Distribution of Two Groups of Bacteria, Oligotrophs and Eutrophs, in the Indian Ocean and the South China Sea

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Abstract

The distribution of two trophic groups of bacteria, oligotrophs and eutrophs, was studied. The former group grows only in nutrient-poor media, while the latter grows in nutrient-rich media. Sea water samples for bacteriological examination were collected from areas in the eastern Indian Ocean and the South China Sea at nineteen stations during a cruise of the R. V. Hakuho-Maru, KH-76-5. Organic nutrient-rich and nutrient-poor media were prepared for counts of viable bacteria. The vertical distribution of oligotrophs was essentially the same as for eutrophs. In general, the concentration of oligotrophs was comparable to that of eutrophs. One of the features of the vertical distribution of bacteria was that the maximum and minimum layers were usually found in the subsurface layers. This minimum layer corresponded to the location of the thermocline. The maximum bacterial layer frequently coincided with the maximum chlorophyll layer, especially in the Indian Ocean. This suggests that one of the important factors involved in bacterial population sizes is the presence of phytoplankton.

Introduction

The determination of the number of heterotrophic bacteria in a marine environment has generally been carried out by counting colonies in nutrient-rich media after a relatively short incubation period. Natural sea water contains an extremely low concentration of organic matter compared with the artificial media usually used for counting the viable bacteria. Heterotrophic bacteria living in a natural marine environment are sufficiently nurtured by this nutrient-poor ambient water. However, the concentration of organic nutrients in natural sea water has often been disregarded in microbiological observations. It has been reported that diluted media can support a higher number of colonies (Jannasch and Jones, 1959), and, recently, several workers (Ishida and Kadota, 1974; Akagi et al., 1977; Mallory et al., 1977; Yanagita et al., 1978) studied a group of bacterial populations in marine and fresh water environments that could not grow in a nutrient-rich medium, such as ZoBell 2216 (ZoBell, 1941).

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The definitions and the methods for isolating this group of bacteria (oligotrophic bacteria) are somewhat different among the studies cited above. The existence of bacteria which grow only in nutrient-poor media would suggest that there is a need to reevaluate ecological studies of bacteria in aquatic environments. Quantitative studies of oligotrophic bacteria in the open ocean are still geographically limited.

Yanagita et al. (1978) defined two trophic groups of bacteria, oligotrophs and eutrophs, living in marine as well as in fresh water environments. The former group can grow only in nutrient-poor media, while the latter can grow in nutrient-rich media as well. The present study describs here of the quantitative distribution of bacteria in the tropical and subtropical open ocean using the method proposed by Yanagita et al. (1978).

Materials and Methods

Water samples and bacterial filtration. Sea water samples for bacteriological examination were collected from nineteen stations in the Philippine Sea, the eastern Indian Ocean, and the South China Sea from December, 1976, to March, 1977, during a cruise of the R. V. Hakuho-Maru, KH-76-5. The station locations are shown in Fig. 1. The water samples were collected with sterilized microbiological samplers attached to Van Dorn bottles or Nansen bottles. The sampling was carried out in four to six different layers at each station. The samples were immediately filtered through sterilized 25 mm HA-type Millipore filters fixed in Swinnex filter holders (Millipore Filter Co.). One to fifteen milliliter portions of raw sea water were filtered

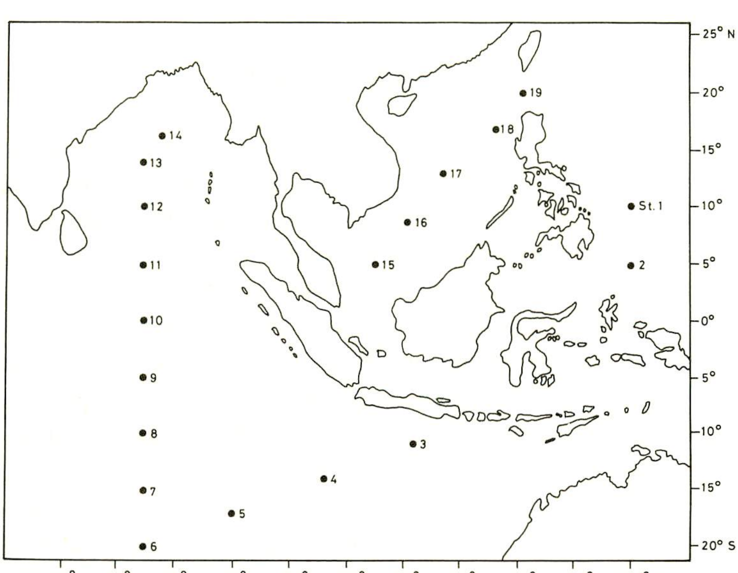

Fig. 1. Location of sampling stations during the R. V. Hakuho-Maru KH-76-5 cruise, December 1976–March 1977.

depending on the bacterial density. The filters were placed on agar plates in Petri dishes as described below.

Counting oligotrophs and eutrophs. Organic, nutrient-rich and -poor media were prepared for counts of viable bacteria. The nutrient-rich medium (medium R) contained 10 g of polypeptone (Daigo-Eiyo Chem. Co.), 5 g of yeast extract (Nissui Pharm. Co.), and 15 g of agar (Difco Lab.), in one liter of filtered sea water, while the nutrient-poor medium (medium P) contained only 10 mg of polypeptone and 15 g of agar. The pH of these sea water media were adjusted to 7.8.

Duplicate filtrations were carried out through Millipore filters and the filters were placed on both the rich and poor media in Petri dishes. The bacteria collected on the surface of the filters were grown for two to three days, for medium R, and usually five to seven days for medium P, at 20°C. After incubation, the number of colonies grown on the filters were counted by the eye, for medium R, and under the dissecting microscope (×40) for medium P. The size of the colonies formed on medium P was generally small (less than 50 μm in diameter) and were transparent.

Yanagita et al. (1978) found that eutrophs isolated in fresh and sea water gave a similar number of colonies on R and P media. This means that the number of colonies formed on medium P contains both eutrophs and oligotrophs. Based on this finding, they assumed that the number of oligotrophs was determined simply by subtracting the total number of colonies on medium R from that on medium P. The author used the method described above for counting oligotrophs in the present study.

The routine oceanographic observations obtained during the KH-76-5 cruise were carried out by a joint effort of scientists and ship's crew, and the results are being published by The Ocean Research Institute, University of Tokyo. The author utilized chlorophyll data as needed.

Results and Discussion

We recognized the presence of two groups of bacteria, oligotrophs and eutrophs, in the tropical and subtropical open ocean. The results clearly demonstrate that using an organic, nutrient-rich medium is improper for enumerating heterotrophic bacteria living in a natural sea water environment because the agar plate method, using a nutrient-rich medium, can miss significant groups of bacteria (oligotrophs) which are actually present. This is due to some groups being obscured by the medium. The colony counts on medium P exceeded those on medium R in 74 samples, or 80 % of the 92 samples studied. The present method assumed that eutrophs could develop colonies on both P and R media, so the colony numbers of medium P should exceed or equal those on medium R. The opposite case was observed in 20 % of the samples. This contradiction was found in coastal eutrophic areas of the Japan Sea (Yanagita et al., 1978). It was thought that eutrophic water contained a group of bacteria that could grow only in a nutrient-rich medium (obligate eutrophs). In the present study, the contradiction can not be explained by the presence of obligate eutrophs. Because of the complexity of the trophic nature of bacteria there are some unsolved problems.

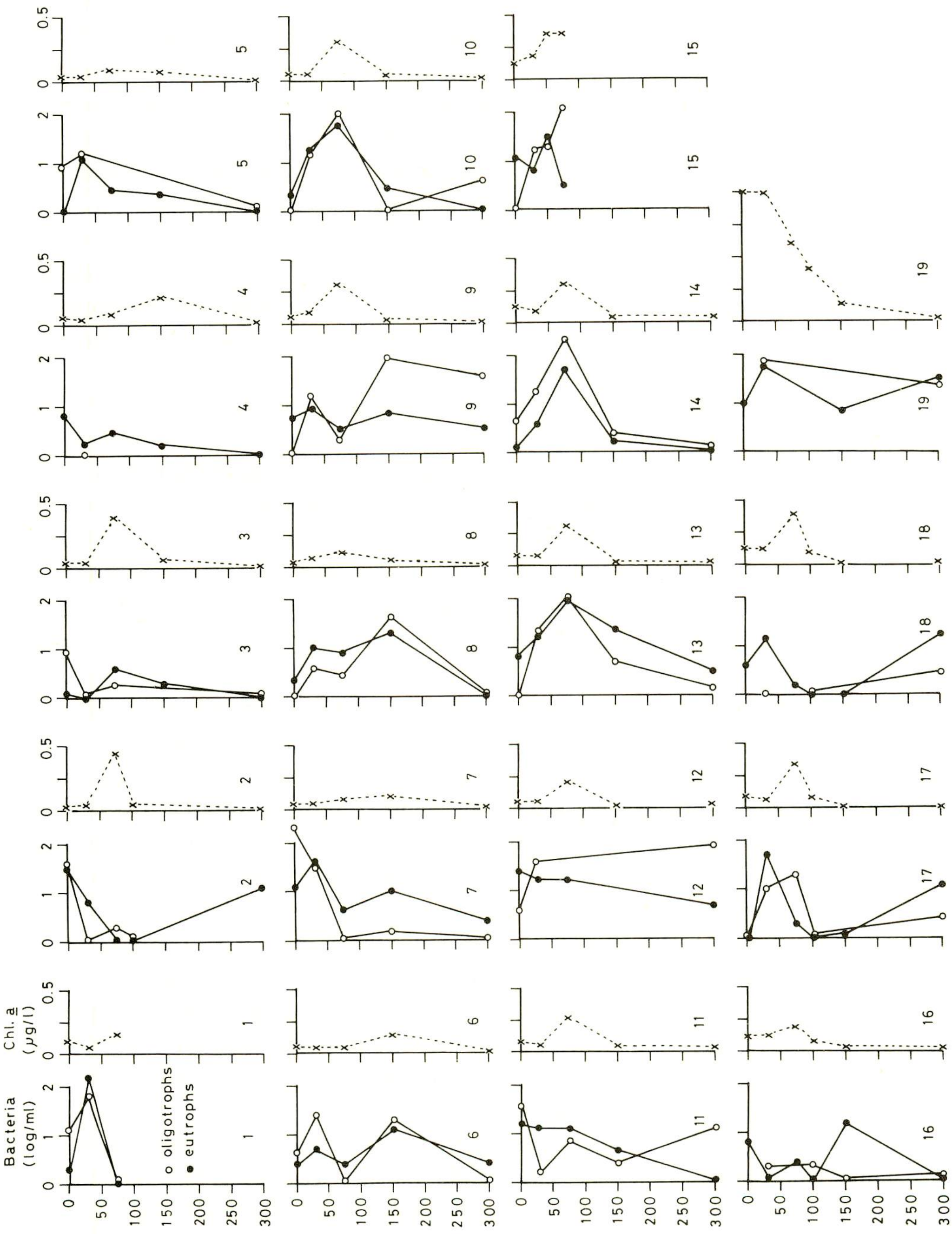

Fig. 2. Vertical profiles of oligotrophs, eutrophs, and chlorophyll *a* at all of the stations studied.

Fig. 2 shows the vertical profiles of oligotrophs, eutrophs, and chlorophyll *a* at all of the stations studied. There were fluctuations in the vertical distribution of bacterial concentrations to a depth of 300 m. However, the pattern of vertical variation for oligotrophs was essentially the same as for eutrophs. In general, the concentration of oligotrophs was comparable to that of eutrophs. One feature of the vertical distribution of bacteria was that the maximum and minimum layers were usually found in the subsurface layers. The subsurface maximum concentrations frequently exceed the surface values (cf. Sts. 10, 13). Below the bacterial maximum layer, the bacterial concentration decreased with increasing depth down to 300 m (cf. St. 5), or the concentration increased again with a pronounced minimum layer (cf. St. 17). This minimum layer corresponded to the location of the thermocline. Minimal counts at the thermocline have been reported by Jannasch and Jones (1959).

Although the general range of the concentrations of oligotrophs and eutrophs in the three regions was of the same order, there were some regional difference worth noticing. The bacterial distributions in the Indian Ocean were characterized by bacterially rich water in the subsurface layer. The largest number of oligotrophs (St. 14, 75 m) for all of the stations sampled were found in this area. The vertical distribution of oligotrophs was nearly identical to that of eutrophs except that, oligotrophs increased at 300 m (cf. 10, 11, 12) while eutrophs decreased. The general concentration of eutrophs was nearly that of heterotrophic bacteria reported by Kriss et al. (1967) in the tropical and subtropical Indian Ocean.

In the South China Sea, the greatest number of oligotrophs was observed on the continental shelf (St. 15, 75 m) while the highest concentration of eutrophs was found in the central part of the area (St. 17, 30 m). A characteristic increase in eutrophs concentration at 300 m was observed in the South China Sea (Sts. 17, 18, 19). This situation was also observed in the Philippine Sea (St. 2) but not in the Indian Ocean. Of all of the bacteria (oligotrophs plus eutrophs) the proportion of oligotrophs differed widely from station to station as well as from various depth within each station The average proportions of oligotrophs at each of the three areas, the Philippine Sea, the Indian Ocean, and the South China, Sea, were 47, 38, and 31% respectively. This is partly due by the tendency of the Philippine Sea and the Indian Ocean to present a somewhat more oligotrophic environment (ex. chlorophyll concentration) than the semi-enclosed South China Sea. The overall mean percentage of oligotrophs did not exceed that of eutrophs using the present method.

It is noteworthy that the maximum bacterial layer frequently coincided with the maximum chlorophyll layer, especially in the Indian Ocean (Fig. 2). Although the positive correlation between bacterial concentration and chlorophyll content for all samples was statistically poor, the coincidence of the bacterial peaks with the chlorophyll maxima was observed in 10 out of 19 stations. This suggests that one of the important candidates controlling bacterial population size is the presence of phytoplankton. While Yanagita et al. (1978), suggested that nitrate concentration was important in limiting the population size of both oligotrophs and eutrophs in sea water, we did not find that to be the case. Akagi et al. (1977) showed that oligotrophic bacterial concentrations correlate with the amount of dissolved carbohydrate.

Bell and Lang (1974) experimentally demonstrated that the growth of marine bacteria was stimulated or inhibited by the release of the extracellular products of *Skeletonema costatum*. This suggests that natural bacterial population sizes in the open ocean are both stimulated and inhibited by the presence of the extracellular products of phytoplankton.

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