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OBSERVATIONS ON THE BACTERIOLOGY OF FRONTS: SOME PROBLEMS OF METHODOLOGY

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RESUME

OBSERVATIONS BACTERIOLOGIQUES SUR DES SYSTEMES FRONTAUX : QUELQUES PROBLEMES DE METHODOLOGIE

Les résultats obtenus lors d'études effectuées pour apprécier les aspects bactériologiques de deux systèmes frontaux, ont montré que l'homéostasie et la complexité du milieu dans ces zones, jointes à l'imprécision des données, conduisent à des valeurs de biomasse difficiles à interpréter en relation avec les facteurs physiques régissant la formation des fronts.

Bien que l'étude de caractères biochimiques permette d'espérer l'obtention d'informations plus fiables et prétant moins à confusion, on souligne la nécessité de développer des méthodes à la fois très précises et rapides, permettant un échantillonnage très fréquent.

INTRODUCTION: The pattern of currents in the oceans have long been known to affect the biology of the seas. In shallow coastal seas as a result of complex interaction of physical phenomena such as solar heat input, wind and tidal velocities and salinity changes, the water of certain areas becomes stratified, the layers being separated by a thermocline or pycnocline. Where the two water masses meet the transitional zone, known as a front or discontinuity, is characterised by a tendency of the water currents to produce a vertical flow. A wide variety of biological forms including cretaceans, fish, sea snakes, birds as well as many planktonic organisms are reported to be influenced by the fronts both as regards their pattern of distribution and their rates of productivity (Owen 1981).

While the effect of discontinuities on many types of organisms has been investigated, the bacteriological aspects have been largely ignored. There are a number of possible mechanisms which could modify the distribution and productivity of bacteria in the frontal area and its associated water masses. It is known that bacterioplankton is patchy in its distribution (Ashby & Rhodes-Roberts 1976). In addition there is a tendency for seston to concentrate at a front, and so increase the local concentration of both bacterial biomass and the rate of mineralisation. Some workers (Savidge 1976; Savidge and Foster 1978) have suggested the possibility that primary productivity may be stimulated at a frontal zone when waters of different origins mix if each supplements the nutritional deficiencies of the other. Finally, the time scale of the bacterial response has also to be considered. The homeostatic tendency of the environment will tend to restore the status quo rapidly, so that without a high frequency sampling programme, the change is not observed. The bacteriologist attempting to investigate the effect of fronts is faced with a number of methodological problems. The only two ways in which the problem can be approached is to count the bacteria and hence calculate their biomass and observe any variations between the stratified and mixed water, or to estimate an "activity" depending on an aspect of their biochemistry. Both these approaches and their associated problems have been recently reviewed (Costerton and Colwell 1977, Floodgate 1980). This paper deals with some surveys using the biomass approach.

<u>RESULTS.</u> During the last few years a number of workers at the Menai Bridge Laboratory have been examining the bacterial aspects of two Fronts; one formed in Liverpool Bay (Floodgate et al., 1981) is mainly dependent on salinity differences in winter and heat input in summer, while another in the Western Irish Sea is found in summer and is due to surface heating. Figure 1 shows the location of both fronts and the sampling stations for Liverpool Bay. Bacterial numbers were determined by ultra-violet epifluoresence microscopy using acridine orange stain, the technique being based on that of Daley and Hobbie (1975), Jones and Simon (1975) and Hobbie et al., (1977). In addition, the bacteria were divided into four arbitary volume classes of "minibacteria" $0.004-0.1 \mu m$ cocci $0.1-0.38 \mu m$, rods $0.03-0.2 \mu m$ and large rods $0.2-0.7 \mu m$. Hence the biomass was calculated in $\mu g C m l^{-1}$. Adenosine triphosphate (ATP) was measured using the luciferin technique and biomass calculated using a conversion factor of $250 \mu g C$ per $\mu g A TP$ (Holm Hansen and Booth 1966). Physical data was also collected to calculate the density σ_t and the stratification parameter \overline{V} (Simpson and Pingree 1978).

Four cruises were made to the Liverpool Bay front during the first four months of 1978 and reported by one of us (Lochte 1979). Only during the mid February cruise was the stratification parameter \overline{v} sufficiently high to indicate well established stratification at all stations, except 12 and 13 which were mixed on all occasions. Bacterial biomass followed a similar pattern to \overline{V} (Fig. 2). During the other three cruises, only weak stratification was found with the point of maximum values \overline{V} varying between stations 14 and 16a. Nevertheless, the distribution pattern of the bacterial biomass continued to show a degree of regularity except for the final cruise in late April. In the first three cruises the lowest biomass and cell sizes were found in the permanently mixed stations 12 and 13 (Fig. 2), suggesting that these represent a different population from those at the more landward stations. The change of bacterial numbers along the transect was constant at about two orders of magnitude, the major part of the change taking place across a comparatively small distance. This change is presumably significant. The distribution of the bacteria tended to be vertically orientated as did the density, salinity and temperature. The area of maximum change in bacterial numbers occurred at approximately the same area as the maximum σ_t change on two occasions but not on the third. On the fourth and final cruise in late April 1978, the bacteria tended to form horizontal rather than vertical strata for reasons that cannot be explained on present evidence. Lochte (1979) tentatively suggested that the front acted as a barrier tending to keep the two populations separate. However, it is impossible to conclude with any certainty from these data that there is a casual relationship between the fronts and the bacterial distribution, but that there is a sufficient consistency of pattern to suggest that there is a probably complex relationship of some sort.

A notable feature was that the ATP values did not in any way correlate with the other bacteriological parameters. We find that the ATP method of estimating biomass works very well with pure cultures but it is more difficult to obtain results that can be interpreted with certainty in the natural environment. Although the algae concentrations as measured by chlorophyll data were at times very low, it seems likely that the major portion of the ITP came from the algal part of the microbiomass.

A contrasting set of results was obtained by one of the authors (B. Egan) in his studies on the Western Irish Sea front. This front has been visited on a number of occasions during 1980 and 1981 both during periods when the front was well established in the summer and when the area was totally mixed in winter. The outstanding feature is the general consistency of biomass on either side of the front and the area of mixing. A typical result is shown in Fig. 3 taken when a front was well established between stations C and G.

During the same cruise as is illustrated in Fig. 3, the ship was kept in the same body of water for 15 hours at a station in the stratified water by following a drifting buoy. The results are shown in Fig. 4 and it will be seen that over this period there was very little variation in the bacterial numbers again suggesting a uniformity of pattern both with regard to time and space. However, it was also found that, on some occasions, the bacterial numbers would rise unexpectedly to a much higher value for no apparent reason. These data indicate that either the front has no effect on the bacteriology or biomass is not a good indicator of these effects as far as this front is concerned. It was thought possible that if biomass measurements are not sensitive enough to detect subtle changes in the bacteria, techniques based on biochemical changes may be more successful and subsequent work indicates this may be so. DISCUSSION. It is reasonable to expect that the distribution of bacteria and biomass will reflect the chemical and physical environment in which it is found. In the case of Liverpool Bay front there was a change in numbers that suggested a relationship, albeit complex, between the biomass and the hydrography, at least during winter, but this was not so in the Western Irish Sea. There are several possible explanations of this. Firstly, the physics of frontal systems is complex, and the simplistic view of the structure of fronts that has been used in these investigations may not be adequate to explain the bacteriological changes. In addition, the counting and biomass assessment methods used may not be precise enough to reveal subtle changes. Biomass variation may be due either to advection concentrating the bacteria by a purely physical process or by a secondary process, as for example, if the effect of the front is to increase the phytoplankton biomass with subsequent effects on the bacteria. Now the precision of the sampling and counting techniques are such that for a significant increase in bacterial biomass to be established a considerable amount of available organic matter has to be produced particularly when it is remembered that the conversion factor of organic matter to biomass is about 0.4. In an area of turbulence moreover the body of water containing the increasing population may have moved into or out of the sampling station. In addition, grazing will also reduce the numbers considerably. In all environments a resilient homeostatic stability tends to revert the environment to its former state after a perturbation. Major changes in the bacteriology may be short lived so that, since data can only be obtained at fairly lengthy intervals, the important variations may be missed. It is clear that in attempting ecological investigations of this kind, it is unwise to use one bacteriological indicator only; but above all there is a requirement for improved methods that will give detailed information on the bacteriology more frequently and more precisely than are available at present.

SUMMARY: The data obtained in surveys to investigate the bacteriological aspects of the biology of two contrasting frontal systems has shown that the inherent homeostasis and the complexity of the environment, together with the imprecision of the data, lead to biomass values that are difficult to interpret in terms of the physical factors governing the formation of fronts. Although biochemical measurements offer some promise of providing more reliable and less confusing information, the need to develop high precision methods that can be made very frequently is emphasised.

LIST OF KEY WORDS

Bacteria, discontinuities, fronts, Methodology.

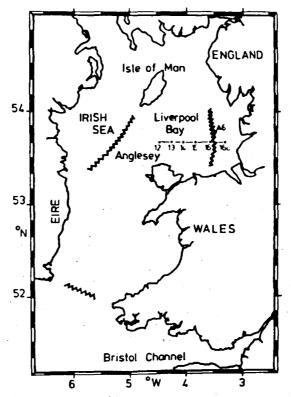


Fig. 1 Position of Liverpool Bay and Western Irish Sea fronts.

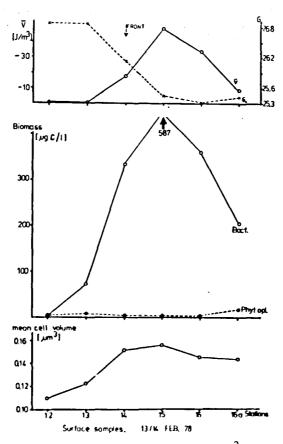
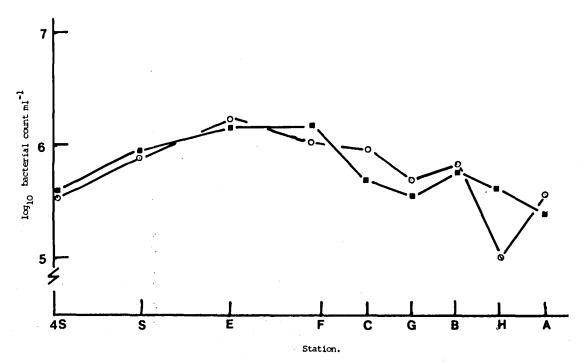
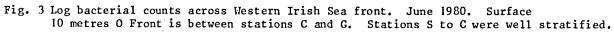
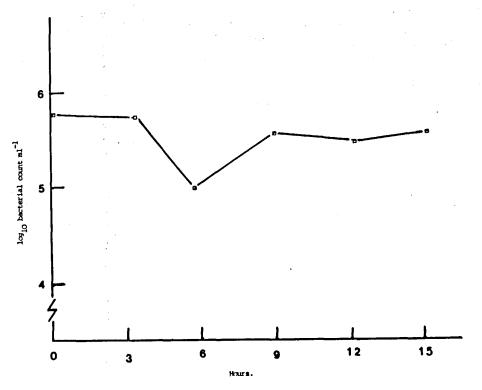


Fig. 2 Top; distribution of stability factor \overline{V} (Joules m⁻³) 0 and surface density $\sigma_t X$ Middle: bacterial 0 and phytoplankton biomass 0 in surface water. Bottom; mean cell volume in surface water along reference line indicated in Fig. 1 on 13-14 February 1978.









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