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Blue-Green algae agglomeration in surface water: a microbiotope of high bacterial activity

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Abstract

Mass accumulations of filamentous blue-green algae in surface waters of the Baltic Sea cause patches of high bacterial productivity within water masses of low microbial activity. Since these algae are apparently not grazed by zooplankton organisms, their degradation in the surface water layer is mainly due to associated bacteria. The network of algae filaments attracts a wide range of small organisms including rotifers, crustaceans, ciliates and flagellates, resulting in a microbiotope of considerable complexity. The succession of bacterial colonization of *Nodularia spumigena* and the activity state of the community was investigated by the INT-staining technique and by microautoradiography. The excretion rates of photosynthesized matter by the blue-green algae agglomerates indicate a high rate of transfer of substrate towards the adherent heterotrophic bacteria. The fate and the final involvement of the agglomerates in the food chain are discussed.

Introduction

Agglomerations of filaments of the blue-green alga *Nodularia spumigena* (Mertens) are the basis for the establishment of a microbiotope with a biology of considerable complexity. Although these algae bloom in the Baltic Sea in the late summer every year and their accumulation at the sea surface is a well known phenomenon, the development of these algae together with their comprehensive epicenosis have only been investigated occasionally as for instance by APSTEIN 1902, VÄLIKANGAS 1926, BURSA 1963, HORSTMANN 1975. Mass occurrences of *Nodularia spumigena* have also been reported from the brackish waters of the Caspian Sea and the Sea of Azov (ZENKEWITCH 1963).

In the study presented here special attention was paid to the role of bacteria within the epicenotic association of the blue-green algae and the mechanisms of functioning and maintaining of the *Nodularia* flock microbiotope.

In the Baltic Sea the greatest bacterial numbers and heterotrophic activity were found to be associated with free-living bacteria (GOCKE 1975, ZIMMERMANN 1977). Heavy colonization of phytoplankton and detritus particles with attached bacteria has seldom been observed in these waters, perhaps because of the short duration of the sedimentation process in the shallow waters of the Baltic Sea and a rapid death and lysis of "unhealthy" algal cells. A contrasting situation is, however, observed for *Nodularia spumigena*, which remains suspended in the upper water layer for weeks even in a stage of progressive decay.

Since there is some evidence that bacteria attached to particles are preferably grazed by microzooplankton it seems to be a useful task for aquatic microbiologists to search for microbiotopes in the sea with a stable constitution where the important functions of

primary production, bacterial growth and predation on bacteria are closely associated in space and time. Unfortunately such compartments of microbiological activity can be seldom recognized in the natural environment (whithout sophisticated techniques) and it is even more difficult to isolate them for separate investigations.

In this contribution microscopic observations of blue-green algae and bacteria interrelationships uring the development of a *Nodularia* bloom and in the flock-like agglomerations of *Nodularia* threads are described. Bacterial orientation on the algal filaments was studied in relation to the photosynthetic and respiratory activity of the blue-green algae. Estimations of *Nodularia* primary production and excretion rates and rates of bacterial incorporation of photosynthesized organic compounds together with biomass estimates of attached bacteria give evidence of the involvement of bacteria in the specific food-chain of a *Nodularia* agglomerate.

Methods

Water samples and net samples for microbiological and planktological observations were collected in fjords and archipelagos of different degrees of eutrophication in Swedish and Finnish coastal waters and from the open Baltic Sea (autumn 1979 and 1980).

Inorganic nutrients and phytoplankton parameters were investigated by means of standard methods.

The aim of the study – following the succession of bacterial attachment to blue-green algae filaments in relation to the physiological stage of the algae – made it necessary

Figure 1

INT-stain of a *Merismopedia* spec. colony from brackish water of low salinity (ca. 5‰) which sometimes accompany *Nodularia spumigena*. Dark cells indicate respiration (red in the original stain), colourless cells are inactive

Figure 2

INT-stain of *Nodularia* filaments. The dark filament to the right shows respiratory activity and is not colonized by bacteria. The filament to the left is inactive and heavily colonized by small coccoid bacteria

Figure 3

Spiralized *Nodularia* filaments partly colonized with epicenotic organisms

Figure 4

INT- stain of a *Nodularia spumigena* filament with active bacterial epicenosis. Note accumulation of bacteria near the heterocyst

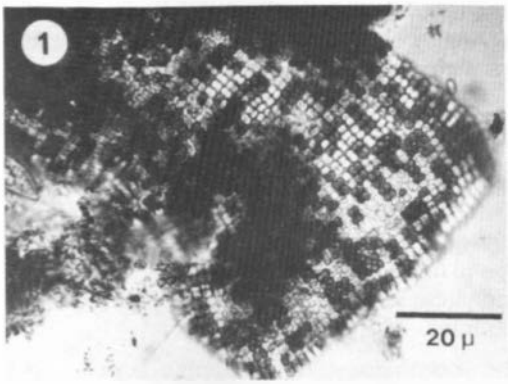
Figure 5

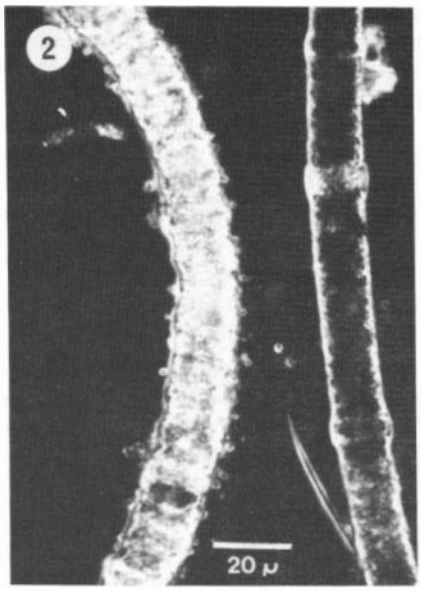
INT-stain of an inactive *Nodularia* filament covered with active coccoid bacteria of the same type as shown in the SEM-photograph in fig. 11

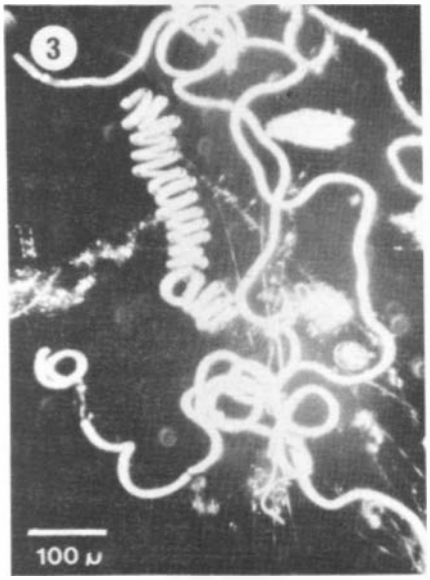
Figure 6

INT-stained *Nodularia* filament showing cells with respiratory activity (dark cells), and deformed cells without respiratory activity. Fungus-like organisms are attached to the inactive section of cells

[1] MAR = Microautoradiography, SEM = Scanning Electron Microscopy
[2] INT = Jodophenyl-nitrophenyl-phenyl-tetrazoliumchloride
[3] DHA = Dehydrogenase-activity, ETS = electron-transport-activity







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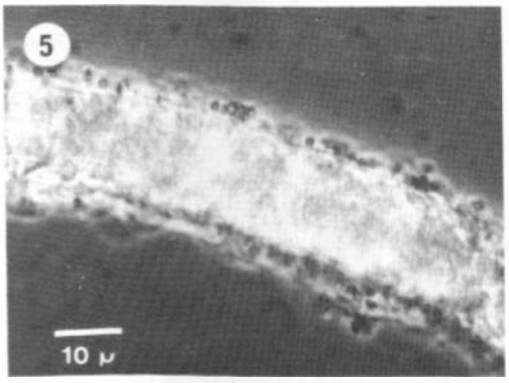
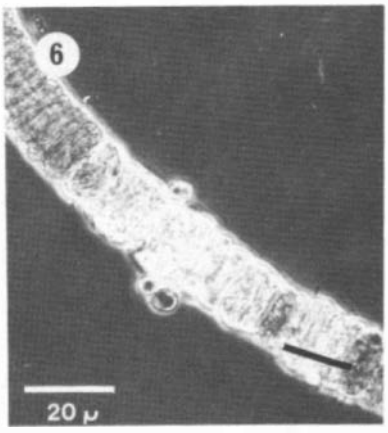




to find a method which provides a quick and reliable picture of the activity stage of the algae, and to apply this method simultaneously with other microscopic methods (MAR[1], SEM[1], Epifluorescence). The INT[2]-staining method was found to be very useful for this particular purpose. The method is a microscopic version of the well-known technique employed for determination of respiration potential (DHA[3], ETS[3]) using tetrazolium salts which are reduced to water insoluble formazans (red) by dehydrogenase in connection with the thyllacoids of the cell (BISALPUTRA et al. 1969). The method was carried out according to ZIMMERMANN et al. (1978) but without combination with epifluorescence. The other alterations to the original method were an increase in the concentration of the dye (0.5 mg INT per 2 ml watersample) and a longer incubation time (2 h). Both alterations caused a greater intensity of the stain (formation of formazan) in most of the samples.

In order to get additional results on the morphology, heterotrophic activity and biomass of the attached bacteria, epifluorescence microscopy according to MEYER-REIL et al. (1978), microautoradiography (HOPPE 1976, 1977) and scanning electron microscopy were applied.

Estimates of the excretion rates of the algae and bacterial uptake of the exudates were performed with the ^{3}H-^{14}C double labelling technique (HOPPE and HORSTMANN unpubl.) together with the method of WOLTER (1980).

Results

In the northern Baltic Sea blooms of the blue-green alga *Nodularia spumigena* appear every year in late summer. Our observations of species distribution of phytoplankton and measurements of inorganic nutrients indicate that the bloom starts in the transition zone between the outer area of an eutrophied fjord and the adjacent inner archipelago where nitrogen supply becomes a limiting factor for diatoms and dinoflagellates but phosphorus is in sufficient quantity (ca. 3 μM PO_4^{3-}). Blooming of *Nodularia spumigena* has also been observed in offshore areas and upwelling areas of the Baltic Sea. Aged *Nodularia* filaments accumulate during calm weather at the sea surface in agglomerates of flock-like appearance which are heavily colonized by bacteria (Figs 1, 3). Exposed to the wind, these microzones are drifted offshore where they cause patches of high biological activity in a rather unproductive environment.

Figure 7

Autoradiograph of an "old" *Nodularia* filament, showing $^{14}CO_2$-assimilation only in some sections of cells within the filament. It has been observed repeatedly that sections with strong photosynthesis correspond to those giving positive results for respiratory activity as detected by the INT staining technique

Figure 8

Autoradiograph of a "young" *Nodularia* filament showing strong photosynthesis $^{14}CO_2$-labelling, heterocysts are only weakly labelled

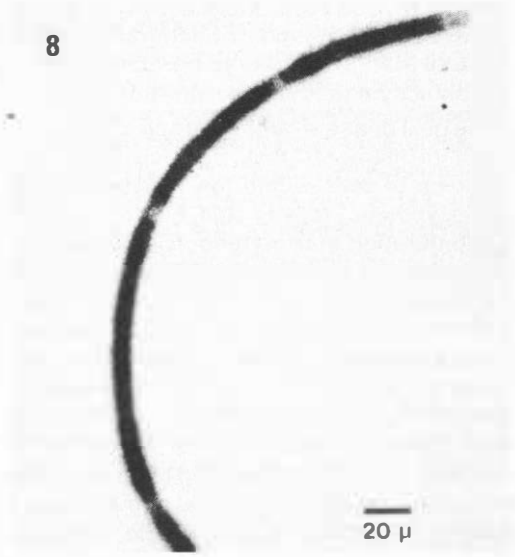
Figure 9

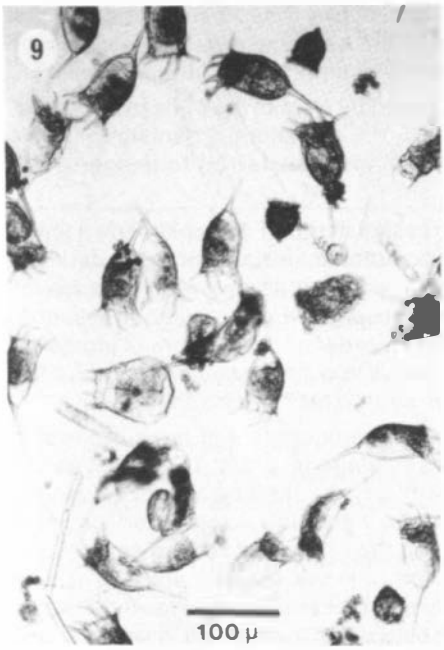
Zooplanktonic organisms frequently found in association with the *Nodularia* microecosystem: *Keratella quadrata*, *Keratella recurvispina*, *Tintinna* and ciliates of different types

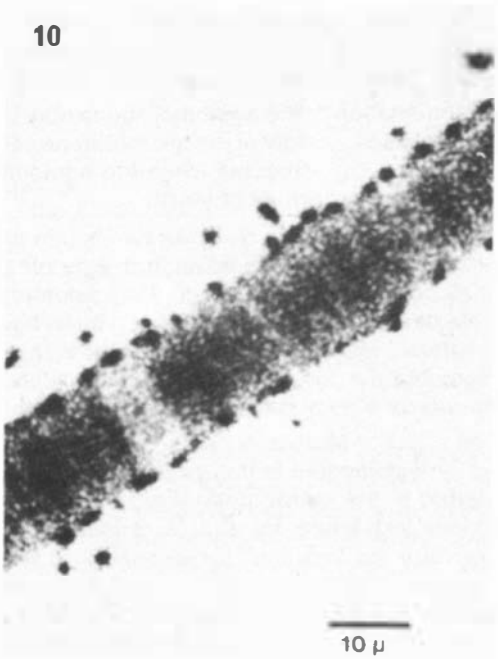
Figure 10

^{3}H-amino-acid mixture microautoradiograph of a *Nodularia* filament with attached bacteria. The bacteria show strong heterotrophic uptake of the label, while the algal filament is only weakly labelled. No increase of bacterial attachment at the heterocysts (nearly unlabelled)

The colonization of *Nodularia spumigena* filaments by bacteria follows a more or less distinct pattern and shows a dramatic increase of the bacterial biomass in the initial stages of flock formation. It has to be emphasized here that bacteria and fungi are the







first members of the complex epicenosis *(Navicula salina, Vorticella marina, Chlorella, Amoeba* spec., *Rhizochloris nodulariae*; BURSA 1963, our own observations), which develops in the *Nodularia* agglomerates.

Young *Nodularia* threads are normally straight and exhibit photosynthetic (detected with MAR[1]) and respiratory activity in all their cells (detected with INT[2]). They seldom show slime formation or bacterial colonization (Figs 2, 7, 8). The microphotographs indicate that $^{14}CO_2$-autoradiography and INT-stain give comparable results in terms of the photosynthetic and respiration activity of individual cells.

In the next phase straight and spiralized filaments of *Nodularia* occur side by side. Both types of filaments may develop slime and may be attached by microorganisms. Sections of cells within the vegetative cells of filaments lose their respiratory and photosynthetic activity and typical morphological structure. In this early stage of agglomeration marine fungi (Chytridiales) may be among the first invaders (Fig. 6). These fungi disappear when bacteria become the predominant colonizers. The first bacteria to appear on the filaments are small cocci in microcolonies (focus of infection) and rods evenly distributed around the filaments (Figs 4, 5, 11, 12). The predominant sites of bacterial colonization seem to be inactive cells along the filaments. Sometimes the slime cover of the heterocysts were found to be the preferred sites of bacterial attachment, supporting PAERL'S (1978) finding (Fig. 4). But this was not always the case as demonstrated by the ^3H-amino-acid mixture autoradiograph of *Nodularia* (Fig. 10).

The next step is characterized by the agglomeration of the *Nodularia* filaments and an immense increase of bacterial numbers and biomass (Fig. 13). In this situation – on the basis of bacteria as a food source – the complex microecosystem of the developing flock is established. Protozoa (*amoeba*), ciliates (sessile and motile e.g. Tintinna), flagellates, *rotatoria* (*Keratella quadrata* and *K. cochlearis*), larvae of crustaceans invade and in some cases reproduce in the protective network of the *Nodularia* agglomerate (Fig. 9). Life within the flock may be maintained as long as it has a continuous input of organic matter through autotrophic or heterotrophic processes and as long as it does not sink to the bottom. In "old" flocks it has been observed that *Nocardia* spec. becomes the dominant species of the bacterial population within the flocks. This species has been reported to cause lysis in blue-green algae cells.

Sedimentation of the agglomerations may be caused by lysis of the cells by *Nocardia*, an increase in weight of the microbiotope caused by the epicenotic organisms or by the collapse of the vacuoles when the agglomerations are transferred to deeper water layers during periods of storm.

The appearance of a *Nodularia* flock in a progressive stage of decay is shown in the photograph which was taken from year old unpreserved material kept in the dark in a BOD bottle (Fig. 14). Though the agglomeration preserved its outer appearance for over one year, microscopic observation however revealed a great number of spores (akinetes) and bacteria in a matrix of amorphous material and a network of structural elements, the remains of the *Nodularia* filaments. This may be the stage from which the *Nodularia* bloom starts again from the seafloor after "rest" during the winter.

The effect of *Nodularia* mass occurrence on the bacteria content of the surrounding water was studied in the Swedish east coast archipelago (Fig. 15). The investigation started in the eutrophied inner fjord (St. 33) with a high standing stock of diatoms. Values estimated for Chl. a, primary production, bacterial activity and bacteria numbers are "normal" for inshore fjord waters. In the offshore direction estimations for most of the parameters measured decreased until we entered an area where *Nodularia* flocks were accumulated in the upper water layer in slick-like formations (St. 36–38). The values from these samples indicate only a low level of Chl. a and primary

production due to the unhealthy appearance of most of the *Nodularia* filaments, but we find a remarkable increase in bacterial numbers and biomass. The same holds true for the saprophytes. Since primary production is low, the high standing stock of bacteria may be supported also by zooplankton excretion products, remains of zooplankton feeding and lysis of dead organisms.

Nutrients for bacteria in the agglomerates may be derived from primary production (particulate material, slime and excretion), and from the zooplankton component. Of these factors only the excretion pattern of living algae is readily accessible to experimental investigation in such a comprehensive system. We therefore studied the exudation of photosynthesized labelled organic matter and the bacterial uptake of these compounds in relation to primary production (Table 1). The first results from the schedule were received from aged *Nodularia* aggregates with only a few living threads. The result is a low primary production coupled with a high rate of exudation and bacterial uptake of the labelled exudates. The exudates are hypothesized to be of low molecular weight and easily degradable by bacteria.

Table 1

Results of experiments from the Swedish archipelago (30–37), the Finnish archipelago (Ex 3) and the Bornholm area (Pr. 2-Pr. 4). Data are presented in dpm and percentage of total exudate from primary production, percentage of primary production incorporated in the bacteria and percentage incorporated exudate from total exudate.

Station	Primary production	Total exudation (^{14}C-labelled)	%	% of primary production incorporated in bacteria	Incorporated exudates	%
30	1.870	1.040	56	40	741	71
31	1.133	646	57	18	202	31
33	3.664	1.805	49	44	1.605	89
34	2.888	631	22	15	426	68
35	3.092	1.377	46	—	—	—
36	1.103	383	35	—	—	—
37	700	323	46	8	193	60
Ex 3	27.798	7.105	26	17	4.618	65
Pr. 2	1.188.828	129.652	11	8	90.756	70
Pr. 3	1.281.966	149.862	12	3	38.964	26
Pr. 4	109.647	42.340	39	17	18.630	44

In the second region we find a higher value of primary production due to a greater number of healthy filaments in the *Nodularia* aggregates. Exudation rates are lower than in the first example, but bacterial uptake of the exudates is still very high. In the third offshore region we find high values for primary production (young flocks) and "normal" values for algal exudation and bacterial uptake rates of labelled exudates.

These data indicate that excretion of photosynthesized matter may be a valuable source of nutrients for attached and freeliving bacteria of the *Nodularia* flock

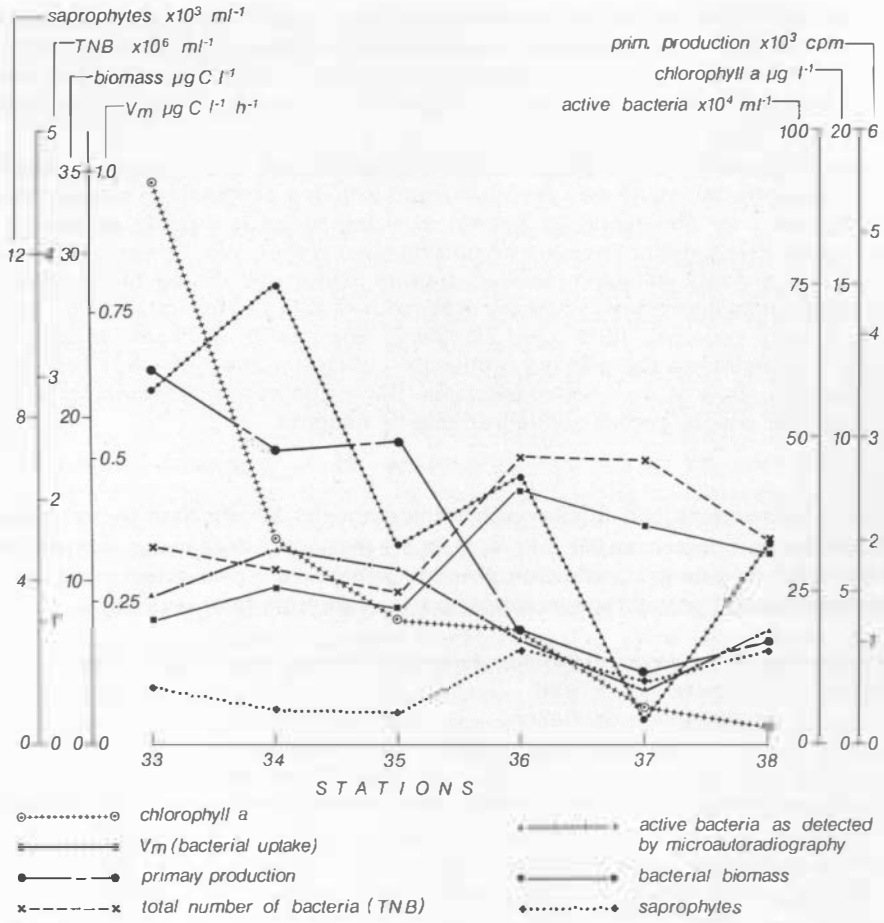


Figure 15

Measurement of different microbiological parameters during a cruise from an "inner fjord" area towards the "outer archipelago". Note the increase of bacterial total biomass and numbers at the stations 36--38 where decaying blue-green algae were present

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Figure 11

SEM-microphotograph of a microcolony of coccoid bacteria attached to a *Nodularia* filament

Figure 12

REM-microphotograph of rod-shaped bacteria attached to a *Nodularia* filament. Note destruction of the slime cover around the bacteria. It is not clear whether this is an artifact or a natural event

Figure 13

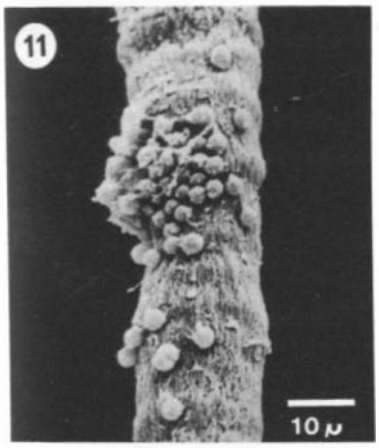
^{3}H-amino-acid mixture microautoradiograph of part of an "old" *Nodularia* agglomeration. The algal filament shows no heterotrophic activity in this late stage of age. It is heavily colonized by active bacteria.

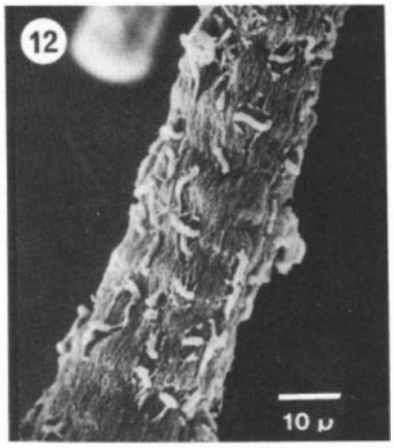
Figure 14

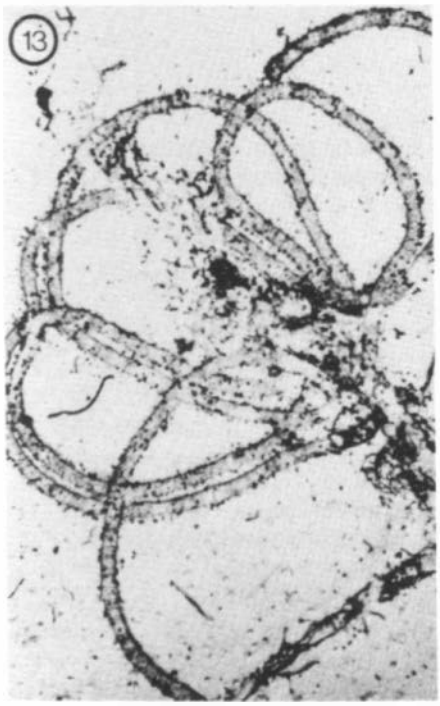
^{3}H-amino acid mixture microautoradiograph of a *Nodularia* agglomeration after one year storage in a BOD bottle. Some structural remains of the algae can be observed which are covered with active bacteria. Note the great number of acinetes which have developed

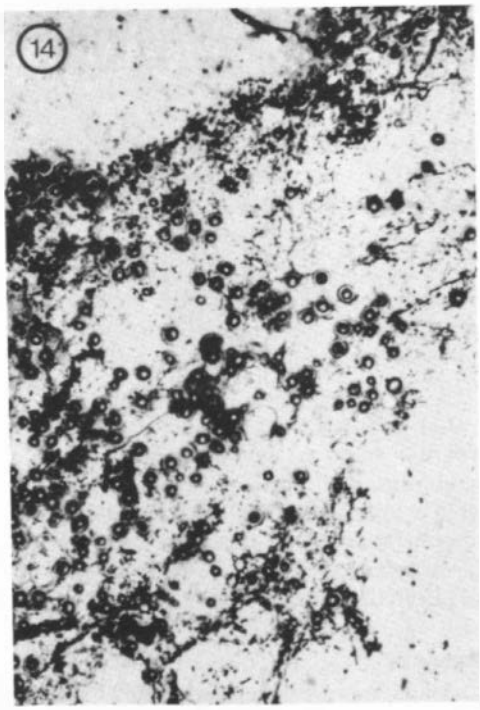
microbiotope, but since primary production is low in aged material it may not be sufficient to maintain the high standing stock of bacteria. In the case of the blue-green alga *Oscillatoria redekii*, HERBST and OVERBECK (1978) reported a rapid turnover of its excretion products by the accompanying bacteria and STABEL (in press) has even used turnover values of this substrate in his determination of the heterotrophic bacterial activity in a lake.

Attachment and concentration of bacteria in a small volume of water can be an advantage for bacteriovorous microzooplankton, suggested by the fact that at least









70 µg/l of organic matter can be efficiently utilized by microzooplankton (JÖRGENSEN 1966). To give a rough impression of the bacterial food available in a *Nodularia* flock, the bacterial standing stock was estimated on the basis of a "normal" flock with a size of 1 cm lenght and 0.5 cm diameter (Fig. 16). This flock contains 4400 filaments, based on the calculation that each filament has a diameter of 15 µm and is separated from others by a 60 µm space. (This is expected to be a conservative estimate.) An average of 45 bacteria was found to be attached to a filament of 75 µm lenght. This calculation arrives at a figure of 7.5×10^8 bacteria per *Nodularia* agglomerate, corresponding to at least 3.8 µg bacterial C if a cell weight of 5×10^{-9} µg is accepted. Compared with the normal bacteria content of sea water of about 2×10^6 mostly freeliving bacteria in 1 ml of water (ca. 10 µg C/l) this would mean that, in a quarter of a ml, the biomass

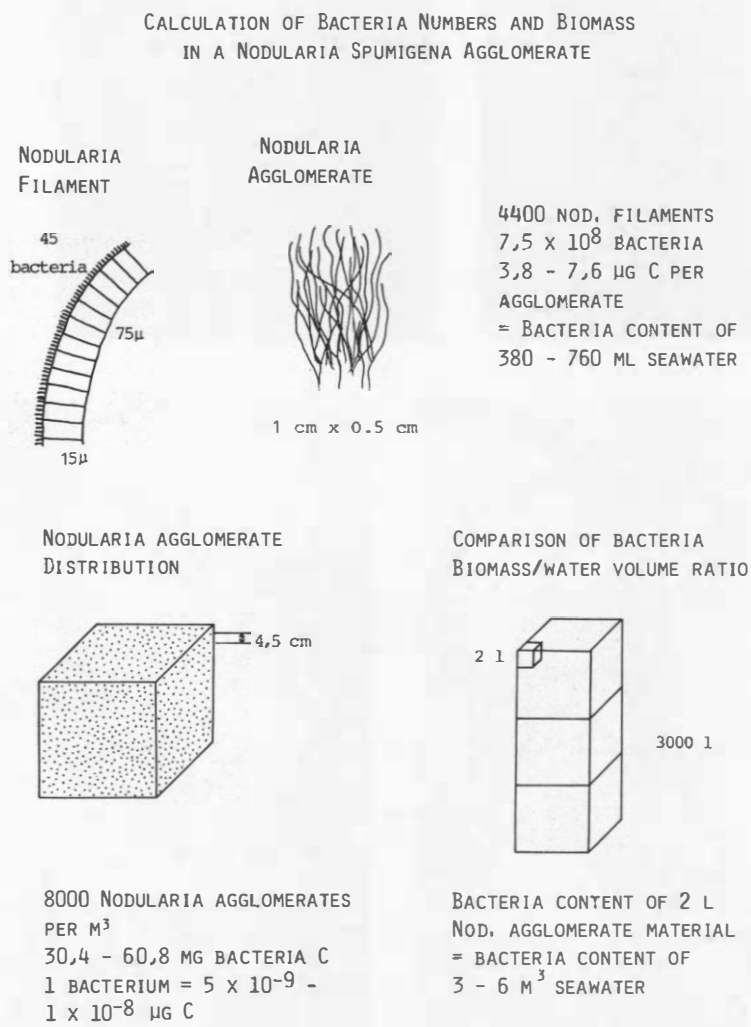


Figure 16
Calculation of bacteria numbers and biomass in a *Nodularia spumigena* agglomerate

equivalent of 380 ml seawater is concentrated. Assuming a distance of 5 cm between the agglomerates (about one flock in a BOD bottle, which is convenient), a bacterial biomass of ca. 30 mg C per m^3 can be calculated, which is concentrated in only 2 l of water (*Nodularia* agglomerate). The standing stock of bacterial biomass in one *Nodularia* agglomerate corresponds to about 760 ciliates of the *Euplotis* spec. type (1 ciliate ca. 0.005 µg C, RIEPER 1981), which is not a production measurement but may at least give an impression of the size of nutrition available for bacteriovorous organisms.

Discussion and conclusions

The *Nodularia* microenvironment turns out to be an agglomeration which does not fit the common definition of living organic material or detritus. It is composed of growing filaments or sections of living cells and dead or inactive threads or sections of cells. The living components maintain their photosynthesis even in heavily colonized agglomerates and supply the biocenosis continously with photosynthesized material (exudation, slime formation). Since photosynthesis is low in the "old" agglomerates it cannot provide sufficient food for the maintenance of the high standing stock of associated bacteria. It is therefore assumed that a recycling of organic matter from microzooplankton towards bacteria takes places (excretion, dead bodies of animals, fecal pellets which are trapped in the filamentous network). The result of this is a more or less "selfmaintaining" system with a balanced in- and output of organic matter. The field observation is that the agglomerates have a stable epicenosis and they may stay for weeks or even months in the upper water layer.

The fauna of microzooplankton found within or associated with the *Nodularia* flock is designed for feeding on bacteria or bacteria predators. We observed protozoa, freeliving (*Tintinna*) and sessil ciliates, colorless flagellates, the rotifers *Keratella quadrata* and *K. cochlearis recurvispina*, larvae and adults of crustaceans. The filaments of *Nodularia* themselves are not eaten by the animals. Evidence for this is given by the size of the filaments and the fact that they accumulate in the surface water to such a high extent. In the literature *Nodularia* has been reported to be toxic to fish, birds and mammals (FITCH et al. 1934, PRESCOTT 1948, SCHWIMMER and SCHWIMMER 1955). We conclude therefore indirectly from our observations that the food chain which is established in the *Nodularia* flocks is mainly based on bacteria and bacteria production.

The mineralization of organic substrates by bacteria and zooplankton may even establish a microzone of enriched inorganic nutrients in and around the *Nodularia* agglomerates, which will supply the living *Nodularia* filaments and the epiphytic diatoms (mainly *Navicula*) with their inorganic nutrient requirements (HORSTMANN, pers. comm.).

The complex microecosystem of the *Nodularia* flock biotope still includes many unresolved problems which need further investigation. I want to point out the controversial observations of a high bacterial activity in the biotope and the stability of the *Nodularia* material towards bacterial attack together with maintenance of a high bacteria standing stock in the presence of high numbers of grazers. Many experiments especially on predation of microzooplankton on bacteria have to be undertaken to prove the hypotheses presented concerning the life cycle of the *Nodularia* biotope in this paper.

Acknowledgements

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