an anaerobic environment consisting of an 0.01 M H_2S solution will certainly show metabolic features differing from those of the well known *Tetrahymena*.

As discussed in this paper, critical studies of the tolerance to or requirement of completely anaerobic conditions have only rarely been carried out; systematic investigations of this kind would be of considerable interest.

Quantitative analysis of the dynamics of the sediment ecosystem will be important in future research. Quantification of the import of organic matter has never been made. A picture of the importance of this import could be obtained by collecting the remains of seaweeds at intervals throughout a year on a given area. Nor have systematical measurements of the respiration and photosynthesis of an undisturbed sea bottom been made through longer periods.

A most promising field seems to be the utilization of labelled compounds in the study of food chains and energy flow in sediments. Sorokin (1964, 1965, 1966) has used this technique for studying bacterial nutrition in the pelagic ecosystem, not least the dark fixation of CO_2 by chemoautotrophic bacteria. With this technique it will be possible to demonstrate and quantify similar processes in sediments and to compare the importance of the different element cycles in the field and in laboratory models and it will probably also be possible to trace food relationships between the microflora and fauna by this method.

Finally, it should be mentioned that general observations on the biology of several important groups of animals (feeding, micro distribution, life cycles, etc.) are needed before we can hope to construct a more complete picture of the sediment ecosystem. Most metazoan groups have in the later years been well treated from a taxonomical point of view and are obvious objects for ecological studies. Other groups, for example the zooflagellates, will also need a taxonomical treatment. Improved methods for quantitative sampling and extraction of the microfauna of sediments will make these organisms useful for studying population dynamics and various properties of communities such as diversity.

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