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The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer® and associated equipment

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Abstract—A brief outline is given of the use of the Technicon Autoanalyzer® for the determination of phosphate, silicate and nitrate and the use of this equipment, together with continuous measurements of temperature and chlorophyll, to record the properties of surface water from a ship underway. The continuous recording of surface properties gives promise of a valuable new method for studying nutrient enrichment and biological production in space and time over large areas of the sea surface in eutrophic waters.

THE USE of the Technicon Autoanalyzer® in seawater analysis has been described by several workers (BREWER and RILEY, 1965; GRASSHOFF, 1965; HENRIKSEN, 1965; ARMSTRONG and LA FOND, 1966; CHAN and RILEY, 1966). LORENZEN (1966) has also shown how a measurement of the red fluorescence of seawater can be made by a specially adapted Model 111 Turner fluorometer, the fluorescence giving a semi-quantitative estimate of the amount of chlorophyll present in the water.

We have used the Technicon Autoanalyzer® and Turner fluorometer during cruises and find that, whereas the equipment and associated chemistry are, in essence, relatively simple, the perfection of a sea-going assembly with proven reliability and easy servicing has been more difficult than might be imagined.

At present we use the Autoanalyzer® for batch analysis, for profiling to a depth of 150 m, using a submerged pump, and for measuring the concentration of plant nutrient in surface waters while the research vessel is underway. The latter approach, sampling continually from the cooling-water intake of the ship, has given promise of a powerful new approach to the study of upwelling and biological activity in eutrophic areas. Some preliminary results to illustrate this are described in the present communication.

A detailed account of the final form of the equipment would be superfluous but " short-hand " notes of the methods for phosphate, nitrate and silicate are given as they may be of interest to other Autoanalyzer® users. These methods were developed independently of those described in the literature and have some advantages in speed and simplicity. The colorimeter and solenoid valves in sample lines were of our own construction; all other equipment was made from standard Technicon components. The incoming sea water was filtered by pumping it at 7·8 ml/min plus air at 0·8 ml/min through the lower side of a dialyzer plate holding a 3-μ pore-size membrane filter and sucking the sample, on demand, from the upper side of the filter. This filter lasted, without changing, for more than a week even in water containing heavy blooms of phytoplankton.

METHODS

Phosphate

Mix 2·5 ml/min filtered sample with 0·32 ml/min of molybdate reagent (34 g ammonium paramolybdate dissolved in 1000 ml of water plus 400 ml concentrated sulfuric acid plus 0·2 g potassium antimony tartrate dissolved in 2500 ml of water). Mix the combined sample and reagent with 0·16 ml/min of reductant (2 g ascorbic acid plus 0·3 ml Levor IV in 100 ml water, prepared by diluting a refrigerated stock solution each day). Levor IV is an anionic surfactant obtainable from the Technicon

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Corporation. Finally inject air at 1·2 ml/min and allow the reaction to proceed in two long coils kept at a temperature above 25°C by placing them immediately beneath a 60-W incandescent bulb with a back reflector. The effluent should be led by the shortest possible length of glass tubing into a colorimeter. Measure the absorbance in a 5-cm cell. Use silicon photocells with 885 mμ interference filters. The Technicon range expander set at 4 × will generally be found most suitable. Standardize with a 1 μg-at. P/l. standard. Use distilled water, 0·075% (w/v) sodium bicarbonate solution or phosphate-free synthetic sea water for blanks.

Nitrate (and or nitrite)

Mix 2·5 ml/min filtered sample with 0·32 ml/min of 8·5% (w/v) ammonium chloride or, if more than 6 μg-at. N/l. is expected, mix 0·6 ml/min sample with 2·5 ml/min distilled water and then with 0·32 ml/min of 2·0% (w/v) ammonium chloride. The ammonium chloride solutions should contain about 0·1% (v/v) of BRIJ® 35, a polyoxyethylene lauryl ether detergent obtainable from the Technicon Corporation. Pass the resulting solution through a small column packed with 30–60 mesh cadmium filings that have first been shaken with ten times their weight of 2% (w/v) copper sulphate pentahydrate solution. (The column is 8 mm dia. by 50 mm in length. One end, upstream, is narrowed down to take plastic connecting tubing and contains a plug of fine copper wool; the other end, downstream, has a small plug of cotton wool and is closed by a rubber bung containing a tube to accommodate the plastic connecting tubing).

After the sample has passed through the reduction column (which can be omitted if only nitrite is required) mix the solution with 1·2 ml/min of air and 0·16 ml/min of 1% (w/v) sulphanilamide in 1·2-N hydrochloric acid. Allow the reaction to proceed in one long coil. Mix with 0·16 ml/min of 0·1% (w/v) *N*-napthylethylene-diamine in water. Allow the reaction to proceed in one long coil. Measure the absorbance in a 5-cm cell. Use selenium photocells with 540 mμ interference filters. Standardize with 5 or 25 μg-at. N/l. solution made in a low-nitrate sea water to avoid salt errors. Use a 0·075% (w/v) sodium bicarbonate solution or nitrate-free synthetic sea water for blanks.

Silicate

Mix 2·5 ml/min filtered sample (or 0·6 ml sample plus 2·5 ml/min of distilled water if more than 12 μg-at. Si/l. is expected) with 0·16 ml/min of molybdate reagent [80 ml 5% (w/v) ammonium paramolybdate solution plus 120 ml of 1·2 N hydrochloric acid] and 1·2 ml/min of air. Allow the reaction to proceed in one long coil. Mix with 0·42 ml/min of 10% (w/v) tartaric acid solution. Allow the reaction to proceed in one short coil. Mix with 0·10 ml/min of stannous chloride solution [1% (w/v) in 1·2 N hydrochloric acid, prepared fresh each day from hydrochloric acid and a stock 40% (w/v) stannous chloride solution in 5 N hydrochloric acid]. Allow the reaction to proceed in one long coil. Measure in a 5-cm cell. Use silicon photocells with 820 mμ interference filters. Standardize with 10 or 50 μg-at. Si/l. solution made in low-silicate sea water to avoid salt errors. Use water, 0·075% (w/v) sodium bicarbonate solution or silicate-free synthetic sea water for blanks.

Chlorophyll

The number 10 or number 3 door was used by us on the Turner fluorometer, as described by LORENZEN (1966). With more than about 4 μg chlorophyll/l. the relationship between fluorescence and chlorophyll was not linear. The conversion factor between fluorescence and chlorophyll, checked at intervals during cruises, was very variable (± 45% from the mean). The "chlorophyll" data, therefore, must be considered only very approximate, especially at high concentrations, but relative changes of more than a few tens of percent indicate a significant variation in the concentration of surface phytoplankton.

Temperature

This was recorded automatically in the ship's cooling water and the equipment was calibrated against mercury thermometer readings of the sea surface temperature.

RESULTS

Our initial attempts at delineating upwelling water were by using nitrate (Fig. 1). A small upwelling area was found off Punta Colnett, Baja California, only a few hundred meters offshore just south

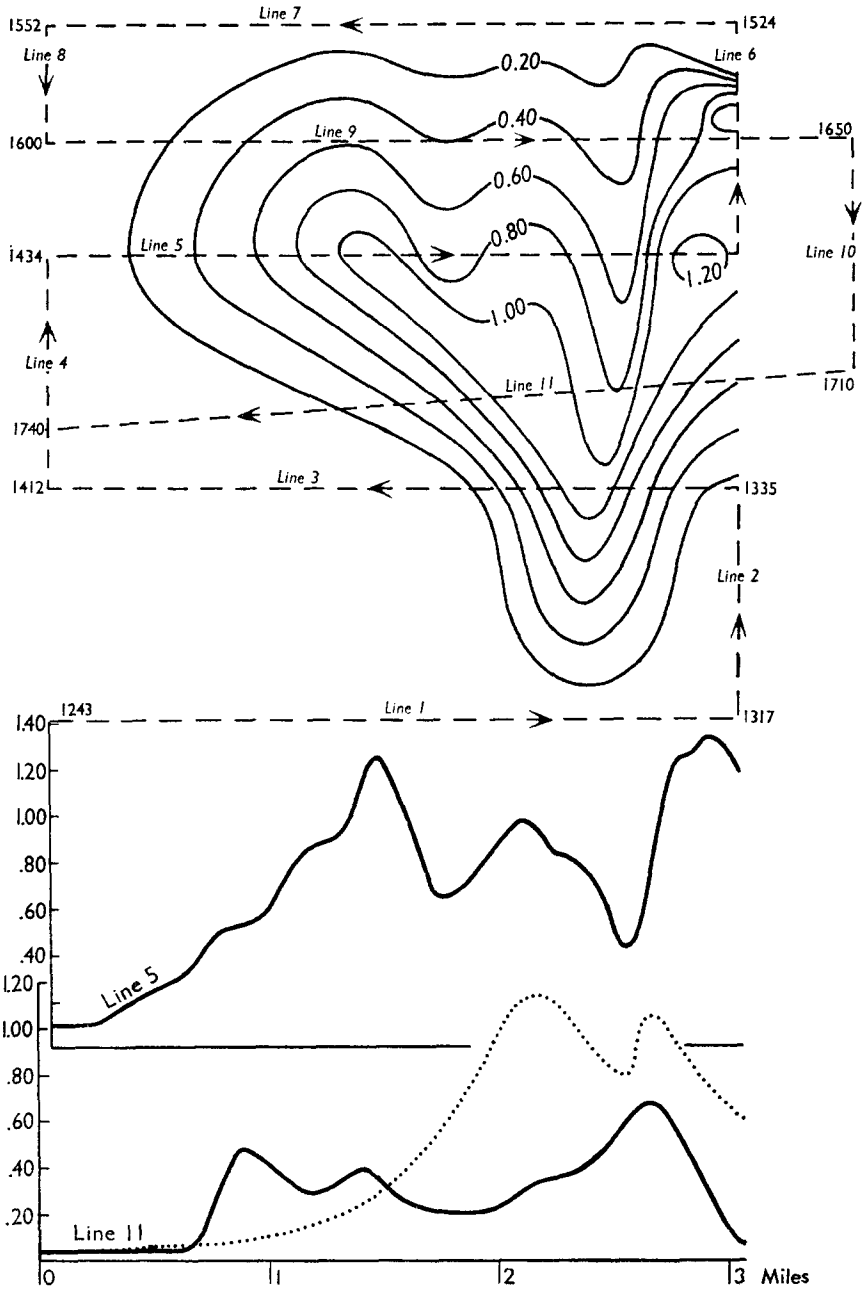

Fig. 1. Nitrate (μg-atom NO_3-N/l.) at surface off Punta Colnett Baja California (29° 57'N, 116° 20'W) 9 July 1965. Above. Horizontal distribution from records of lines 1–8 assuming stationary phenomenon and ignoring line 11. Below. Solid lines; specimen records. Dotted line, record predicted for line 11 assuming contouring correct.

of the Punta. The region was discovered in the early morning by noting a slight drop (about 1 deg C) of surface temperature but later in the day even this indication was lacking. However, a slight but measurable increase of surface nitrate was detected by the Autoanalyzer® and the patch was contoured by the east-west transects (Fig. 1). The patch undoubtedly moved south to north during this operation, however, as the record obtained on the final transect (line 11) showed a discrepancy with values calculated from the earlier data on the assumption that the surface water did not move. The ship was travelling at only 8 knots and it would have been better to have cruised faster and perhaps to have made traverses more nearly parallel to the direction of surface current flow rather than at right angles to it.

Two transects (Stas. 16–18 and 19–21, Fig. 2) were made perpendicular to the Peru coast off Pisco (Figs. 3 and 4). The ship was steaming at 10·5 knots. The water in the bay (right hand side of Figs. 3 and 4) was rich in plankton which decreased seaward but with no obvious correlation with

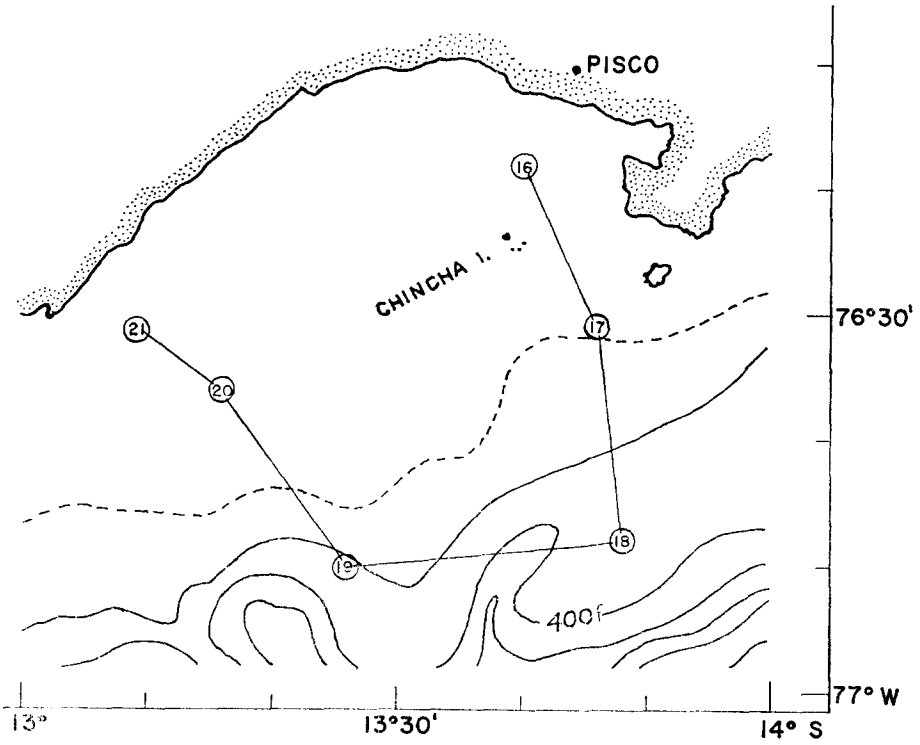

Fig. 2. Position of transects off Peru coast near Pisco. 21 March 1966. Contours at approximately 100, 200, 400 and 600 fms etc. are chart records of doubtful precision.

bottom topography. In the southern transect (Fig. 3, Stas. 16–18) large and variable concentrations of chlorophyll were also encountered over a distance of 10 miles as one left the bay and entered the north-flowing surface coastal current but these patches were absent 30 miles further north along the transect (Stas. 19–21) (Fig. 4). The surface water was appreciably (4 deg C) warmer in the bay than immediately outside and, where measured, changes of nitrate concentrations followed changes of chlorophyll concentration. Chlorophyll and temperature traces alone are not, however, very informative.

Analyses of surface chlorophyll, temperature and nitrate (Figs. 5 and 6) were carried out about 50 miles off the Peru coast just south of Pisco. The trace in Fig. 6 is continuous with that in Fig. 5 but the ship changed direction from WNW to NNE. The start of each leg is arbitrarily called mile 0. Concentrations of surface nitrate and pigment are relatively low but show marked changes. Where the ship left an area of cool water with nitrate present (Fig. 5), the water warmed through a degree

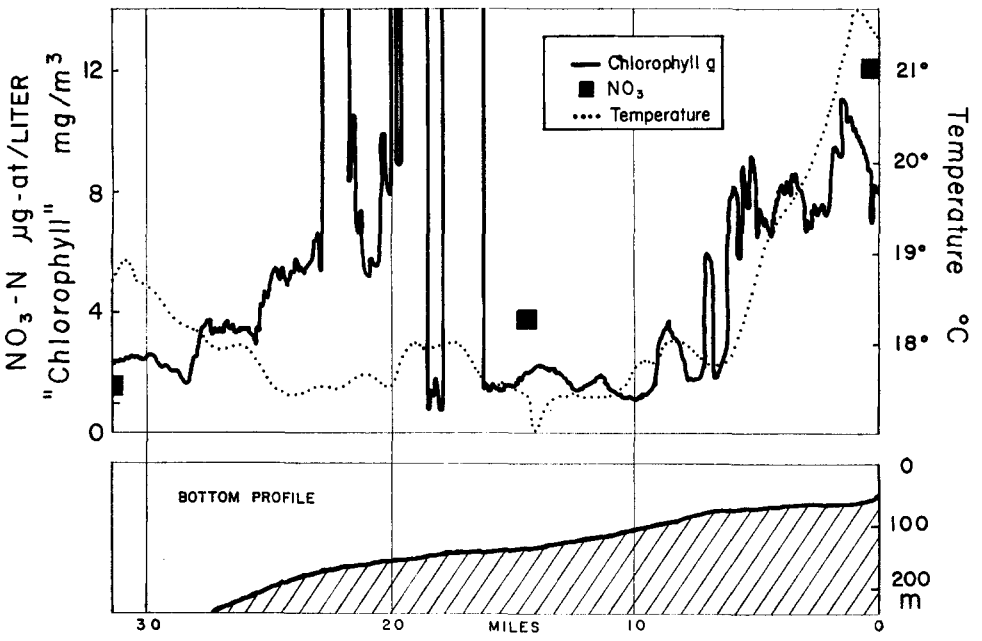

Fig. 3. Surface temperature, chlorophyll and nitrate, Stas. 16–18. Bottom topography from acoustic depth recorder.

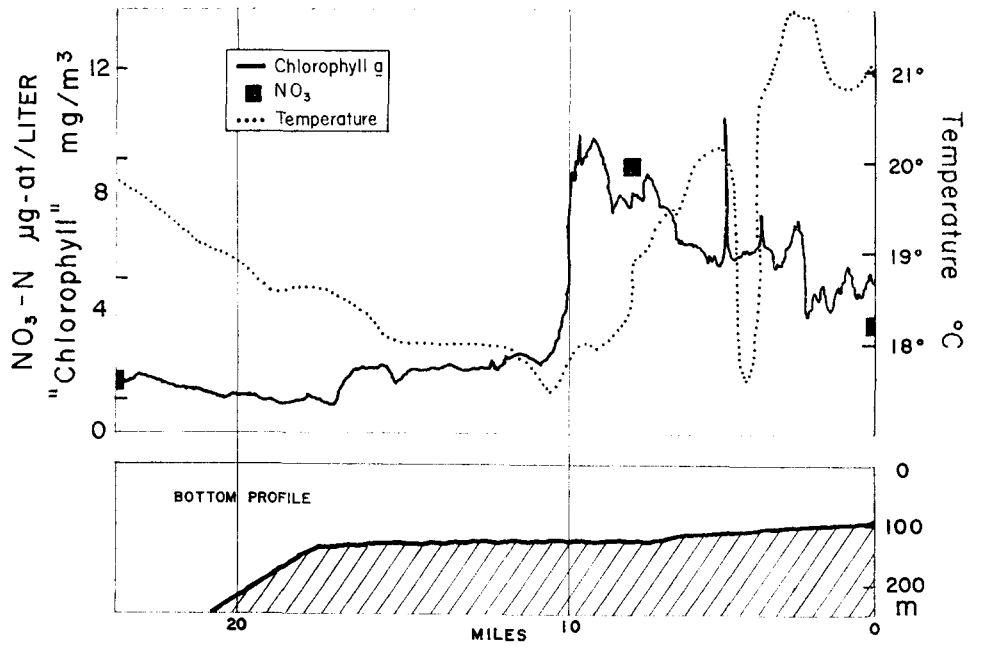

Fig. 4. Surface temperature, chlorophyll and nitrate, Stas. 19–21. Bottom topography from acoustic depth recorder.

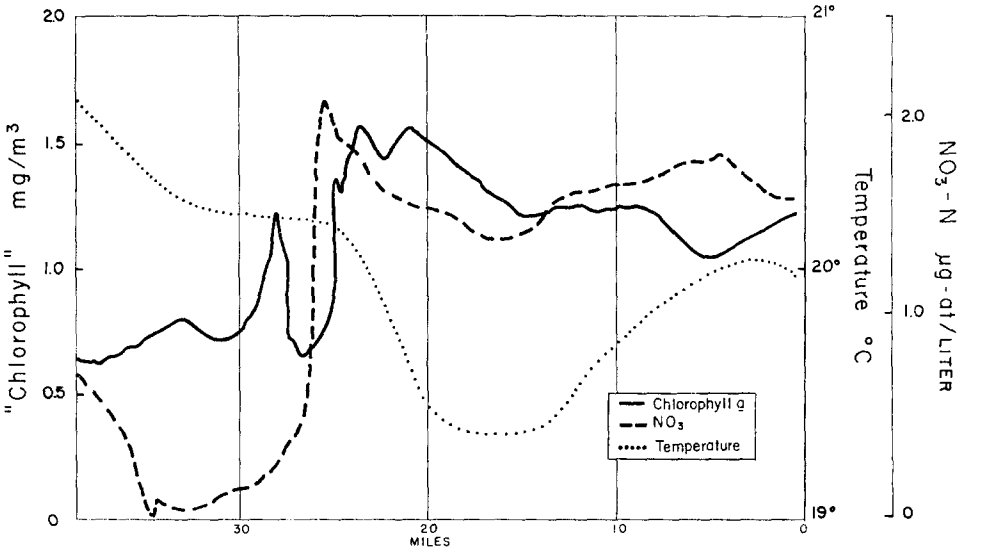

Fig. 5. Surface temperature, chlorophyll and nitrate off Peru coast (see text).

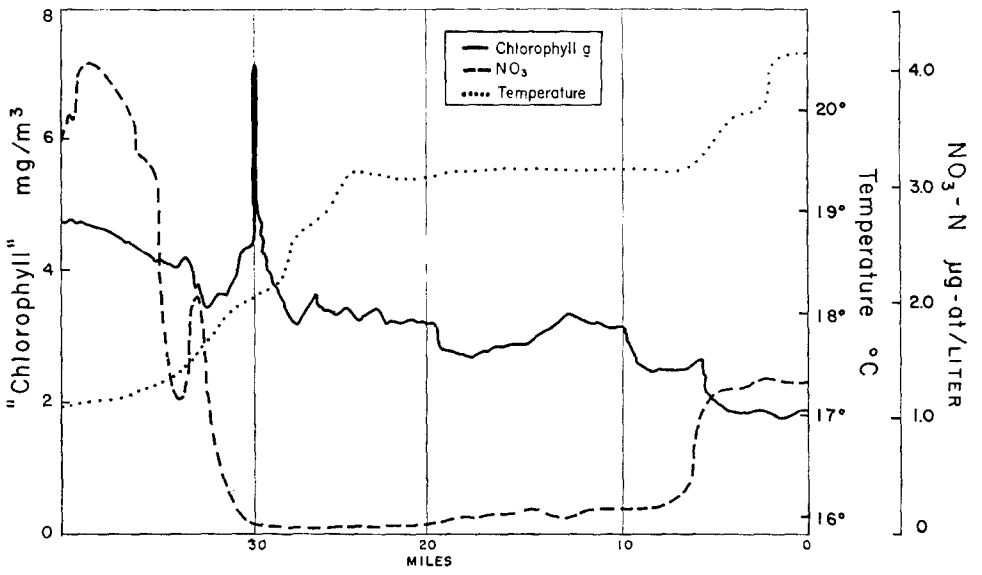

Fig. 6. Surface temperature, chlorophyll and nitrate off Peru coast (see text).

before a decrease in nitrate concentration was brought about by plant growth. Whereas when the next area of cooler nutrient-rich water was encountered (Fig. 6) the temperature drop more nearly coincided with the nitrate increase. However, as will be seen from the commencement of the trace in Fig. 5, surface warming can occur and every decrease in temperature is not reflected by a concomitant change of surface nutrient concentration. Chlorophyll values, which reflect an integration of biological activity over several days, may not reveal much in a single trace. In the middle of Figs. 5 and 6 one can recognize a rise of chlorophyll accompanied by the decrease of an approximately equivalent amount of nitrate but the picture is disturbed at the edge of patches.

In Fig. 7 we show a record of surface chlorophyll, temperature, nitrate, silicate and phosphate. The special fluorometer was inoperative at this point so the chlorophyll had to be determined at discrete

intervals by direct analysis of extracted surface samples (HOLM-HANSEN *et al.*, 1966). The ship was travelling from south to north parallel to the Peru coast, near to Punta Atico (Fig. 8), about 10 miles offshore and along an extensive upwelling patch where surface nitrate concentrations were in excess of 24 μg-at. N/l. A transect perpendicular to the coast, made in the center of this patch but not shown here, indicated that the patch was about 20 miles wide. At either end (Fig. 7) sea surface temperatures

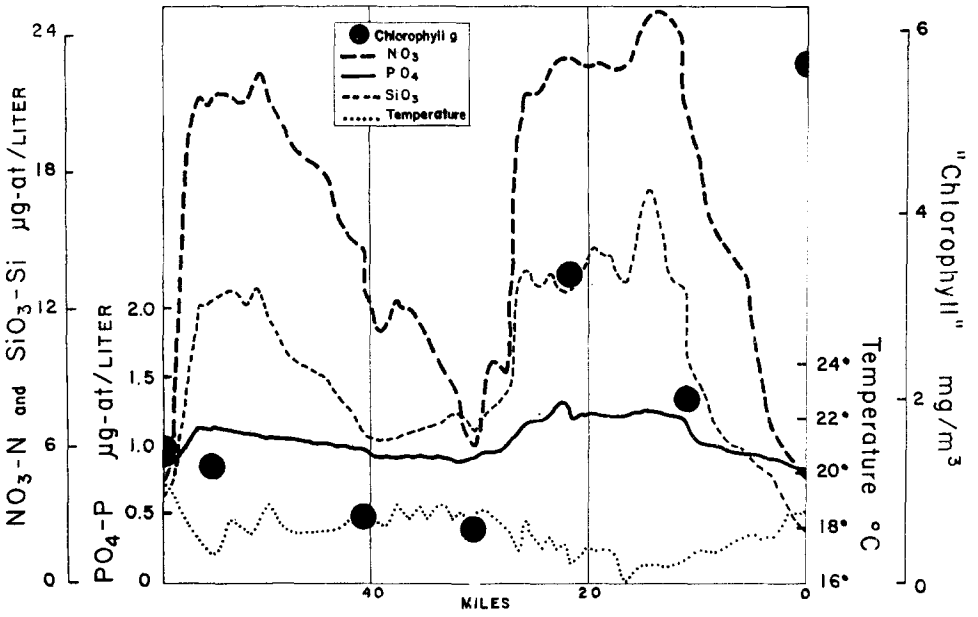

Fig. 7. Surface temperature, chlorophyll, nitrate, silicate and phosphate off Peru coast near Punta Atico, 14 March 1966.

increased 2 deg C or more and the pigment concentration was high, notably to the south (right side of arbitrary mile 0 in Fig. 7). It is interesting to note that the surface nitrate concentration, which was effectively zero outside the upwelling patch, increased exponentially with distance, into the patch, until it was 50% or more of its maximum value. The effect is not shown accurately by the traces in Fig. 7 but can be seen on the original Autoanalyzer® records as a very close approximation to exponential. The distance over which an exponential change of surface concentration was noted was 2 to 3 miles.

The 60-mile-long area (Figs. 7 and 8) consisted of two separate but nearly coalesced patches, indicated only slightly by the temperature and phosphate traces but well marked by silicate and nitrate. Phosphate will be seen to be a poor surface indicator of upwelling as only small changes occur on a "base line" of about 0·8 μg-at. P/l. Nitrate, on the contrary, is excellent and should be used if only one element is employed for detecting upwelling. The silicate trace paralleled that of nitrate but some slight variations in the N/Si ratio are outside experimental error and reflect different histories of regeneration in the upwelled water. Surface chlorophyll *a* concentrations were variable in the upwelling area and not correlated with variations of nutrient concentration in the same water. The concentration of phytoplankton, however, was less within the area than on either side of it due to one or more of three factors, (1) grazing, (2) insufficient time since upwelling for phytoplankton growth, (3) sinking of plant cells from the top few meters of the sea. The latter is the least likely.

The surface current off Punta Atico was moving south to north parallel to the coast and along the axis of the trace shown in Fig. 7, at about 20 miles a day. It is reasonable to suppose, therefore that, the features shown in Fig. 7 would have been identifiable the next day had the transect again been made at a similar distance from the coast but starting further north. Operations with several ships some miles apart (with vessels moving parallel to the coast for 24 hr, and then back-tracking to repeat the operation several times) should allow recognition and tracing of patches of upwelling water. Biological

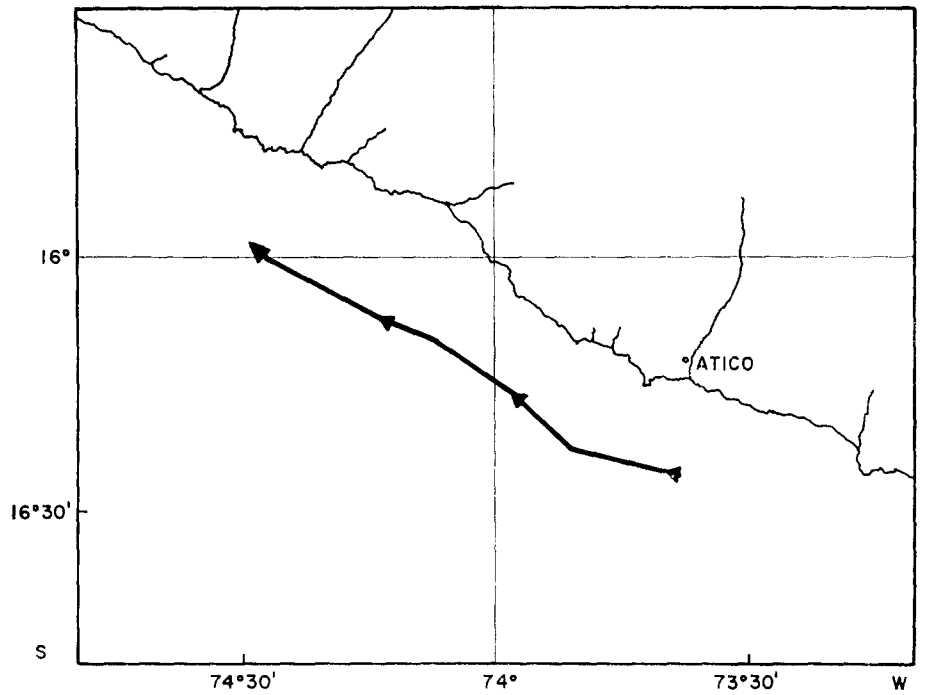

Fig. 8. Position of track through upwelling area off Punta Atico Peru. Start and finish of line correspond with start and finish of traces in Fig. 7.

changes could be studied and related to chemical changes, and the vagaries and uncertainties which seem to accompany the use of parachute drogues could be avoided. For some small scale work, the addition to the water of marker dyes which could be detected by an auxiliary fluorometer would have advantages.

It is suggested that some such method would be of value in areas where upwelling effects are important, and that a large scale trial would be worthwhile.

Several difficulties remain. The analytical output requires digital storage and the greatest care is needed to incorporate reliable time and navigational data concurrent with the analytical results. The successful delineation and following of an upwelling patch necessitates previous knowledge of the direction and velocity of the surface currents which transport the upwelled water from its place of initial detection.

Once upwelling patches are recognized and can be followed for several days a meaningful sampling programme of more detailed biological and chemical work can be incorporated to improve our knowledge of the productivity of coastal waters.

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