

RELEASE OF DISSOLVED ORGANIC MATTER BY MARINE PHYTOPLANKTON IN COASTAL AND OFFSHORE AREAS OF THE NORTHEAST PACIFIC OCEAN¹

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ABSTRACT

The rate of release of dissolved organic matter during phytoplankton photosynthesis was measured in eutrophic and oligotrophic areas of the Northeast Pacific Ocean. A method using liquid scintillation counting techniques is described that allows sample preparation to be done simply and accurately at sea. The absolute amounts of dissolved organic matter release were generally greatest near the surface and in eutrophic waters, but relative to total production were greatest in oligotrophic areas. A close correlation was found between the production of particulate organic matter and the release of dissolved organic matter.

INTRODUCTION

The magnitude of release of dissolved organic matter by phytoplankton during photosynthesis in the ocean is largely unknown. Most work has been done in the freshwater environment where losses exceeding 50% of the recently assimilated carbon have been reported as common (Fogg, Nalewajko, and Watt 1965; Nalewajko and Marin 1969; Watt 1966). Few measurements have been made in natural populations from the marine environment, but those values reported indicate comparably high values (Hellebust 1965; Horne, Fogg, and Eagle 1969). Apparently release is relatively lower where photosynthesis is high and is therefore of a more serious nature in oligotrophic areas.

During the past decade, production processes have been investigated off the Washington and Oregon coasts in connection with studies of the effect of the Columbia River on the Northeast Pacific Ocean (e.g., Anderson 1964; Small and Curl 1968). The region encompasses zones of highly productive nutrient-rich waters near the coast including areas of intensive upwelling during summer and compara-

tively unproductive nitrate-depleted offshore areas both within the influence of the Columbia River and seaward of it. The purpose of this study is to measure in these areas the magnitude of dissolved organic matter release by phytoplankton relative to the primary production as measured by the ¹⁴C method, which usually accounts only for the particulate organic fraction. The study also contributes to the methodology for measurement of release of organic matter by phytoplankton.

We thank Miss L. Matson for advice on preparation of scintillation solutions for liquid scintillation counting.

METHODS

Observations were made at seven stations during a cruise of RV *Thomas G. Thompson*, 15–31 July 1968 (Fig. 1). Particulate organic matter production, as measured by ¹⁴C (Steemann Nielsen 1952), was determined in the euphotic zone at depths representing 100, 55, 23, 15, and 4% of surface illumination. We inoculated 50-ml samples with about 2.5 μ Ci of ¹⁴C and incubated them in a deck incubator from local apparent noon to sunset. We used wire mesh screens to simulate underwater light intensity. After exposure, the samples were filtered with HA Millipore filters (0.45- μ pore diam) at a suction pressure not exceeding 0.5 atm. Filters were fumed over concentrated HCl, stored in desicca-

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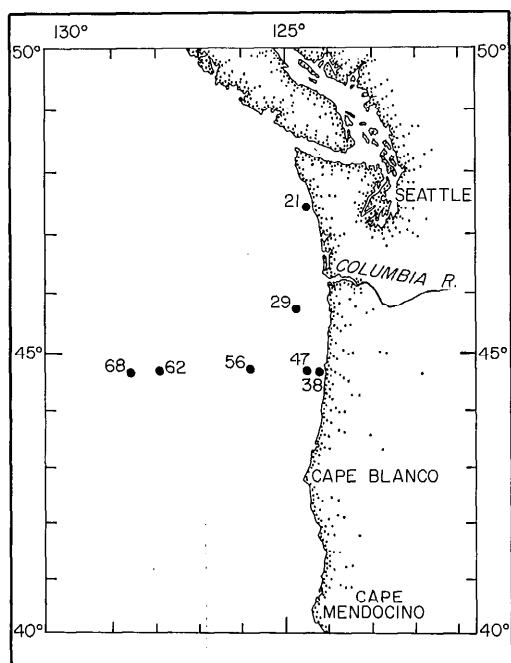


FIG. 1. Station locations off the Washington and Oregon coasts, 15–31 July 1968.

tors, and later counted by Geiger techniques in the laboratory. No corrections for dark uptake were made. Chlorophyll *a* was measured in acetone extracts (UNESCO 1966) and nitrate by the method of Wood, Armstrong, and Richards (1967).

The release of organic matter by phytoplankton was determined at sea by a modification of the method described by Nalewajko, Chowdhuri, and Fogg (1963). Filtrate from the samples used for measurement of particulate matter production was acidified with 0.1 N HCl to a pH of 2.8 and bubbled with air (250 ml/min) for at least 15 min, resulting in complete removal of inorganic ^{14}C . Aliquots (2 ml) of the acidified filtrate were then transferred directly to 20-ml glass vials containing 10 ml of a scintillation solution for counting with a liquid scintillation spectrometer (Packard Tri-carb model 3310) (see Wang and Willis 1965 for discussions of liquid scintillation counting). Results are expressed both as rates of release and

as per cent release relative to total organic matter production.

We needed to develop a scintillation (fluor) solution with a high counting efficiency yet capable of incorporating large amounts of seawater relative to the volume of fluor solution because we expected to find low levels of radioactivity. The earlier methods of plating and planchet counting (Hellebust 1965; Watt 1966) were not considered sufficiently accurate or convenient for routine shipboard use. The Van Slyke wet combustion method described by Nalewajko et al. (1963) has problems with chloride interference when used with seawater, in addition to being a complex procedure, and therefore is undesirable for work at sea.

The composition of the scintillation solution used here is as follows: To 7.8 ml of toluene solution containing 5 g/liter of PPO and 0.5 g/liter of dimethyl POPOP, add 2.2 ml of Bio-Solv solubilizer formula BBS-3 (available from Beckman Instr. Inc.). This solution accepts 2 ml of seawater per 10 ml of fluor and has a counting efficiency of 75% as determined by internal standards. An unquenched ^{14}C standard has a counting efficiency of 92% with the present instrument. Upon addition of the seawater sample, the solution becomes turbid and milky-white, and the water settles out. Refrigeration (8.5°C) is necessary so that the water can be mixed into solution and so that the preparation will clear. The low temperature must be maintained during counting. However, the samples can apparently be stored indefinitely at room temperature if a refrigerated counter is not immediately available. It is possible to increase the amount of solubilizer and thus increase the water-holding capacity of the solution, but 2 ml of seawater contained sufficient radioactivity for our purpose. For ambient temperature liquid scintillation counters, the volume of seawater added to the fluor must be reduced to a point where the water remains in solution and the mixture does not become turbid at room temperature.

The possible loss of volatile organic

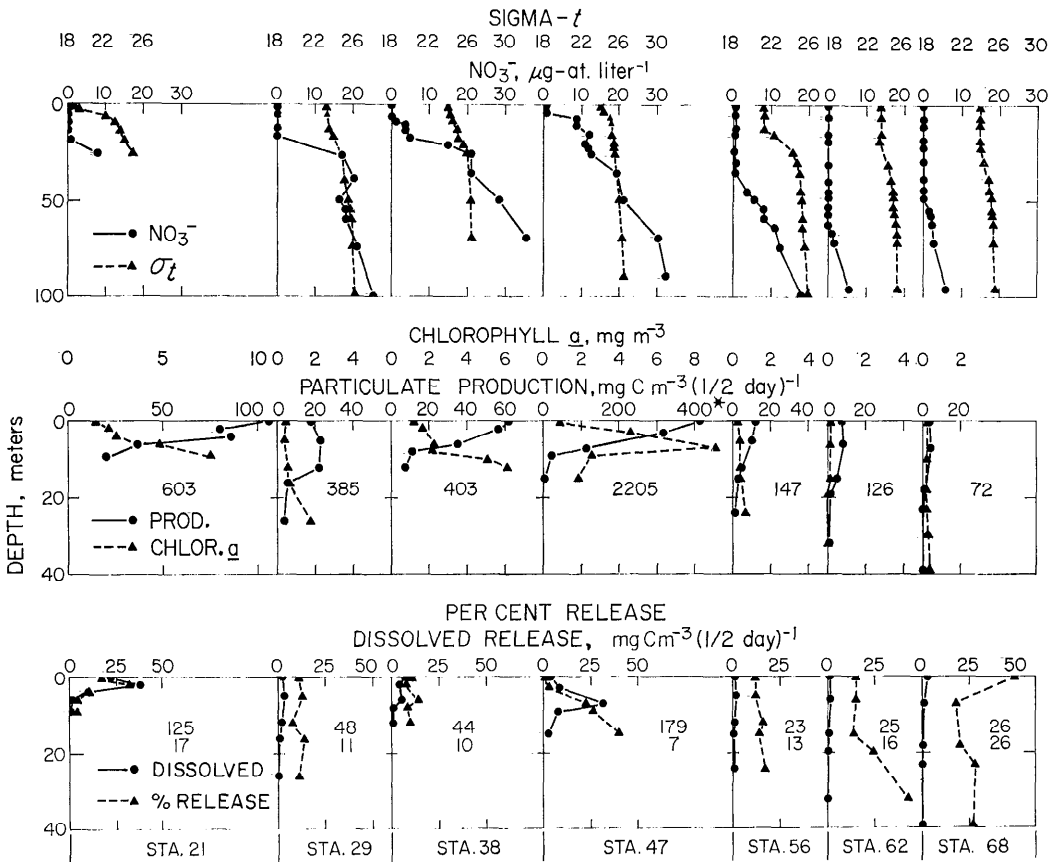


FIG. 2. Vertical distribution of density (σ_t), nitrate, production of particulate organic matter, chlorophyll a , release of dissolved organic matter, and per cent organic matter release relative to total production at stations off the Washington and Oregon coasts, 15-31 July 1968. The numerals in the middle panels are for total particulate matter production in the water column ($\text{mg C m}^{-2} \frac{1}{2} \text{ day}^{-1}$), whereas the two sets of numerals in each of the bottom panels are water column values for release of dissolved organic matter ($\text{mg C m}^{-2} \frac{1}{2} \text{ day}^{-1}$), and per cent release, respectively.

* Note the change in scale for particulate matter production.

compounds during acidification and bubbling was investigated. Carbon-14-labeled compounds of sodium acetate, sodium glycollate, succinic acid, glucose, and a purified protein hydrolysate from cells of *Chlorella vulgaris* containing a mixture of 16 amino acids were added to samples of filtered seawater, and recovery was measured before and after acidification and bubbling. The treatment resulted in losses of less than 1% for succinic acid (0.6%), glucose (0.5%), and the amino acid mixture (0.3%), whereas the more volatile

compounds had losses of less than 2% (glycollate 1.2%, acetate 1.5%).

Bacterial uptake of labeled dissolved organic materials during incubation could lead to an underestimation of the release rate. The significance of this effect was investigated on three separate occasions in a eutrophic area of Puget Sound, where absolute values of release were comparable with those measured in the coastal areas. Bacterial densities were not estimated, but it might be expected that they would be at least as great as those in the coastal areas.

Each time, six samples were obtained from depths within the euphotic zone. Before inoculation with ^{14}C , penicillin and streptomycin were added in concentrations of 100 and 50 mg/liter, respectively. The samples were incubated *in situ* at their original depths from local apparent noon to sunset. Controls consisted of samples inoculated with ^{14}C but without antibiotics. No statistically significant difference in release of organic matter was found between the control samples and those containing antibiotics.

RESULTS AND DISCUSSION

The vertical distributions at each station of density, nitrate, chlorophyll *a*, production of particulate and dissolved organic matter, and the per cent of organic matter release relative to total production are shown in Fig. 2. These stations included waters near the coast (stations 21, 38, and 47) where productivity was high. At station 47 the effect of upwelling on productivity was especially evident ($>4 \text{ g C m}^{-2} \text{ day}^{-1}$). Stations 29 and 56, less productive, were within the main axis of the Columbia River plume, whereas stations 62 and 68 were in oligotrophic waters ($<0.2 \text{ g C m}^{-2} \text{ day}^{-1}$) beyond major influence of the plume.

The rate of release of organic matter generally decreased with depth, as did particulate matter production, except at stations 21 and 47 where the maximum release occurred at middepth. At station 47 the subsurface maximum in release coincided with a large chlorophyll maximum at the same depth. Otherwise there appeared to be little relationship between release of organic matter and chlorophyll concentration, nor was there an apparent relationship with nutrient concentrations as measured by nitrate. The highest release values both for individual measurements and for total amounts within the euphotic zone were found in the most productive areas and the lowest values in oligotrophic waters.

The per cent of organic matter release relative to total production ranged from 1% for surface waters of the most productive

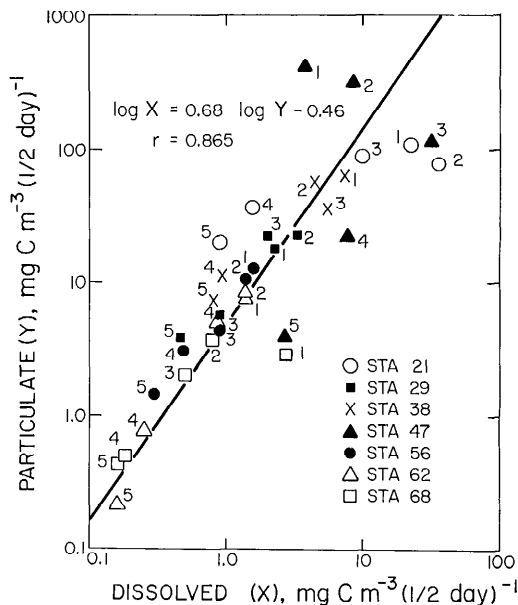


FIG. 3. Relationship between production of particulate organic matter and release of dissolved organic matter from individual measurements made within the euphotic zone at stations off the Washington and Oregon coasts, 15-31 July 1968. The numeral for each point refers to the depth at which samples were taken expressed as per cent of surface light: 1 = 100%, 2 = 55%, 3 = 23%, 4 = 15%, 5 = 4%.

station (station 47) to 49% for oligotrophic surface waters (station 68). However, the remaining values for surface waters ranged only between 11 and 17% and included both eutrophic and oligotrophic areas. At three stations (47, 62, 68), a general increase with depth occurred. The per cent release for the whole euphotic zone ranged from 7% (at the nutrient-rich upwelling station 47) to 26% (at the oligotrophic station 68).

Clearly the best relationship that can be demonstrated in these data is between the rate of production of dissolved organic matter and that of particulate matter. However, the relationship is not linear; a log-log expression best fits the data (Fig. 3). Individual values from all stations showed a highly significant correlation ($r = 0.865$). The greatest scatter results from values for station 47. The same log-log

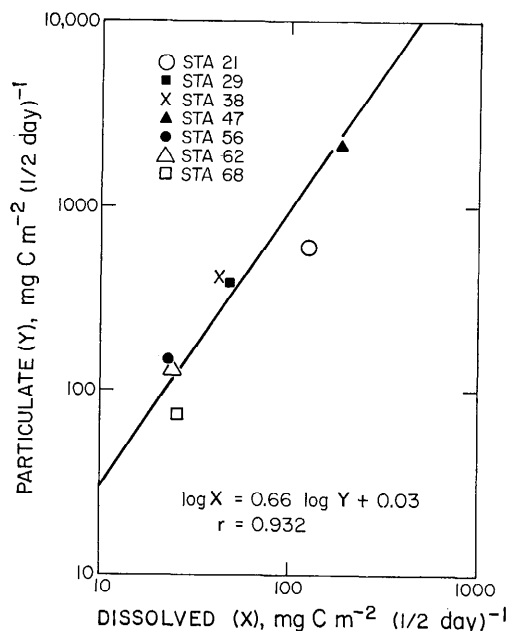


FIG. 4. Relationship between production of particulate organic matter and release of dissolved organic matter for the euphotic zone at stations off the Washington and Oregon coasts, 15–31 July 1968.

relationship of release of organic matter with production of particulate matter was found for the total values in the water column ($r = 0.932$; Fig. 4). Although clear relationships are not apparent between release of organic matter and other factors such as light, nutrients, or chlorophyll concentration, we are in general agreement with other workers that this release, on a relative basis, is greatest in oligotrophic areas.

The rates of release recorded here may be minimal because of possible simultaneous uptake of dissolved organic matter by bacteria and the algae themselves during incubation. Some of the extracellular organic carbon may not be measured if it originates from previous nonlabeled products of photosynthesis (Eppley and Sloan 1965). Conversely, grazing by zooplankton and subsequent release of ingested materials would contribute to the measured rate of production of labeled dissolved or-

ganic matter. Also, these values indicate only the release occurring during photosynthesis and do not account for uptake or further release during the night.

The failure of bacteria to show a significant uptake in the Puget Sound samples suggests that the population density is probably low, although the substrate concentration may be below the threshold level for uptake (see Jannasch 1967). Also, it is possible that short-term uptake rates of organic substrates may not be affected by the antibiotics even though bacterial growth is inhibited. Alternatively, the lack of a significant change by bacterial activity might also suggest that most of the dissolved organic matter is released during separation of the cells from the seawater. This idea is strengthened by the close correlation found between particulate matter production and release. However, we used low suction pressures to minimize cell damage; Fogg and Nalewajko (1964) have presented evidence that argues strongly against labeled compounds being derived from broken cells when precautions are used.

The measurement of release of dissolved organic matter by phytoplankton involves many factors and processes that are incompletely known. Our results, in agreement with previous work on the subject, emphasize the importance of measuring release of organic matter when making estimates of primary production, especially in oligotrophic areas.

Samples for liquid scintillation counting can be simply and accurately prepared at sea. The scintillation solution appears well suited for counting of radiotracers associated with particulate matter filtered onto membrane filters, as in the procedure used in measuring primary production. Most other solutions developed for this purpose (e.g., Wolfe and Schelske 1967) require prior desiccation of the filter; wet filters can be used with the current preparation (as well as that reported by Schindler 1966). Immediately after filtration, the filter is fumed with HCl and added to the scintillation solution, capped tightly, and

stored for subsequent counting. This eliminates the serious problem of loss of ^{14}C activity from filters stored in desiccators (Wallen and Geen 1968). Because of its large water-holding capability, the solution should be useful for counting of other aqueous solutions containing radiotracers and is now routinely used here for calibration of inorganic ^{14}C solutions used in primary productivity work.

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