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12

GROWTH PATTERNS OF NATURAL SEAWATER BACTERIAL COMMUNITIES INCUBATED *IN SITU*

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ABSTRACT - Using *in situ* incubations in dialysis bags of natural bacterial communities, the change in growth rates over a 2-day period has been studied for the community as a whole and for different morphological groups within the community. A sequence of events during growth of both communities well above (4m) and well below (60m) the thermocline in the stratified waters, close to a shallow sea front in the Irish Sea, was noted. There was evidence not only of a periodic pattern of growth of the whole bacterial community but also of synchronous cell division of groups within the community. An increase in mean cell volume of both 4 m and 60 m bacterial communities preceded an increase in specific growth rate which usually coincided with a decrease in mean cell volume. The 4 m community as a whole and both the coccoid and rod forms had doubling times close to one day. However, more coccoid forms divided during the night while more rod forms divided during the day. Conversely, at 60 m, more rods divided during the night while having the same doubling times as those found at 4 m. More of the coccoid forms divided during the day and they had a slower doubling time (nearly 3 days) than that found at 4 m. This decreased the doubling time of the whole 60 m community to 2 days. There are distinct differences in numbers of phytoplankton, protozooplankton, macrozooplankton and fish between the waters above and below the thermocline. Some of these organisms exhibit diurnal rythms and their possible influence on the growth rates and rhythms of bacterial communities in each water mass is discussed.

Key words : Growth rates, bacteria, diel changes, *in situ*, fronts.

RÉSUMÉ - Les modifications du taux de croissance d'une communauté naturelle de bactéries, prise dans son ensemble, et des différents groupes morphologiques la constituant sont étudiées sur une période de 2 jours par incubations *in situ* dans des sacs à dialyse. Les phénomènes successifs survenant durant la croissance de la communauté sont étudiés à la fois nettement au-dessus (4m) et au-dessous (60m) de la thermocline dans des eaux stratifiées, à proximité d'un front côtier en mer d'Irlande. Il est mis en évidence, non seulement des phases périodiques de croissance sur toute la communauté bactérienne, mais également une division synchrone des cellules dans les différents groupes de la communauté. Une augmentation du volume moyen des cellules des deux communautés des 4 et 60 m précède une augmentation du taux de croissance spécifique, qui coïncide généralement avec une diminution du volume cellulaire moyen. La communauté présente à 4 m, dans son ensemble, mais aussi les deux groupes morphologiques (cocci et bâtonnets) qui la composent, ont des temps de génération proches de la journée. Toutefois, la plupart des formes coccoïdes se divisent pendant la nuit, et la plupart des formes en bâtonnet se divisent pendant la journée. Réciproquement, à 60 m, la plupart des bâtonnets se divisent pendant la nuit, tout en ayant le même temps de génération qu'à 4 m. La plupart des cocci se divisent pendant la journée et ont un temps de génération plus faible (presque trois jours). Ceci fait passer le temps de génération de l'ensemble de la communauté des 60 m à 2 jours. Des différences nettes sont observées dans les numérations de phytoplancton, protozooplancton, macrozooplancton et poissons entre les eaux situées au-dessus et au dessous de la thermocline. Plusieurs de ces organismes présentent des rythmes diurnes et leur

influence possible sur les taux de croissance et rythmes des communautés bactériennes dans chacune de ces masses d'eau est discutée.

Mots clés : Taux de croissance, bactéries, variations journalières,*in situ*, fronts.

INTRODUCTION

There have been only a few reports of diel changes in the growth of planktonic bacteria. Diurnal variation of bacterial biovolumes and cell numbers (Krambeck, 1978 & 1984; Krambeck *et al.*, 1981) and bacterial uptake of glucose and algal exudates (Straskrabova & Fuksa, 1982) have been found in freshwater environments. Meyer-Reil *et al.*, (1979) examined one water body in the Baltic Sea and found fastest glucose turnover times in the evening. Lochte (1985), however, found no distinct diel patterns in the heterotrophic turnover of glucose in a water body in the Irish Sea marked with a drogue. Both authors point out the difficulty of being sure that the same water body is sampled.

Rieman *et al.*, (1984) found diel changes in bacterial growth by directly monitoring water samples enclosed in bottles. However, enclosure in bottles can result in nutrient limitation, the degree of which varies with the water sample (Turley & Lochte, 1985).

By enclosing natural bacterial communities in dialysis bags and incubating them *in situ* we have attempted to overcome both the problem of following the same water body and that of nutient limitation in 'closed' glass enclosures. Assimilable dissolved organic carbon diffuses through the dialysis membrane to allow exponential growth of the bacterial community. This represents a simple and reproducible method of studying the *in situ* growth and production rates of marine planktonic bacteria (Turley & Lochte 1985).

The western Irish Sea front (Fig. 1a) is a shallow sea tidal mixing front. The waters to the northwest become stratified during spring and summer due to increased solar heating while the waters to the southeast remain tidally mixed throughout the year. The front is the gradient interface between the stratified and mixed water masses. The distinct water masses (Fig. 1b) associated with the front during spring and summer can therefore be sampled repeatedly.

Since aspects of the physical, chemical and biological oceanography of the experimental site have been intensively studied over the last years (Fogg *et al.*, 1985 a & b ; Lochte, 1985, Turley, 1985 ; Scrope-Howe & Jones, 1985 ; Egan & Floodgate, 1985 ; Kassab *et al*, 1985) it may be possible to relate any changes in bacterial growth to the environment.

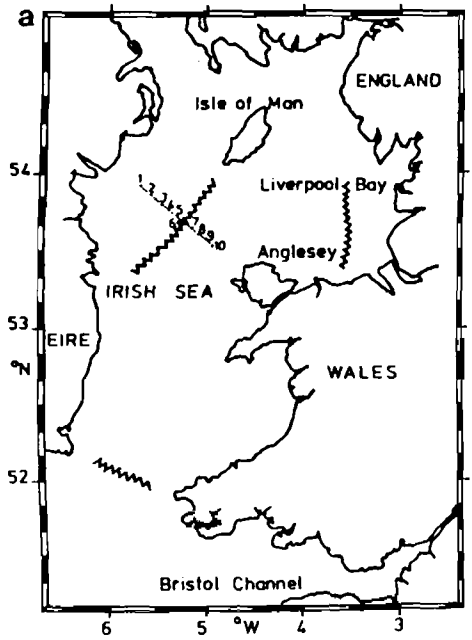
In this paper we present some of the data published by Turley & Lochte (1986) in combination with other data collected in the study site both during the course of the above experiment and at other periods. This highlights the complexity of the environment the bacterial community is exposed to, and the variety, the spatial and temporal variability in the sources of potential substrates available for microheterotrophs.

METHODS

Sea water samples were taken aseptically well above (4 m) and below (60) the thermocline in the vicinity of the front (station 5. Fig. 1a) on 6 July 1982. A subsample was filtered under sterile conditions through 3 μm pore size Nuclepore polycarbonate filters to remove grazers. One litre dialysis bags were filled with the filtered and unfiltered subsamples and incubated *in situ* for 48 h. At intervals samples were removed for enumeration of bacteria and determination of their cell volume and shape by an epifluorescent direct count technique (Daley & Hobbie, 1975 ; Hobbie *et al.*, 1977). At the end of the experiment sections of the dialysis tubing were examined by SEM. No colonization or pitting of

the dialysis tubing surfaces was found after the 48 h incubation. Detailed descriptions of the sampling, incubation and counting and SEM methods are given in Turley & Lochte (1985).

Specific growth rates (μ_n) (1/day) over the whole of the incubation time of the bacteria in the dialysis bags were calculated by regression ($\ln N_t = \ln N_0 + \mu_n.t$) taking bacterial numbers into account, where N_0 is the number at the beginning of the experiment, N_t is the number at time t (days). Specific growth rates (μ'_n) (1/day) were also calculated between sampling times (t_1 and t_2) by $\mathrm{LnN}t_2 - \ln N t_1 / t_2 - t_1$. Doubling times were calculated by $0.693/\mu_n$.



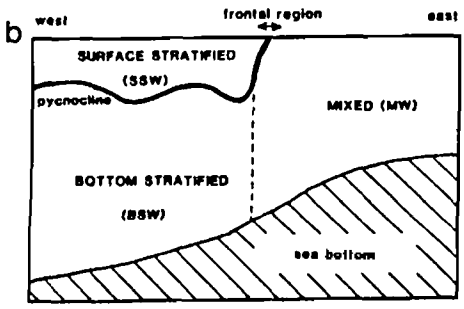


Figure 1 : a) Map of the Irish Sea showing the position of fronts (jagged lines) and the transect across the western Irish Sea front.
b) Schematic diagramme showing a section normal to the front and the differentiation of the three water masses.

Novitsky & Morita (1976 & 1978) and Amy *et al.* (1983) found that cultures of a starved marine vibrio can change cell size and shape. While we do not rule out the possibility that the natural bacterial community may undergo similar transformations, until such time that the natural bacterial community can be divided or "tagged" on a basis of substrate specificity, the differentiation of "rod" and "coccoid" morphologies is a convenient way of separating potentially different microbial communities. Both μ_n and μ'_n have therefore also been calculated for the two major morphological groups, the coccoid and the rod shapes.

Primary productivity and heterotrophic uptake of phytoplankton exudates were determined by uptake of ^{14}C-bicarbonate (Strickland & Parsons, 1972) on subsamples incubated *in situ* for different times over the 24 h of the dialysis bag experiment (Lochte & Turley, 1985). The amount passing a 1 μm pore size filter and retained on a 0.2 μm filter was taken to be the heterotrophic uptake of phytoplankton exudates (Larsson & Hagström, 1982).

The number of protozooplankton was determined by inverted microscopy according to Utermöhl (1958). For each sample 200 ml was settled. Protozooplankton >50 μm in diameter were counted at 100x magnification in the whole chamber. Smaller organisms were counted at 400X magnification in two transects of the counting chamber.

RESULTS AND DISCUSSION

At 4m the mean cell volume, although generally increasing over the duration of the experiment (Fig. 2a), had periods of no increase or, in the case of the unfiltered dialysis bags, even a decrease in cell volume during the night. Also synchronous changes in the specific growth rate of the whole community were noticable (Fig. 2b). Rapid increases occurred in the mid afternoon or early evening which stabilized at night. This indicates that there is a sequence of events during the growth of the bacterial community at 4m. From morning to early evening (of the first day) there was an increase in mean cell volume. During this period there was no significant change in the specific growth rate of the whole population. Just after dusk the specific growth rate increased dramatically while the mean cell size remained constant or even decreased. During the rest of the night and the following day the specific growth rate remained at this elevated rate. By the late afternoon another rapid increase in the specific growth rate occurred and the pattern of events was repeated. It should be remembered that many people have found, using microautoradiography (Hoppe, 1976), that not all the bacterial population is active - at least not at the same time. These results indicate that a significant part of the population was undergoing synchronous division, certainly sufficient to superimpose this periodic step pattern of specific growth rates on the whole bacterial population.

At 60 m, in contrast to the surface waters, specific growth rates for the whole bacterial community did not increase with time but rather had periods of rapid growth and periods of little growth (Fig. 2c). During the daylight of the first day there was an increase in the mean cell volume of the bacterial community (Fig. 2a) but no detectable increase in cell numbers (Fig. 2c). During the night cell volumes decreased and stayed low for the next 24 hours when specific growth rates were high. In the last 12 hours mean cell volume increased while change in specific growth rates decreased rapidly. This latter change in mean cell volume, although small compared to that seen at 4 m, was significant and due to a small shift in the percentage of bacteria in the size class "minibacteria" into "large cocci" and a slightly larger shift in the percentage of bacteria in the size class "small rods" into "large rods" (Turley & Lochte, 1986).

This relationship, seen at 4 m and 60 m, between specific growth rate and mean cell volumes seems to indicate that cells increase in size before dividing (Krambeck 1984). It must be remembered that the specific growth rate for the whole population is influenced by the growth of the individual members of the community.

The specific growth rates for the 4 m whole bacterial community (Table 1) was over twice as high as that at 60 m. Indeed, it was found that bacterial productivity was 4 times greater at 4 m than at 60 m (Turley & Lochte, 1985). At 4 m both rods and cocci grew at the same rate (Table 1). At 60 m, while the rods had a similar growth rate to those at 4 m, the cocci were growing at a much slower rate. There is, therefore, an indication that there was a difference in the growth rates of different members of the community.
At 4 m rods had higher specific growth rates during the day while more coccoid forms divided at night (Fig. 3a). Therefore, part of each morphological group must have exhibited synchronous cell division. It would seem that the periodic step pattern, seen in the specific growth rates for the bacterial community as a whole (Fig. 2b), reflects the combination of growth rates for both coccoid and rod forms. It is interesting that both groups, the cocci and the rods, had doubling times of very close to one day (Table 1) besides having diel periods when more cells divided (Fig. 3a). This may indicate that the growth rates and even the times of most rapid growth are influenced by diurnal rythms of other physical, chemical or biological variables around them. Indeed, diel variations of biologically labile organic compounds have often been observed (Sieburth *et al.*, 1977,

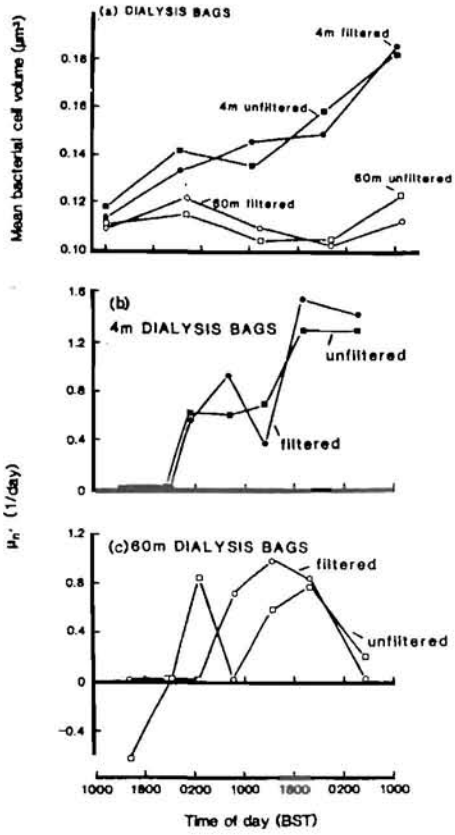


Figure 2 : a) Change in the mean cell volume and b) & c) specific growth rates of natural bacterial communities incubated in dialysis bags *in situ*, from 6-8 july 1982, above (4 m) and below (60 m) the thermocline in stratified waters (station 5, fig. 1a) of the western Irish Sea.

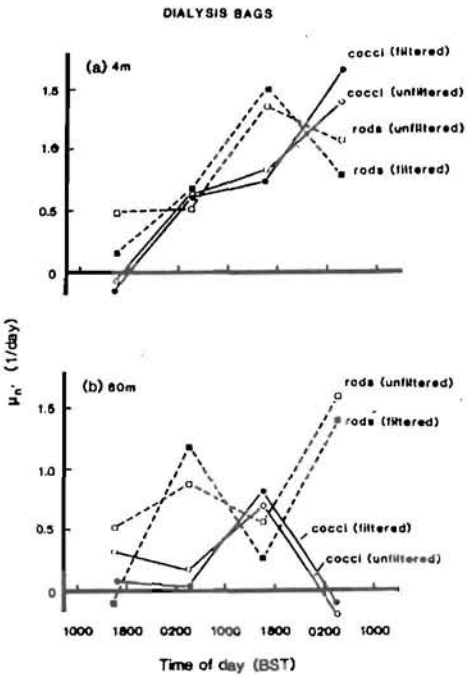


Figure 3 : Changes in specific growth rate (μ_n') of different morpholigical groups of natural bacterial communities incubated in dialysis bags *in situ*, from 6-8 july 1982, above (a) and below (b) the thermocline, at 4 m and at 60 m respectively, in the stratified waters (station 5, fig. 1a) of the western Irish Sea.

Burney *et al.*, 1981a; 1981b & 1982, Meyer-Reil *et al.*, 1979; Sellner 1981, Mopper & Lindroth, 1982) and diel release and uptake of these compounds has been ascribed to coincidentally related biological processes.

At 60 m, the coccoid and rod groups (Fig. 3b), like those at 4 m, were out of phase with each other. However, the synchronous growth of each of these groups was at different times of the day when compared to the morphological groups at 4 m.

These data highlight three pertinent questions. Firstly, why were the growth periods of the coccoid and rod forms out of phase with each other? Secondly, what were their changes in growth rates synchronised to? Thirdly, why were their periods of rapid growth at different times of the day at 4 m and at 60 m? To attempt to answer any of these questions it seems essential to examine the possible sources of assimilable organic matter in each water mass.

The primary production and the phytoplankton exudate uptake by microheterotrophs during the first day of the experiment is shown in Figure 4. Exudates were mainly taken

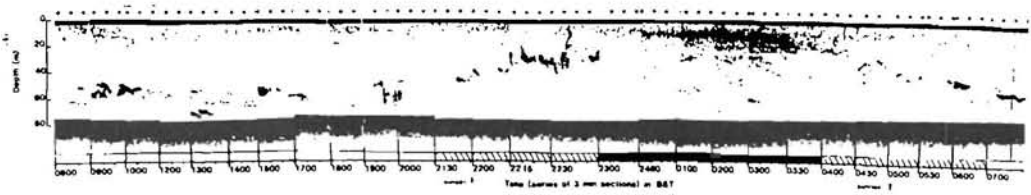


Figure 6 : Photomontage of 3 minute sections of an echosound trace to indicate the movement of the deep-scatter layer over 24 hours on an anchor station (Figure 1, station 5) in july 1982, in the stratified waters close to a shallow sea tidal mixing front in the western irish sea

We have presented evidence which indicates that there is diel growth of bacteria, that this is due to synchronous growth of different parts of the bacterial community and that these patterns of growth depend on the characteristics of the water masses under investigation. Finally, we have proposed, using circumstantial evidence, that this may be due to the bacteria being synchronized to the diel rhythms of dissolved organic carbon release by other organisms. Indeed, it would seem that if this hypothesis were so the biochemical events within a bacterium, such as enzyme production etc., may be synchronized to the endogenous rhythms of an organism it may never have any direct contact with and several fold its size.

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