Heterotrophic Activity in the Mauritanian Upwelling in March 1973: Assimilation and Mineralization of Amino Acids

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1. Introduction

The heterotrophic bacteria in natural waters are extremely difficult to study because of difficulties encountered in biomass measurement and in activity measurement.

Firstly, the bacteria are known to be the smallest living organisms in seawater, and they cannot be fully separated by differential filtration because the smallest phytoplankton cells and the larger bacteria are equal in size. Moreover, the bacteria are commonly associated with the detrital particles, which are larger than bacteria, so they are retained by membrane filters with greater porosity than the true size of the bacteria. Also, it is not possible to measure directly the carbon or nitrogen of bacteria in a sample of seawater.

Secondly, the bacteria do not contain a specific compound, such as chlorophyll in phytoplankton cells. Although the carbon-chlorophyll relation is not clear, the chlorophyll measurement associated with other parameters (e.g., specific determination) allows the comparison of phytoplankton abundance in two oceanic regions. This is not possible for bacteria.

Thirdly, in contrast to photosynthetic algae, which use only carbon dioxide or bicarbonate, the heterotrophic bacteria may use many different sources of carbon that are not exactly known, extremely diversified, and that vary with the ambiant populations of phytoplankton and zooplankton. While the $^{14}CO_2$ added in primary production experiments behaves like natural CO_2 (except for isotopic discrimination), the simple labeled organic compounds added in the heterotrophic activity measurements do not reflect the overall organic matter present, and the uptake of labeled compounds does not preclude the behavior of the organic matter. Hence, the rate of production of bacterial cellular material and the rate of decomposition of organic matter under natural conditions are almost impossible to measure.

However, the study of the uptake of single substrates gives valuable information about the substrates that are being used and about the activity of bacteria, which are certainly the main organisms responsible for uptake of organic solutes at natural concentrations (Wright and Hobbie, 1966), although it is not possible to distinguish this from algal heterotrophic activity.

This paper deals with the uptake of amino acids in an upwelling area, during a bloom of phytoplankton. Comparisons are made with glucose uptake under the same conditions, a year before in the same area.

2. Methods

The satisfactory results obtained in March 1972 by following a drogue in the Mauritanian upwelling between Cape Timiris and Nouakchott (Herbland et al., 1973) led us to undertake a similar study in 1973 in the same area during the same season. The coldest water (15°C), the richest in nutrients (20 µgat l NO_3), and the poorest in oxygen (60% saturated) and chlorophyll (2 µg/l) were determined by surface survey, with continuous measurements. The area was east of a little canyon south of Cape Timiris where the depth of the bottom is 25-30 m. The drogue was set at a depth of 3 m, and followed for six days. Three times each day (6 h, 12 h, and 18 h) the ship was brought to the drogue, and a series of observations was made. Each series has a separate station number.

The methods used for measuring oxygen, nitrate, ammonia, organic phosphorus, and chlorophyll have been given in detail elsewhere (Herbland and Voituriez, 1974). The organic excretion of phytoplankton was measured after filtration of particulate matter, followed by the removal of $H^{14}CO_3^-$ by acidification (pH 2.5), bubbling (10 min), and direct

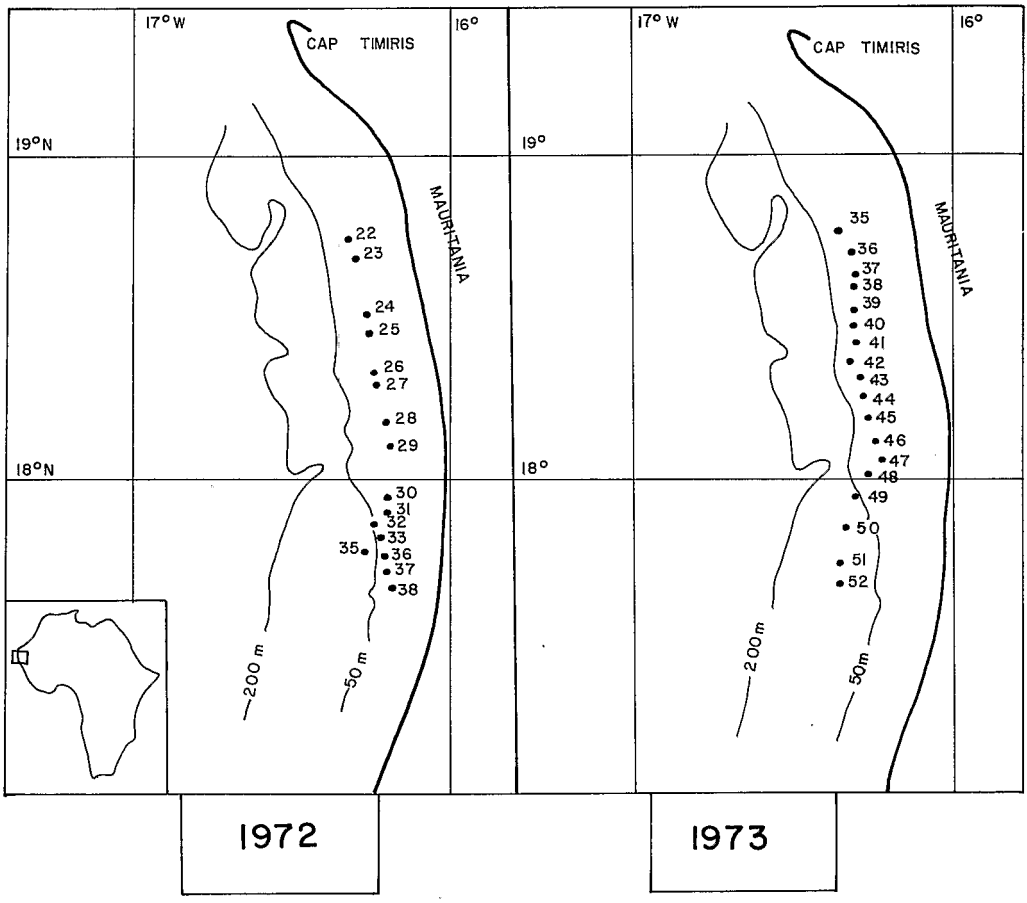


Fig. 1. Position of stations as determined by the location of the drifting buoy in 1972 and 1973

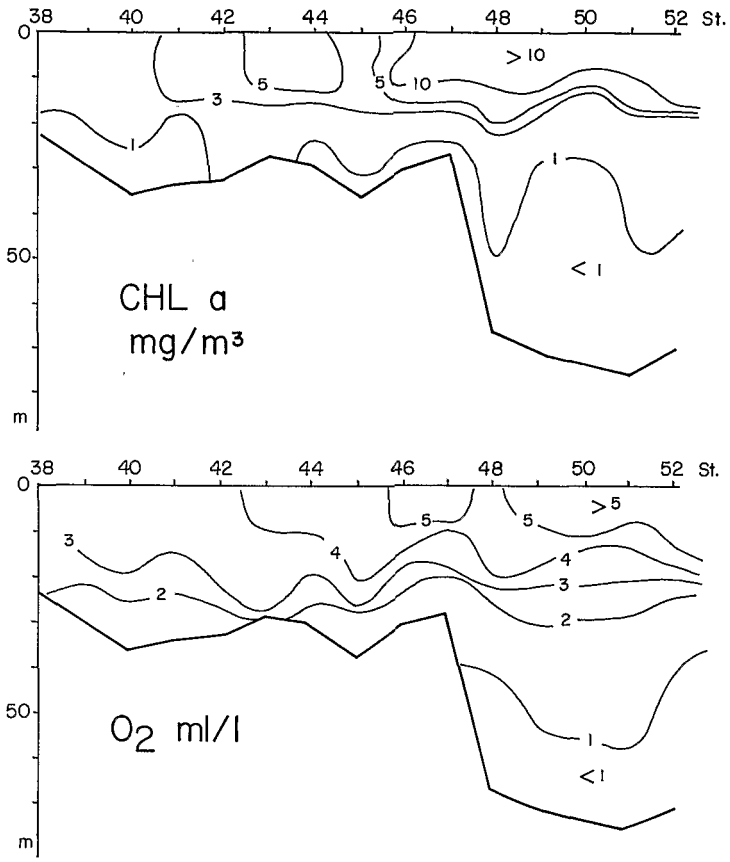


Fig. 2. Chlorophyll a and oxygen evolution during the drift of the buoy in 1973

counting of the remaining labeled organic compounds by liquid scintillation (Anderson and Zeutschel, 1970). The heterotrophic utilization (assimilation and respiration) was measured by 3-H incubation experiments, in the dark, with a ^{14}C amino acid mixture. The mixture contained glycine (11%), serine (29%), lysine (27%), aspartic (18%) and glutamic (15%) acids, at a total concentration of 15 µg C/l. After incubation, the filtration was made with low suction pressure (200 mmHg) on Millipore membrane filters. The CO_2 respired was trapped directly in a scintillation vial, suspended at the top of the conic incubation flasks, with one ml of hyamine hydroxide dropped on a glass fiber filter. The radioactivity was counted with a liquid scintillation spectrometer. For details, see Herbland and Bois (1974).

3. Results and Discussions

The tracks of the drogues were very similar from one year to the next (Fig. 1). They followed a course parallel to the shore, but in 1973, the drogue veered offshore on the last day.

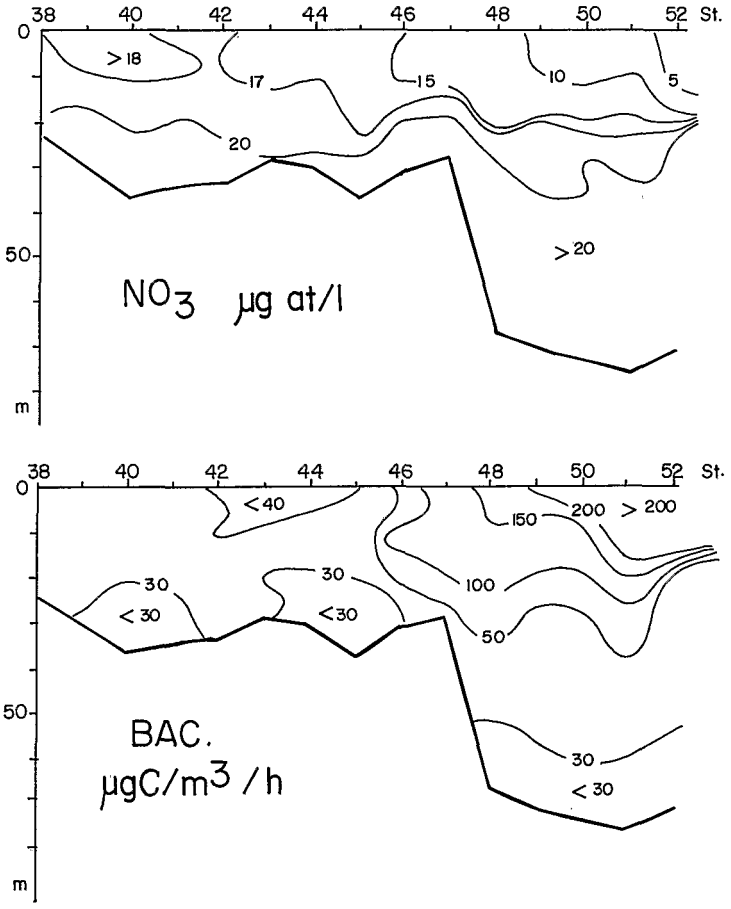


Fig. 3. Nitrate and heterotrophic activity evolution during the drift of the buoy in 1973

3.1 Heterotrophic Activity Evolution Versus Other Biologic Parameters

Where upwelling takes place (cold water), the chlorophyll a values are relatively low (2 µg/l). They increases regularly and reach 10 µg/l the third day and are higher than 30 µg/l the fourth and fifth days (Fig. 2). The oxygen concentrations follow parallel pattern. Initially the water is undersatured (60%). Saturation is reached the third day. The biologic production of O_2 is not sufficient to explain the increase of oxygen in the water both years. There is evidence of atmospheric enrichment (Herbland and Voituriez, 1974). During the same period, nitrate decreased from 18 to 5 µg at/l (Fig. 3). The heterotrophic activity is very low during the first days. It increases sharply on the third day and remains very high during the bloom of phytoplankton (Fig. 3).

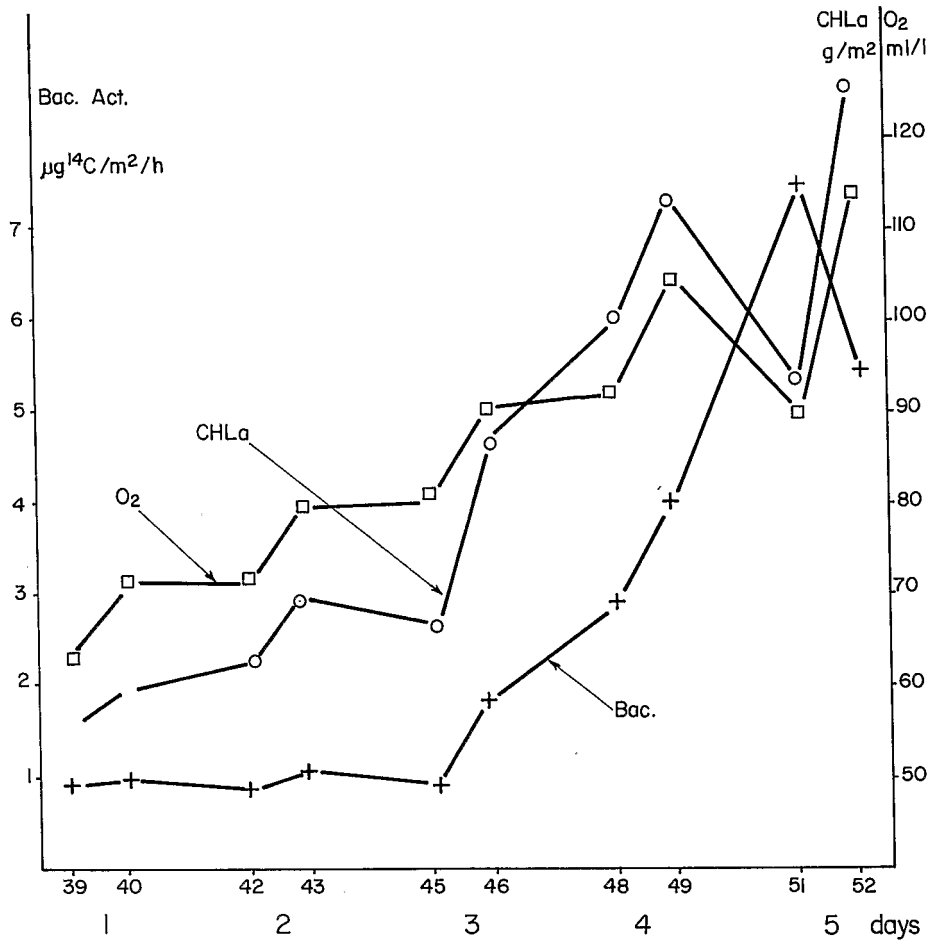


Fig. 4. Evolution of integrated values (0-20 m) during the drift of the buoy in 1973

The evolution of integrated values (Fig. 4) shows clearly the simultaneous increase of chlorophyll and oxygen, and later, the increase of heterotrophic activity. The amino acid uptake bloom seems to occur immediately after the phytoplankton bloom. Ammonia increases subsequent to heterotrophic activity (Fig. 5). Then we have the successive evolutions in the water mass: (1) oxygen increase with simultaneous phytoplankton increase and nutrient decrease, (2) heterotrophic activity increase, and (3) ammonia increase.

Phytoplankton cells are certainly not yet decomposed because nutrients are available. Some authors have recently found that excretion products of phytoplankton can be taken up by marine bacteria in culture (Wright and Shah, 1975) or in natural populations (Tanaka et al., 1974; Herbland, 1975).

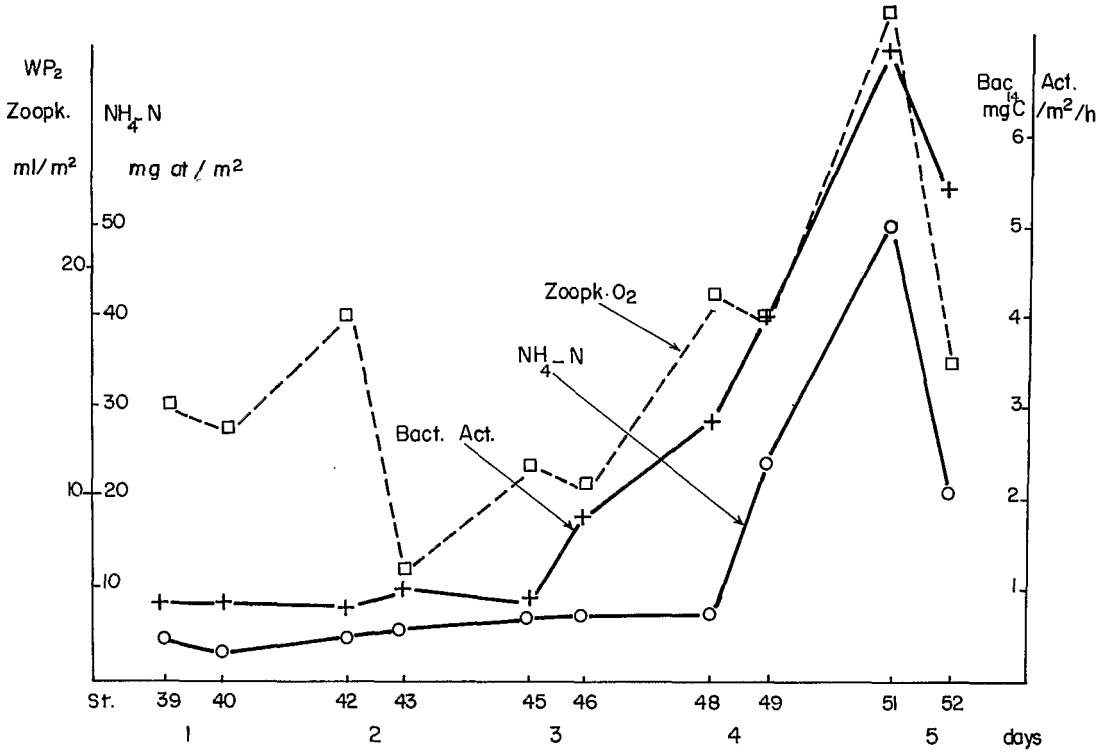


Fig. 5. Evolution of integrated values (0-20 m) during the drift of the buoy in 1973

In this drogue study, the excretion of phytoplankton was measured. There is a simultaneous increase of heterotrophic activity and excretion of phytoplankton (Fig. 6). It does not prove that there is a trophic link but it is a strong indication. The vertical distribution of heterotrophic activity shows that, at the beginning, in recently upwelled water, it is homogeneous (stations 39 and 42 in Fig. 7). At the end, however, during the bloom of phytoplankton, heterotrophic activity is located essentially in the upper fifteen meters, where the phytoplankton and organic matter (measured by organic phosphorus) are abundant (stations 51 and 52). The same result was obtained in 1972 (Fig. 8) with glucose uptake.

3.2 Growth Yield or Growth Efficiency

A certain amount of amino acids was taken up, and a percentage of this amount was oxidized to carbon dioxide. The growth yield is the ratio of amino acid assimilated/assimilated + oxidized. This ratio has sharp variations: An average value of 78% was found by Williams (1970) for an amino acid mixture. Hobbie and Crawford (1969) reported results in natural fresh-water populations taking up sugars, amino acids, and acetate; most of their values lay in the range 70-80%, the overall range being 43-92%. Despite these variations, the two amino acids leucine and lysine showed the highest growth yields while aspartic and glutamic acids had the lowest (Crawford et al., 1974; Gocke, 1976).

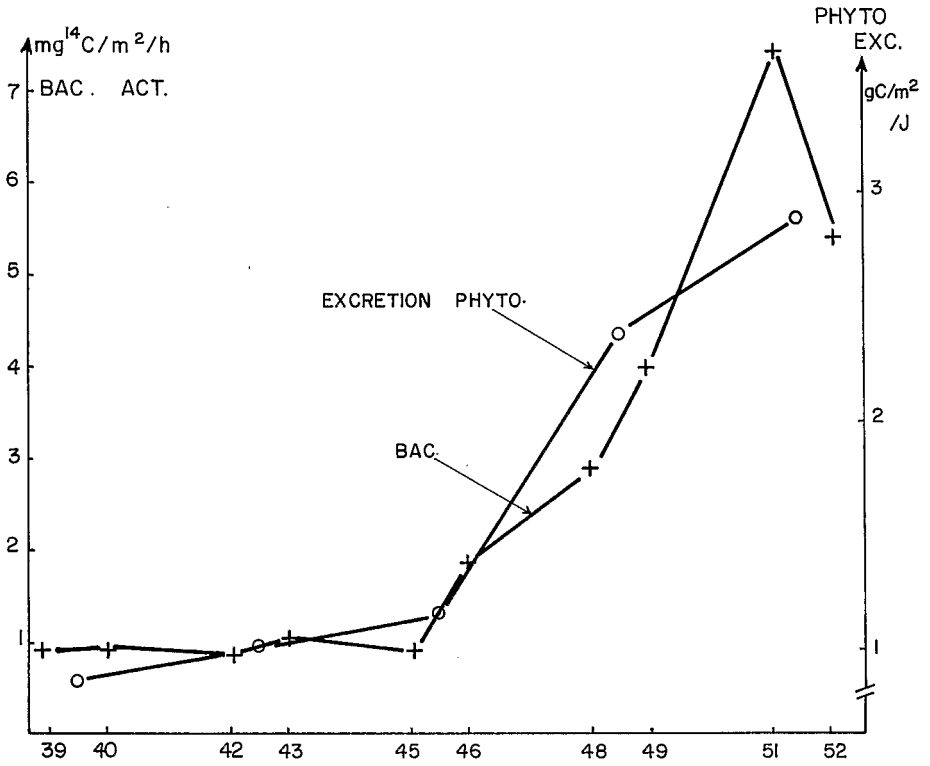


Fig. 6. Comparison between the phytoplankton organic excretion and heterotrophic activity (integrated values), during the drift of the buoy in 1973

We measured simultaneously assimilation and respiration during incubation. There is a direct relation between the carbon assimilated and the carbon respired (Fig. 9). For all samples, the mean value of growth efficiency is 50%. In 1972, the mean value with glucose as substrate was 35%. These results are low compared with those in the literature. This ratio was not constant over the track of the drogue (Fig. 10). It was low, about 40%, at the beginning, and reached a maximum of 60% the fourth day when the bacteria and phytoplankton are actively growing. It can be seen that the yield values below 18 meters are lower than the values between 0 and 10 meters. This result cannot be fully explained, but it is possible that oxygen concentration (oxygen is the last electron acceptor in respiration processes) can affect the yield: The oxygen concentrations are low near the bottom and at the beginning of the experiment. However, many parameters other than oxygen can influence the metabolism of bacteria.

3.3 Geographic and Nychthemeral Variations

In 1972, after the drogue study, a transect of 120 miles was done from the coast to the open sea to try to measure the extent of the upwelling area. Except for station 50, where chlorophyll and heterotrophic

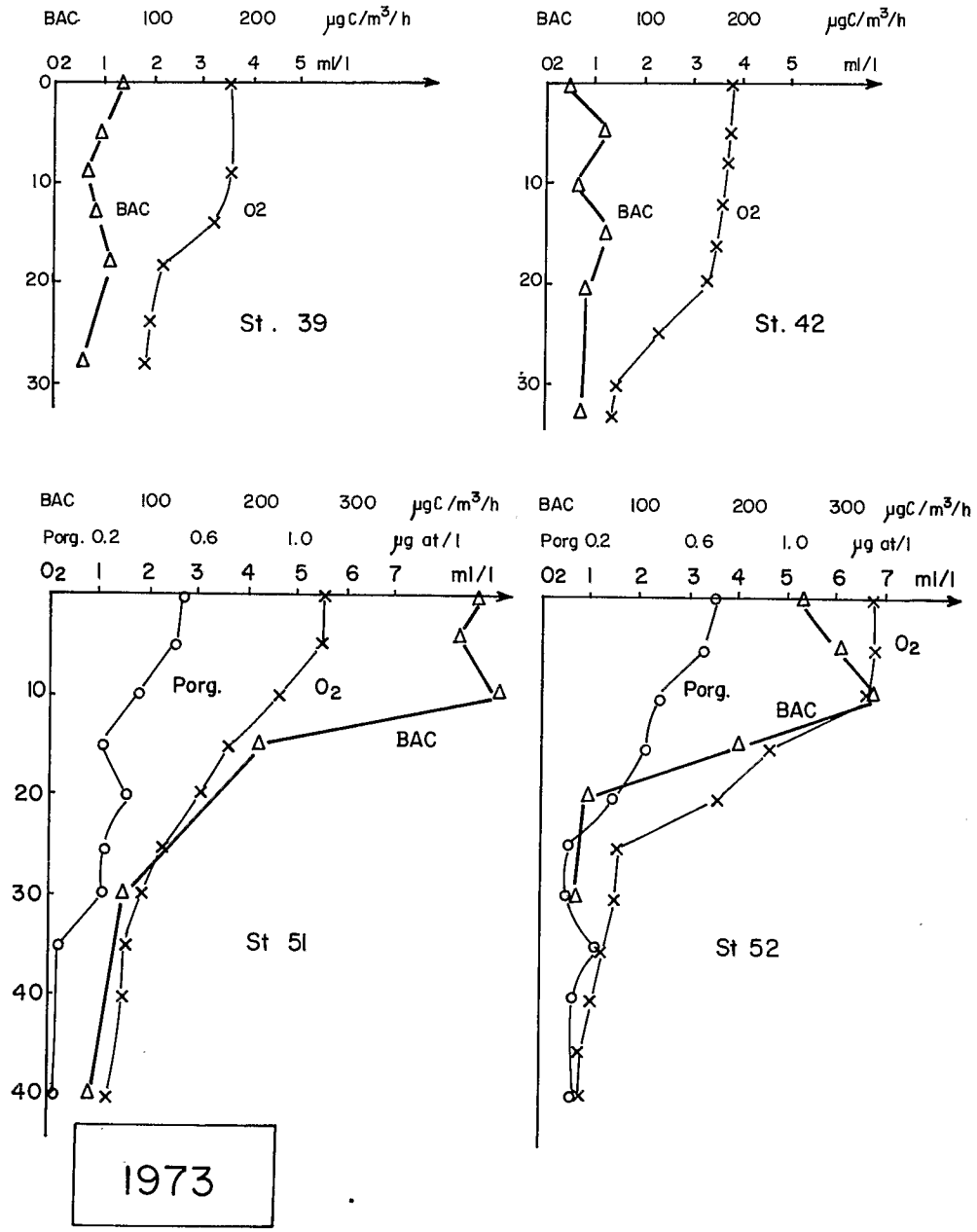
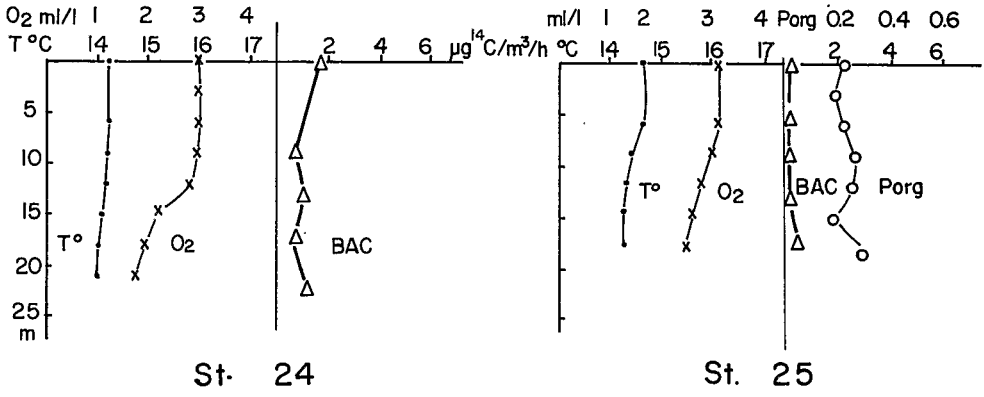
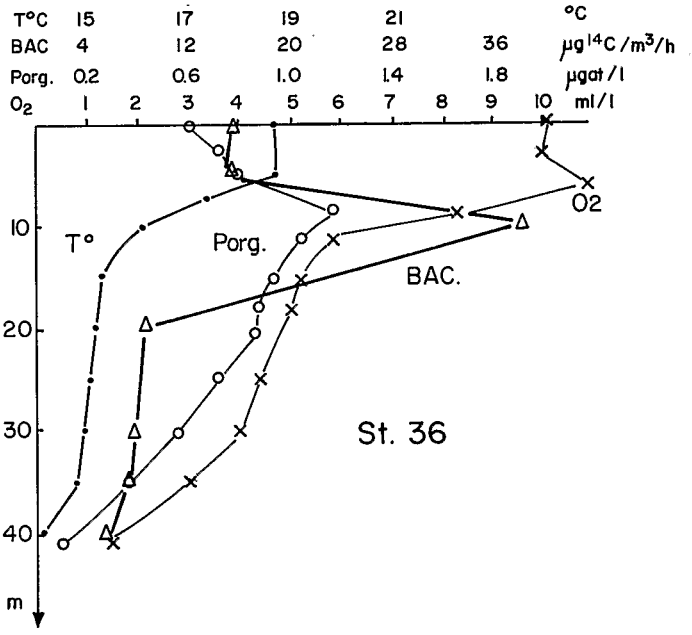


Fig. 7. Vertical distribution of organic phosphorus (Porg), oxygen (O_2), and heterotrophic activity (Bact) at two stations in recently upwelled water (Sts. 39 and 42), and at two stations after the phytoplankton bloom (Sts. 51 and 52) in 1973





1972

Fig. 8. As in Figure 7, but in 1972. Stations 24 and 25 are in recently upwelled water, and 36 is in old upwelled water

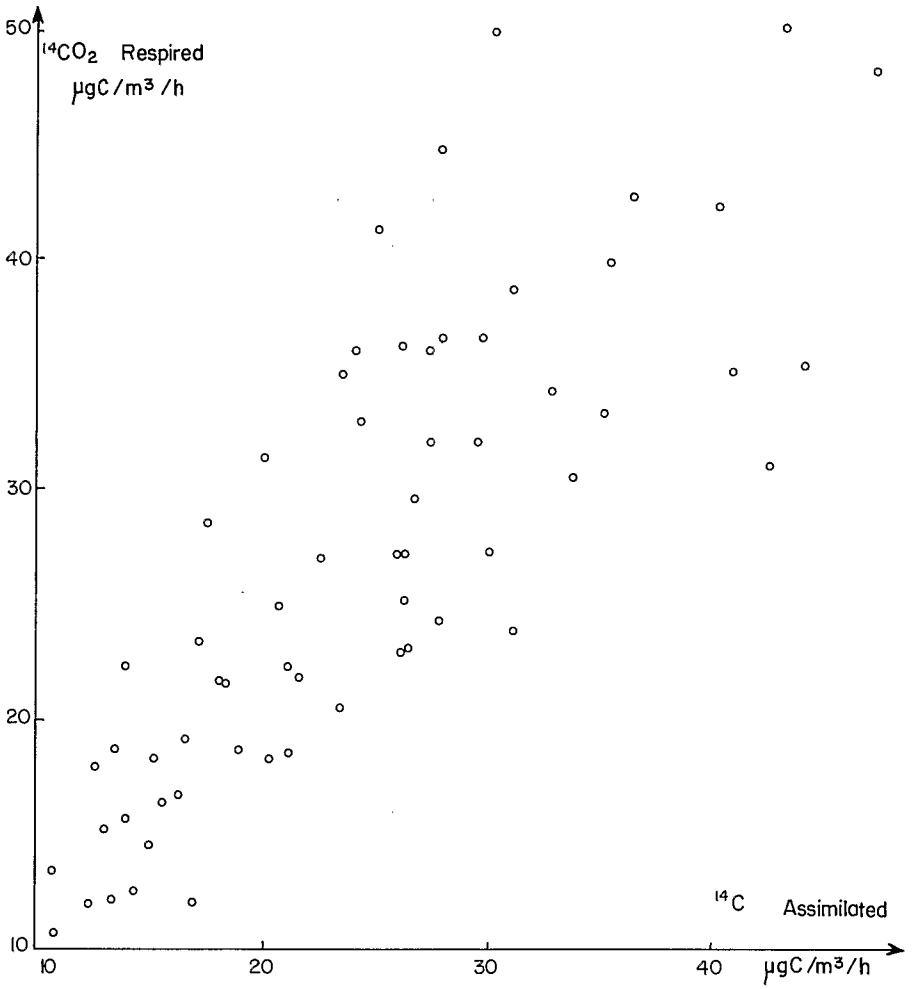


Fig. 9. Relation between carbon of amino acid "assimilated" by the cells, and carbon "respired" during the 3-h experiments

activities were high (probably a divergence area), there was a general decrease from the shore to the open sea (Fig. 11).

Daily variations were seen. Incubations made during the morning (M) gave higher values than incubations made during the afternoon (AN). It is possible that during the night, heterotrophic activity is more important, hence, the bacteria are more numerous in the morning sample than in the afternoon one. It could be the result of a competition between autotrophic and heterotrophic organisms, the "autotrophs" being active during the day and perhaps inhibiting the "heterotrophs."

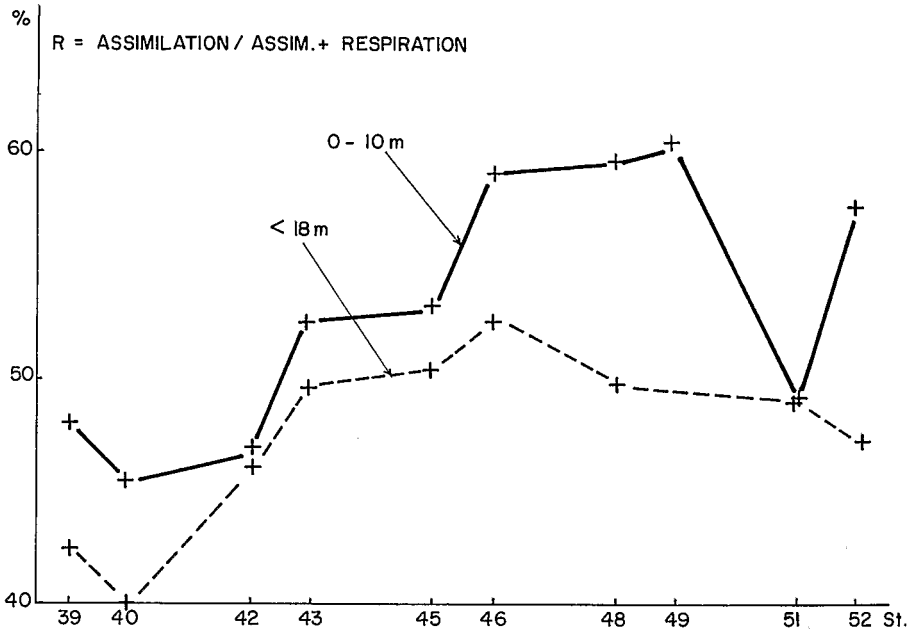


Fig. 10. Change of growth yield in two layers of water, during the drift of the buoy in 1973

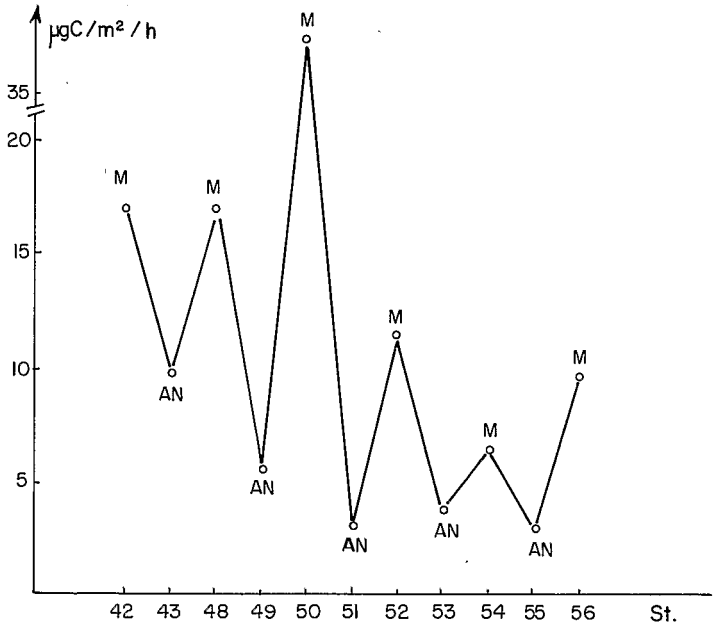


Fig. 11. Geographic and nychthemeral variations of bacterial activity: St. 42 is near the shore, and 56 is in the open sea. The distance between Sts. 42 and 56 is approximatively 120 miles. M morning stations; AN afternoon stations

4. Conclusions

Amino acids are well taken up by heterotrophic organisms (probably bacteria) in this upwelling area.

The same results were found in 1972 with glucose and in 1973 with an amino acid mixture:

1. Same time succession: upwelling water rich in nutrients and poor in oxygen and chlorophyll, phytoplankton bloom, heterotrophic bloom, and ammonia enrichment.

2. Growth yields are somewhat higher with amino acids than with glucose (50% against 35%). This average yield seems to be affected by the environmental conditions (oxygen concentration and an undetermined factor).

3. Nychthemeral variations have been observed, but not satisfactorily explained.

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