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Marine Bacteria as Indicators of Upwelling in the Sea

By

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ABSTRACT

THE 'oxidase reaction' (using p-amino-dimethyl-aniline oxalate as the reagent) has been used to distinguish oxidase-negative from oxidase-positive bacteria from the sea, when grown on membrane filters. By this means it has been shown (*a*) that under conditions of stable stratification of the sea as in the tropics, a relationship exists between the percentage incidence of oxidase negative bacteria in the flora and the depth of the water; (*b*) that the maximum value for this percentage incidence (100) is reached at or immediately below the upper limit of the oxygen minimum layer; (*c*) that this percentage value (expressed as Ox^n values) may be used to demonstrate the movements of water masses during upwelling.

Such upwelling as indicated by theoretical findings and by temperature determinations along two transects off the west coast of Ceylon during the north east monsoon, has been confirmed by the distribution of Ox^n values at these transects.

Introduction

Many of the classical techniques used at present for the study of oceanic upwelling have been found, especially under tropical conditions, to be of limited value. These techniques are based on chemical estimations of chlorides, phosphates, nitrates, silicates and oxygen, as well as physical measurements of temperature, of the water of the deeper layers of the sea and that of the surface. However many of these techniques suffer from the same kind of disadvantage as that pointed out by Cooper (1960) in the use of salinity determinations during studies of deep-sea oceanography—that the interpreted differences are not much greater than the errors of measurement and that an exact measure of the statistical significance of this error cannot readily be made. The type of difficulty in demonstrating upwelling with these techniques is evident, for instance, in the work of Rochford (1962). He found that though phosphate determinations indicated two regions of upwelled water off North West Australia, south of Timor and in the East Arafura Sea, in both instances determinations of salinities and temperatures were very variable and gave no indication of upwelling.

The phenomenon of upwelling is of special significance under tropical conditions, particularly due to its correlation with regions of increased productivity in the sea. In addition there is reason to believe that coastal upwelling of cold deeper water influences fish behaviour significantly; for fish are known to react to temperature differences of even 0.03°C. (Bull, 1952).

Theoretical calculations based on the work of Sverdrup and his colleagues (Sverdrup, 1930; 1938; Sverdrup & Fleming, 1941) and of Yoshida (1955) indicated that conditions of wind force and direction during the North East monsoon were favourable for upwelling on the west coast of Ceylon. An attempt was therefore made to confirm these theoretical findings, especially by using certain characteristic properties of marine bacteria as indicators of such water movement.

Methods

At about 20 stations between 50 and 100 miles off-shore, bacterial sampling of the water was done at various depths from the surface down to 200 meters. A similar number of stations was chosen between 25 to 30 miles off the shore. These stations were chosen for correlation between depth and bacterial flora after bathythermographic readings had indicated a stabilised layer of discontinuity at depths between 200 to 250 meters.

For the study of upwelling, sampling stations were chosen along two transects at right angles to the shore, one off Balapitiya (6°38′N), and the other off Chilaw (7°38′N) on the west coast of Ceylon. The sampling stations were so located on these transects that about half of them were within and about half beyond the edge of the continental shelf. Each transect itself was sited as far away as possible from major river mouths to minimise fluviatile interference with hydrological conditions. At the Balapitiya transect the North East Monsoon current system had stabilised itself after rounding Dondra Point at the southern tip of the island. At the Chilaw transect this current system had swung North West and had weakened considerably.

Water was collected at intervals down to depths of 200 meters at sampling stations beyond the continental shelf and to appropriate lesser depths at the inshore stations, as indicated in Figs. 2, 3, 4 and 5. Nansen bottles were used and, immediately they were hauled in, temperatures were recorded and the water sampled for bacterial flora. Bacterial counts were done using the millipore membrane filter technique (Kriss, Rukina & Novozhilova, 1952) on 25 mls. of water (or on appropriate dilutions of surface and other samples down to depths of 50 m., sterile sea-water being the diluent). The filtration was carried out within 5 to 10 minutes of sampling. The membranes were incubated in aluminium tins, one in each, on filter pads impregnated with ZoBell's liquid medium, 2213 (ZoBell, 1946), with aged sea water as base.

The colonies were counted after incubation at 20°C. for 3 days, and at 28-30°C. for another 3 days. Colonies were picked up at random from duplicate samples for generic determinations of the bacteria. The " oxidase " reaction of the colonies on the membrane was determined by placing the membrane, with colonies intact, on a drop (about 2 mls.) of a freshly prepared 5% soln. of p-amino-dimethyl-analine oxalate (Difco) in distilled water for about a minute. When the positive colonies had taken on a dark pink colour (which later turned to black, especially in the centre) the membrane was placed on a clean glass dish and both types of colonies counted at a magnification of 10 times with a Zeiss stereoscopic microscope. The relative abundance of oxidase negative and oxidase positive colonies was calculated and expressed as the percentage of oxidase negative colonies in the total count. An average of two or more duplicate counts was used for computing the final value of this percentage.

Results

From samples of sea water at locations where stable stratification occurred as indicated by bathythermographic readings, it was possible to demonstrate a relationship, which is statistically significant, between the proportion of oxidase negative bacteria in the total viable count and depth, down to and below the layer of discontinuity (Fig. 1). This relationship can be used for estimating the depth of the discontinuity layer—the depth at which the percentage of oxidase negative bacteria in the flora (Ox^n value) reaches 100 corresponding approximately to the upper limit of this layer.

Temperature determinations along the transects showed upwelling (Figs. 2 & 4). A comparison of Figs. 2 & 3 and 3 & 4, of the temperature profiles at the transects with the distribution of the Ox^n values on those same transects, showed that these latter values are an even clearer indication of this upwelling.

From both temperature and Ox^n values at the Balapitiya transect (Figs. 2 & 3), a picture emerges of a stream of water in cross-section, upwelling obliquely across the face of the steep continental slope. Lowest temperatures and highest Ox^n values are found in the core of the stream. Temperature measurements did not clearly indicate the emergence of this stream at the surface, but the Ox^n values do, very markedly. The transect off Chilaw presents a somewhat different picture, of a stream of water rising almost vertically from about 100 m. below the surface.

Discussion

Since the pioneering work of Ekman in 1902 on upwelling, several other workers have examined the basis of this phenomenon (Thorade, 1909; McEwen, 1912; Defant, 1936; Sverdrup, 1930; 1938; Sverdrup & Fleming, 1941; Yoshida, 1955). An application of recent views, those of Yoshida (1955) arising out of his study of upwelling off the Californian coast, to condition in Ceylon, led us to the conclusion that there should be upwelling of water in narrow bands within relatively small distances from the shores.

In view of the limitations already referred to in the classical techniques for the demonstration of movements of water masses, the possibility of using bacteria as indicators for this purpose was explored in this study.

The use of bacteria as indicators of water movements is relatively recent (Kriss, 1960). It has been shown that even slight variations in the stratification of the water can be detected by determining differences in the abundance of these microorganisms. Kriss and his colleagues, however, have relied, except for their broad division of the bacterial flora into ' autotrophs ' and ' heterotrophs ', upon the total numbers of bacteria rather than on the presence of particular groups to characterise the different water masses. They did consider the possibility of using morphological types, but reported that " due to the scarcity of microbial forms, chiefly rods and cocci, the morphological characteristics of bacteria could not be relied on as indicators in in hydrological studies " (Kriss, 1960). This difficulty need not be expected as regards the biochemical and physiological properties of bacteria. These not only show a wide range of variation but they are also sufficiently specific to permit of easy differentiation. It is precisely such a property of the bacterial flora, namely the nature of their cytochromes, that has been successfully used in the present investigation to characterise water masses.

Earlier studies in another connection had shown that the cytochromes, which mediate in electron transfer during cellular metabolism in a majority of bacteria, are sensitively adapted to the oxidation-reduction conditions of the environment. Even within a single group of bacteria (the fluorescent pseudomonads) such environmentally determined variations could be demonstrated (de Silva & Swain, unpublished).

Kriss, Lebedeva and Mitzkevitch (1960) have indicated from the numbers of heterotrophs, that the deep waters of the tropical Indian Ocean are probably rich in organic matter. With a relatively stable thermocline which has been shown to occur under tropical conditions (Watts, 1958), it is to be expected that large amounts of decaying organic matter will accumulate at or below that layer, producing a zone characterised by a deficiency of oxygen and a low oxidation-reduction potential. Vinogradov and Voronina (1961) have in fact demonstrated the existence in the Arabian Sea of a sharply defined O_2-minimum layer with its upper limits about 125 to 200 meters below the surface. This limit probably coincides with the layer of sudden density change reported by earlier workers (Carruthers *et al*, 1959; Neiman, 1960); and it is therefore to be expected that the bacteria characteristic of these waters will have cytochromes adapted to operating under conditions of low oxygen tension. The results of the present investigation confirm this.

The validity of the observed relationship between Ox^n values and depth will, of course, have to be confirmed by further investigations in widely different oceanographic conditions: but it is interesting to note that extrapolation of curves A & B in Fig. **1** to the Ox^n value of 100, in the absence of upwelling, corresponds with fair accuracy to the expected depth of the upper limit of the oxygen minimum layer. It is also noteworthy that the maximum Ox^n values appear inshore in much shallower waters than they do away from the continental shelf. This therefore might be an indication of the upward slope of the oxygen minimum layer near the continental shelf observed by Carruthers *et al* (1959) off the Indian coast. If a straight line relationship obtains between Ox^n values and depth-under conditions of stable stratification, then determination of Ox^n values at a few depths will provide, by graphical extrapolation, a fair estimate of the upper limits of the layer of discontinuity and oxygen minimum.

The work reported here is the first experimental demonstration of upwelling induced by monsoon winds off the coast of Ceylon. La Fond has also reported similar upwelling along the east coast of India and has corelated it with the replenishment of plant nutrients in the sea (La Fond, 1954; Bhavanarayana & La Fond, 1957). Recently Wyrtki (1962) has demonstrated that upwelling occurs along the coast of Java and Sumatra on the right flank of the South East Equatorial Current, during the South East Monsoon. These investigations along with the data now presented, appear to indicate that monsoon wind induced upwelling is one of the main factors responsible for the seasonal enrichment of the sea in the tropical and sub-tropical regions of the Indian Ocean.

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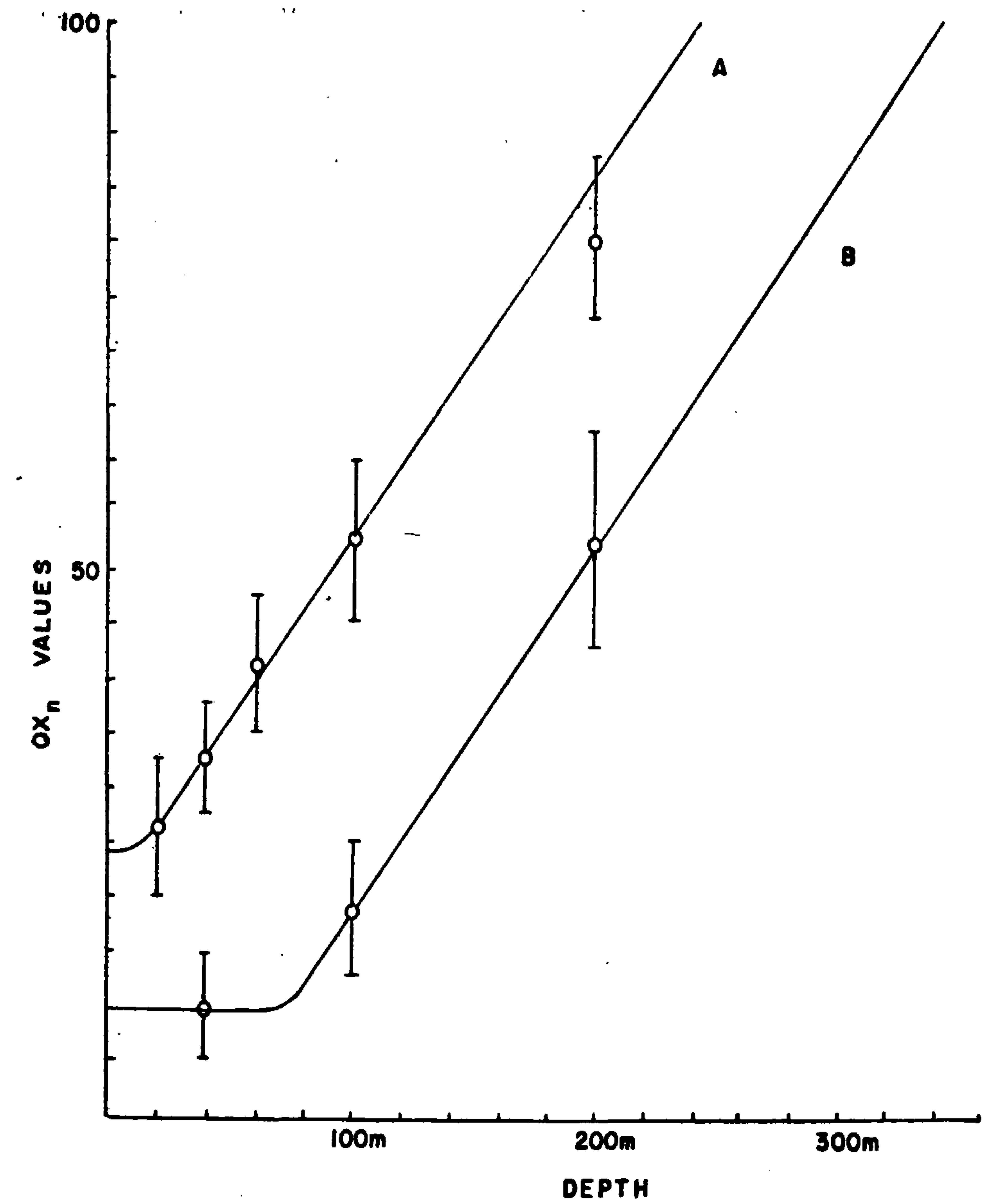

Fig. 1. Correlation of Ox^n values (=percentage of oxidase negative bacteria in the total viable count) with depth of the sea. A=Inshore stations, 25–30 miles from the coast; B=Off shore stations, 50–100 miles from the coast.

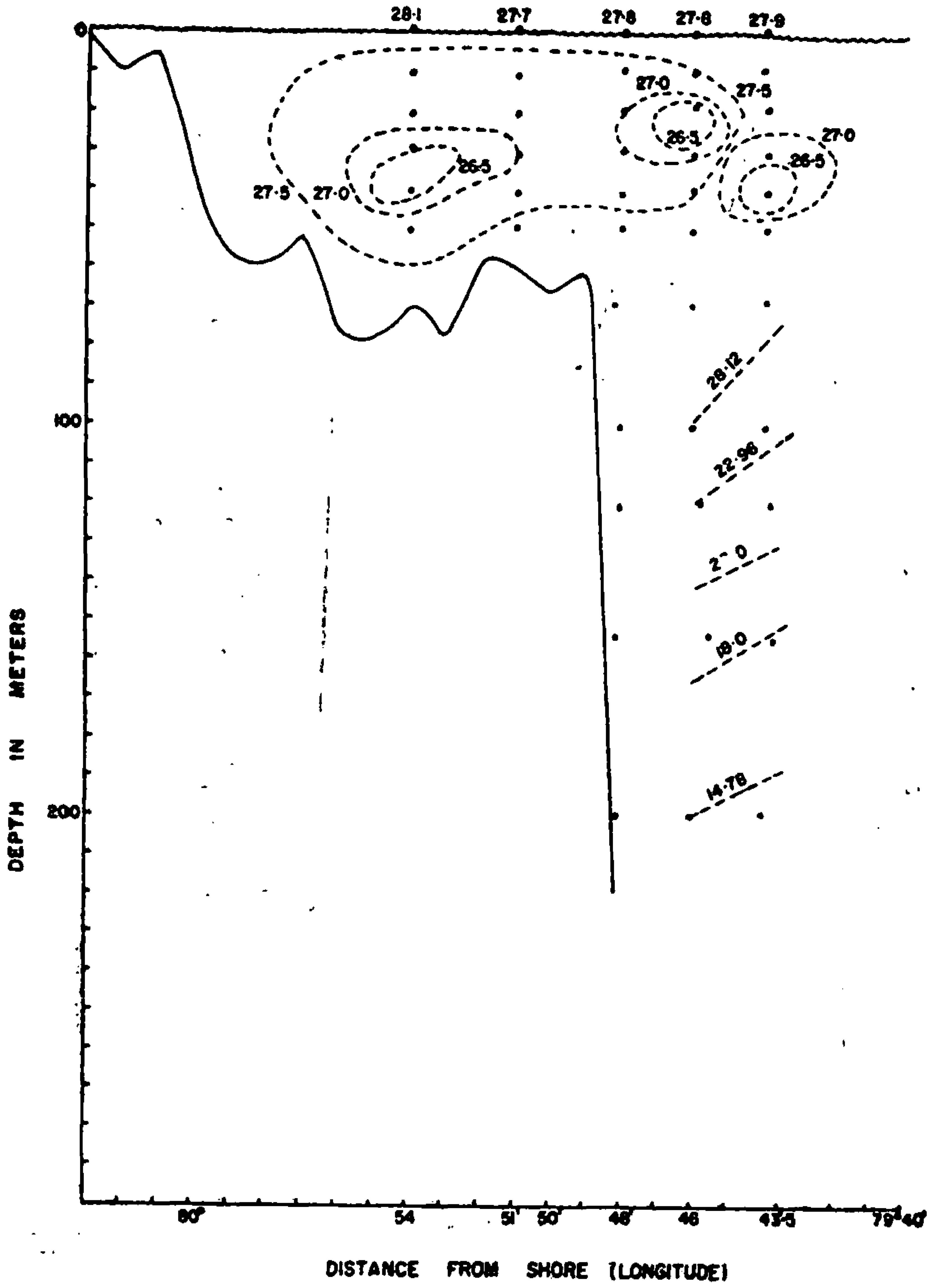

Fig. 2. Distribution of temperature measurements at the transect off Balapitiya.

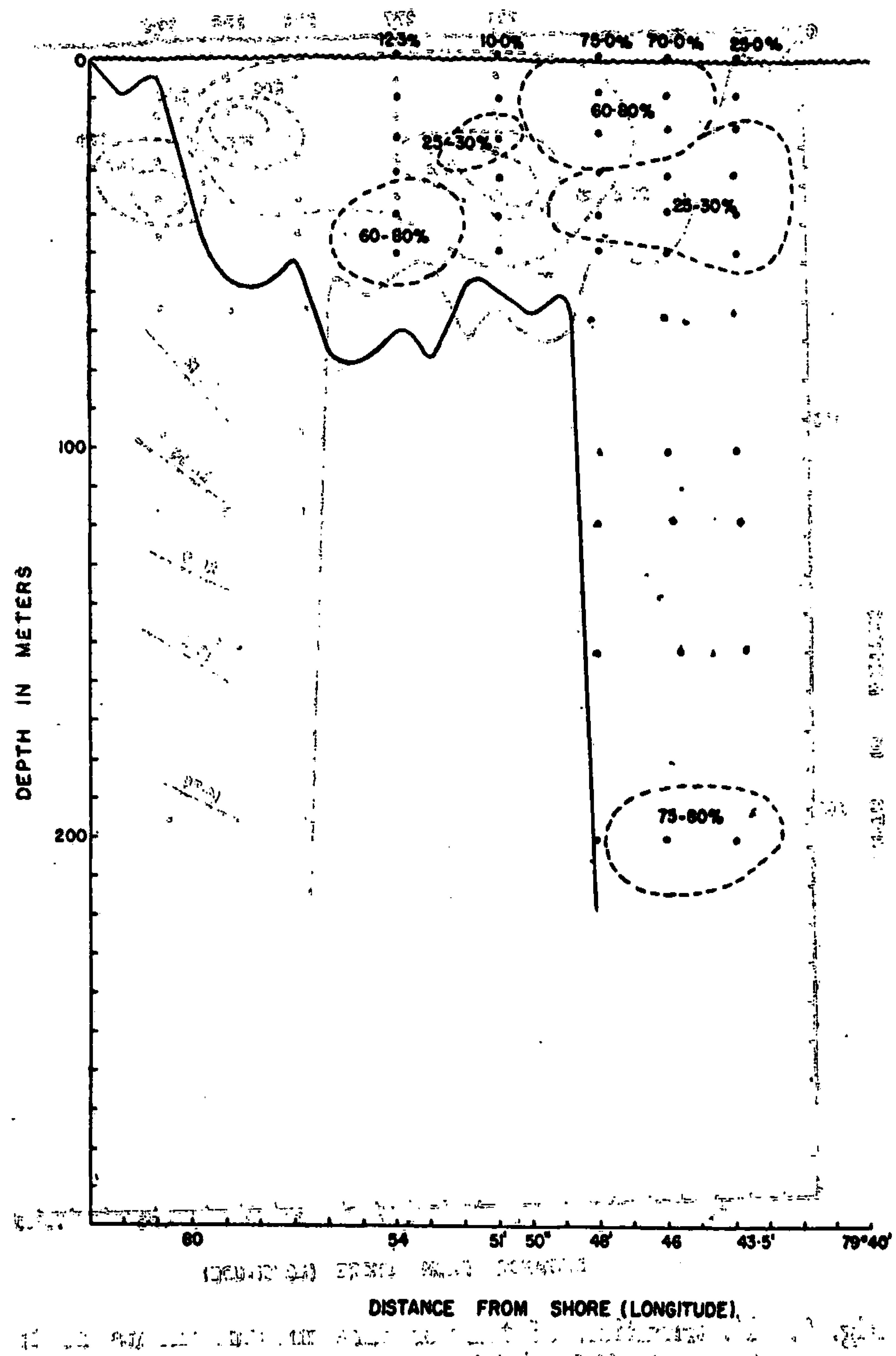

Fig. 3. Distribution of percentage oxidase negative bacteria (Ox^n values) at the transect off Balapitiya.

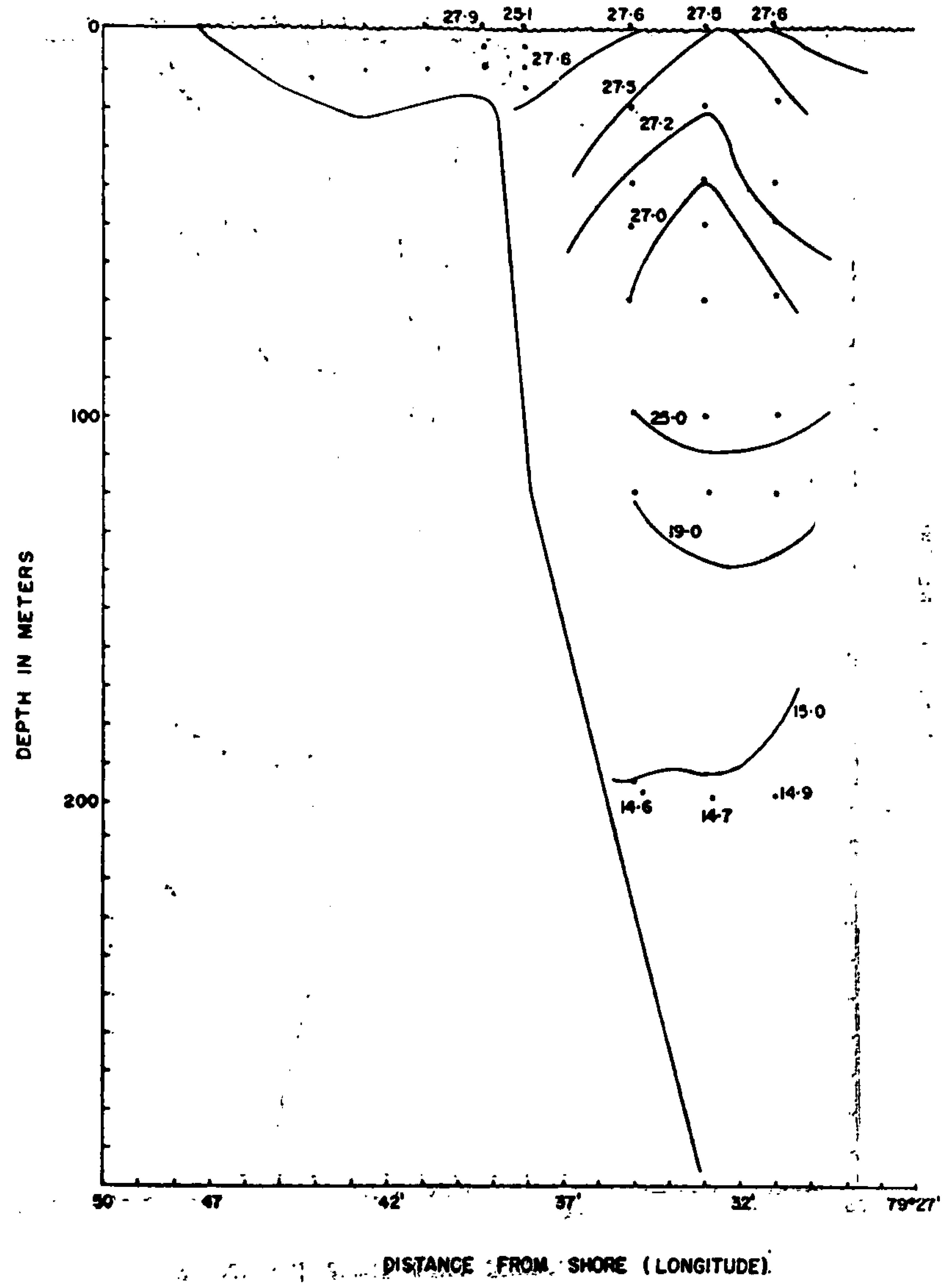

Fig. 4. Distribution of temperature measurements at transect off Chilaw.

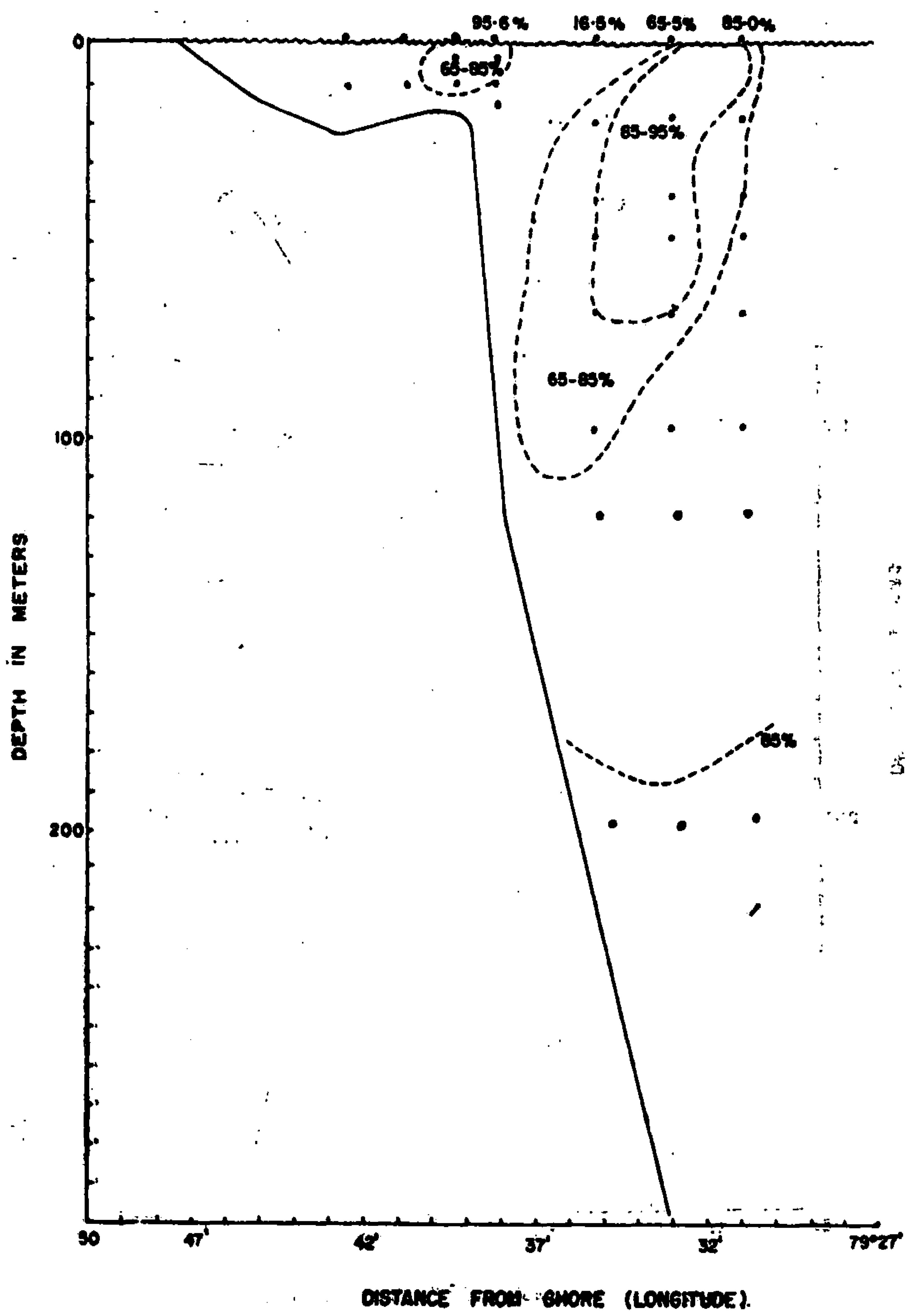

Fig. 5. Distribution of percentage oxidase negative bacteria (Ox^n values) at the transect off Chilaw.