

SOME ASPECTS OF MARINE MICROBIOLOGY

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INTRODUCTION

MARINE sediments have been studied by geologists and palaeontologists and the benthos, particularly bacteria and animals, have received attention from bacteriologists and biologists. There has, however, been insufficient examination of the sea bottom, its fauna and flora, as a part of the marine biological system. The studies made give no clear indication of the extent of the interaction between the bottom and the sea itself.

MICROBIOLOGY OF MARINE MUDS

Wood (1956) showed that the bottom muds collected from the ocean deeps by the Danish research ship *GALATHEA* included diatoms, some of which still contained protoplasm. Moreover, these diatoms were benthic species, and no planktonic forms were observed in any of the samples. All of the species found in the *GALATHEA* material except *Ethmodiscus rex* have also been collected from estuaries of Australia and New Zealand (see Crosby and Wood, 1959 ; and Wood MS.).

More recently a study has been made of the sea bottom on the continental shelf east of Port Hacking, Australia, at depths from 60 to 120 metres. Samples taken weekly over six months invariably showed pennate diatoms with a few benthic discoid species. The diatoms contained chloroplasts and many of the pennate forms e.g. *Navicula* spp., *Pleurosigma* spp. were actively motile, although the one per cent. surface illumination level did not exceed 87 metres in 33 observations made in 1955-7 by Jitts (1959), i.e. the diatoms were in many cases below the theoretical euphotic zone. Cultures in Allen and Nelson's medium (Allen and Nelson, 1910) yielded growths of *Navicula*, *Nitzschia*, and *Pleurosigma* species. Table I gives a list of the diatoms found in the various benthic environments for comparison.

It will be seen that there is a continuity between the estuarine bottom flora through the continental shelf flora to that of the ocean deeps, and this would appear to confirm Wood's (1956) suggestions that these benthic floras are ecologically related to each other, and that they constitute a separate and distinctive biocoenosis. The diatoms of the estuarine muds are reinforced by epontic species which fall on to the muds and are included in the mud flora. This, and the large number of samples may account in part for the comparative diversity of the estuarine flora, but it is probable that the number of species in the plant communities diminishes seaward. Although the studies on the bacteria of marine sediments in deeper waters are few, the evidence suggests that the bacterial flora in such places does not differ materially,

TABLE I

Diatoms of the Estuarine Muds, Continental Shelf and Ocean Deep*

<i>Estuaries near Sydney</i>	<i>Shelf</i>	<i>Deep</i>
<i>Synedra fulgens</i>	<i>Synedra</i> sp.	<i>Synedra tenera</i>
<i>S. frauenfeldii</i>	<i>Grammatophora marina</i>	<i>Navicula</i>
<i>S. ulna</i>	<i>Achnanthes brevipes</i>	<i>Cocconeis pediculus</i>
<i>Grammatophora marina</i>	<i>Achnanthes dispar</i>	<i>Pinnularia dactylis</i>
<i>Licmophora abbreviata</i>	<i>Cocconeis placentula</i>	<i>Trachyneis aspera</i>
<i>L. grandis</i> v. <i>divisa</i>	<i>Navicula hennedyi</i>	<i>Pleurosigma normanii</i>
<i>L. flabellata</i>	<i>N. lyra</i>	<i>Diploneis crabro</i>
<i>Terpsinoe musica</i>	<i>N. lyra</i> v. <i>australis</i>	<i>Nitzschia panduriformis</i>
<i>Striatella unipunctata</i>	<i>N. gracilis</i>	<i>Melosira granulata</i>
<i>Achnanthes longipes</i>	<i>N. brasiliensis</i>	<i>M. moniliformis</i>
<i>A. brevipes</i>	<i>N. luxuriosa</i>	<i>Coscinodiscus centralis</i>
<i>Cocconeis heteroidea</i>	<i>N. sulcifera</i>	<i>C. marginatus</i>
<i>Cocconeis placentula</i>	<i>Diploneis crabro</i>	<i>C. pacificus</i>
<i>C. scutellum</i>	<i>Trachyneis aspera</i>	<i>Ethmodiscus rex</i> (fragments)
<i>Navicula subcarinata</i>	<i>Diploneis smithii</i>	
<i>N. cyprinus</i>	<i>D. fusca</i>	
<i>N. granulata</i>	<i>D. finnica</i>	
<i>N. brasiliensis</i>	<i>D. rostrata</i>	
<i>N. lyra</i>	<i>D. pseudobombiformis</i>	
<i>N. yarrensii</i>	<i>Amphora graeffii</i>	
<i>N. grevillei</i>	<i>A. proteus</i>	
<i>N. lyra</i>	<i>A. bigibba</i>	
<i>N. lyra</i> v. <i>elliptica</i>	<i>A. cuneata</i>	
<i>N. lyra</i> v. <i>australis</i>	<i>A. weissflogii</i>	
<i>N. peregrina</i>	<i>Pleurosigma balticum</i>	
<i>N. radiosa</i>	<i>P. angulatum</i>	
<i>N. spectabilis</i>	<i>Amphiprora alata</i>	
<i>Neidium affine</i>	<i>Nitzschia distans</i>	
<i>N. amphigomphus</i>	<i>Campyloneis grevillei</i>	
<i>N. iridis</i>	<i>Campylodiscus biangulatus</i>	
<i>Trachyneis aspera</i>	<i>Mastogloia lineata</i>	
<i>Pinnularia major</i>	<i>Skeletonema costatum</i> (spores)	
<i>P. brevicostata</i>	<i>Melosira sulcata</i>	
<i>P. gibba</i>	<i>Hyalodiscus stelliger</i>	
<i>P. splendida</i>	<i>Pyxidicula cruciata</i>	
<i>P. stauroptera</i>	<i>Coscinodiscus nitidus</i>	
<i>Diploneis crabro</i>	<i>Coscinosira</i> sp.	
<i>D. fusca</i>	<i>Triceratium</i> sp.	
<i>D. chersonensis</i>		
<i>Dictyoneis marginata</i>		
<i>Pleurosigma angulatum</i>		
<i>Pl. balticum</i>		
<i>Pl. formosum</i>		
<i>Anomoeoneis serians</i>		
<i>A. sphaerophora</i>		
<i>Mastogloia cribrosa</i>		
<i>M. quinquecostata</i>		
<i>M. gracilis</i>		
<i>M. smithii</i>		
<i>M. rostellata</i>		
<i>Tropidoneis lepidoptera</i>		

* Port Hacking, Botany Bay, Lake Macquarie, New South Wales

TABLE I—(Contd.)

<i>Estuaries near Sydney</i>	<i>Shelf</i>	<i>Deeps</i>
Amphora robusta		
A. hyalina		
A. proteus		
A. lineolata		
A. ostrearia		
A. gigantea		
A. rhombica		
A. javanica		
A. arcta		
Epithemia argus		
Campylodiscus ornatus		
C. adriaticus		
C. undulatus		
Melosira moniliforme		
M. sulcata		
M. nummuloides		
M. granulata		
M. juergensii		
Coscinodiscus marginatus		
C. pacificus		
C. radiatus		
C. argus		
Actinoptychus senarius		
Aulacodiscus margaritaceus		
Auliscus sculptus		
Biddulphia pulchella		
B. aurita		
Triceratium favus		
T. robertsonianum		
T. alternans		
Isthmia nervosa		

except in relation to hydrostatic pressure, from that of the continental shelf and of estuaries (see Waksman *et al.*, 1933; Zobell, 1946, 1952; Wood, 1953; Kriss and Rukina, 1953; Baas Becking and Wood, 1955).

THE RELATION BETWEEN THE BENTHIC FLORA AND THAT OF THE NERITIC PLANKTON

In addition to the benthic diatoms of the continental shelf bottom, spores of planktonic forms may occur. Thus, on one occasion, in August, just prior to the spring maximum, a culture from the continental shelf mud at 120 metres yielded a unialgal culture of the planktonic diatom *Skeletonema costatum*, of which the spores had been previously observed in the mud. It is interesting to note that, a week later, the surface water samples from the same station yielded a bloom of this diatom, while an old culture of *S. costatum* which had become overgrown by *Navicula grevillei*, when subcultured on the same date as the mud sample was collected, gave an apparently unialgal culture of *S. costatum*. The question arises, does this suggest a biological rhythm in phytoplankton. Diurnal, tidal rhythms have been demonstrated in diatoms by Callame and Debuyser (1954), and a lunar rhythm for *Goniaulax* has been suggested by Sommer *et al.* (1937) and Wood (1954).

Further evidence in this connection is given in Figure 1. In this experiment a sample of sea water from the laboratory pond was placed in glass and plastic jars in the laboratory, one of each in the daylight, one under artificial light, and one in the dark at room temperature. Direct counts of the diatoms in the jars and in the pond

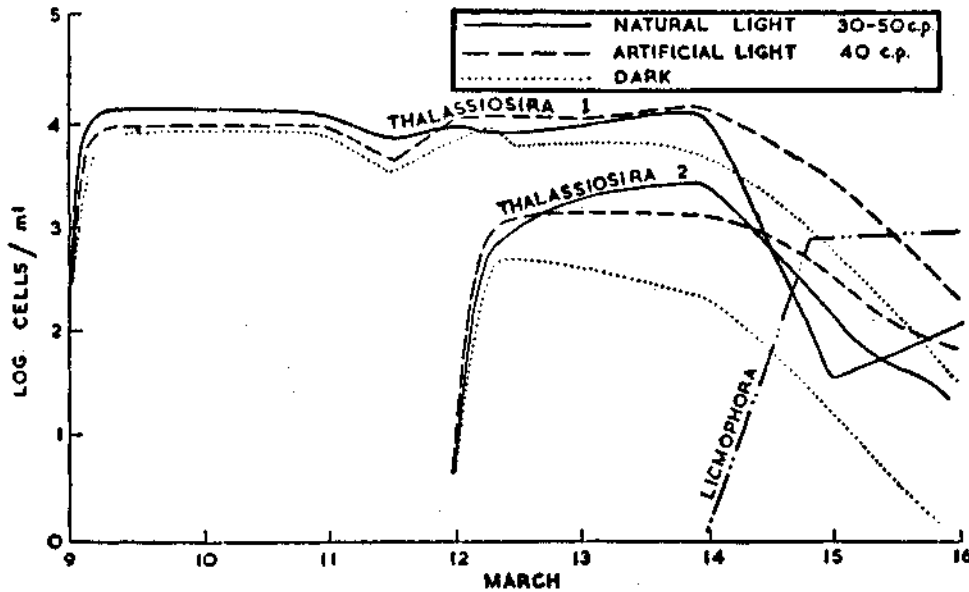


FIG. 1. Growth curves for diatoms expressed as log. cells/ml. in sea water samples in natural light, in artificial light, and in darkness. Series 1 represents the original, series 2 the later isolation.

were made at regular intervals and the growth of the predominant diatom *Thalassiosira aestivalis* plotted as Log_{10} cells. There was no significant difference in the growth rates in plastic and glass bottles, and only a relatively slight decrease in the growth rate in the darkened bottles.

The count of *T. aestivalis* in the pond remained approximately constant between 400 and 500 cells per ml. In the jars however, there was an immediate and very rapid logarithmic increase for the first four hours, after which the rate tapered off to the stationary phase which lasted for over four days and was followed by the death phase. It is interesting to note that natural light gave slightly better growth than artificial light of the same candle power, while the darkened bottles multiplied slightly more slowly and did not reach quite the same maximum population. When *Thalassiosira* was declining, *Licmophora abbreviata* grew logarithmically for ten hours.

A second isolation of *T. aestivalis* made from the pond on the third day increased logarithmically, but to a lower population level, and entered the death phase at the same time as the original culture. Samples taken from the pond after the fifth day failed to grow. These facts suggest a rhythm, but whether due to external or internal factors is not known.

The appearance of *Skeletonema costatum* in the surface water off Jibbon Cape following the appearance of the species in culture is open to two interpretations: 1. the diatom emerged from the spores *in situ* and floated to the surface, or 2. there was a turnover and mixing of the mud and water, the bloom actually occurring at the surface.

Further studies on the muds and surface waters gave evidence on this. The Eh of the surface muds over the six month period was + 170 mV, ranging from + 50 to + 250 mV at an average pH of 7.0 (6.6 to 7.4). During this time, on three occasions, the sulphate reducing bacterium *Desulphovibrio desulphuricans* was isolated from the surface water (Eh + 430 mV), and on two of these occasions a Chlorobium and a Chromatium (two photosynthetic sulphur oxidizing bacteria) were isolated. All these bacteria are strict anaerobes, and grow only at Eh values far below that of the water or most of the surface muds (see Baas Becking and Wood, 1955). These organisms could only have been derived from the bottom in a region where the Eh value was below + 110 mV for active *Desulphovibrio* and below -110 mV for the Chlorobium, though these organisms can exist but not assimilate at higher Eh values.

Putting the facts together, we may conclude that vertical mixing of surface muds and the waters of the continental shelf is responsible in part for the neritic blooms of phytoplankton, and possibly contributes nutrients as well as microorganism. We know (Baas Becking and Macay, 1956) that sulphate reduction can cause release of phosphate in the muds, while nitrogen fixation by many anaerobes has been established.

I was somewhat surprised at the high Eh values of the surface of the muds at the Port Hacking stations, but this would account for the fact that I failed to find many true (as distinct from facultative) anaerobes in this region on the continental shelf. Prevot (1958) has criticised my technique (Wood, 1953) on account of the findings of Callame (1951). However, Callame's samples were taken in lagoons and estuaries with a considerable amount of pollution where one would expect low Eh values and a correspondingly increased possibility of true anaerobes occurring. One would not expect true anaerobes, except adventitiously, at Eh values of + 100 mV or more.

PHOTOSYNTHETIC MICROORGANISMS BELOW THE PHOTIC ZONE

One other point arising from the observations here recorded is the repeated finding of photosynthetic microorganisms at depths below the euphotic zone. These include diatoms, flagellates and photosynthetic bacteria. For instance Bernard (1942) recorded coccolithophores at 4,000 metres and Kriss and Rukina (1953) recorded active photosynthetic sulphur bacteria at depths of 2,500 metres in the Black Sea. The Russians gave experimental evidence that the purple and green sulphur bacteria were actively photosynthetic *in situ*, and as there was no light at such depths, they suggested that the bacteria were using radioactive energy. While I find this explanation hard to accept, I have no alternative to offer. Another possibility for the growth of photosynthetic microbes below the photic zone is that they are growing heterotrophically. We know (Lewin, 1953, and Wood, 1956) that some diatoms are capable of heterotrophic growth, even at pressures up to 500 atmospheres, but Kriss and Rukina gave good evidence that their photosynthetic bacteria were

incapable of heterotrophic growth. Because of the number of records of photosynthetic microorganisms occurring in quantity below the photic zone, we cannot assume that the organisms are inert or are living a heterotrophic existence. Perhaps the organisms below the euphotic zone are in the stationary or death phases of their growth curves, perhaps those autotrophic microbes which are found below the compensation point as normally determined, have lower light requirements or a lower katabolic rate than others, or perhaps they are using sources of energy other than sunlight for the reduction of carbon dioxide.

The observations and experiments recorded in this paper pose more problems than they solve. They are given in order to stimulate thought, and to warn against over simplification of problems in marine microbiology.

REFERENCES

- ALLEN, E. J., and NELSON, E. W. 1910. On the artificial culture of marine plankton organisms. *J. Mar. biol. Ass. U.K.*, **8** : 421-474.
- BAAS BECKING, L. G. M., and MACKAY, MARGARET. 1956. Biological processes in the estuarine environment, VI. The influence of *Enteromorpha* on its environment. *Proc. Kon. Ned. Akad. Wetensch.* **B59** : 109-123.
- and WOOD, E. J. F. 1955. Biological processes in the estuarine environment I and II. Ecology of the sulfur cycle. *Proc. Kon. Ned. Akad. Wetensch.* **B58** : 160-181.
- BERNARD, F. 1948. Recherches sur le cycle de *Coccolithus fragilis* Lohm. flagellé dominant des mers chaudes. *J. Cons. int. Explor. Mer.*, **15** : 177-188.
- CALLAME, B. 1951. Recherche sur les bactéries anaerobies du Golfe de Guinée et de la lagune Ebrié. *Ann. Inst. Past.* **80** : 438.
- and DEBUYSER, J. 1954. Observations sur les mouvements des diatomées a la surface des sédiments marins de la zone intercotidiale. *Vie et Milieu*, **5** : 243-249.
- CROSBY, L. H., and WOOD, E. J. F. 1959. Studies on Australian and New Zealand Diatoms II. Normally epontic and benthic species. *Trans. roy. Soc. N.Z.*, **86**.
- KRISS, A. E., and RUKINA, E. A. 1953. Purple sulfur bacteria in the hydrogen-sulfide containing depths of the Black Sea. *Dokl. Akad. Nauk. U.S.S.R.*, **93** : 1107-1110.
- LEWIN, JOYCE C. 1953. Heterotrophy in diatoms. *J. Gen. Microbiol.*, **9** : 305-313.
- JITTS, H. R. 1959. Measurement of light penetration in the Tasman Sea, 1955-57. *C.S.I.R.O. Div. Fish. Oceanogr. Tech. Paper* 6.
- PREVOT, A. R. 1958. Utilité de bactériologie marine dans le present et l'avenir. *Bull. Inst. Ocean. Monaco.*, 1114.
- SOMMER, H., WHEDON, W. F., KOFOID, C. A., and STOHLER, R. 1937. Relation of paralytic shell fish poison to certain plankton organisms of the genus *Goniaulax*. *Arch. Path. Lab. Med.*, **24** : 537.
- WAKSMAN, S. A., HOYCHKISS, M., and CAREY CORNELIA, L. 1933. Marine bacteria and their role in the cycle of life in the sea. *Biol. Bull. Woods Hole*, **65** : 137-167.
- WOOD, E. J. F. 1953. Heterotrophic bacteria in marine environments of eastern Australia. *Aust. J. Mar. Freshw. Res.* **4** : 160-200.

- WOOD, E. J. F. 1954. Dinoflagellates in the Australian region. *Aust. J. Mar. Freshw. Res.*, **5** : 171-351.
- 1956. Diatoms in the ocean deeps. *Pacif. Sci.*, **10** : 377-381.
- Studies on Australian and New Zealand diatoms IV. (MS.).
- ZOBELL, C. E. 1946. *Marine Microbiology*. Chronica Botanica Co.
- 1952. Bacterial life at the bottom of the Philippine trench. *Science*, **115** : 507-508.