

Research Note

The Influence of Dissolved Petroleum Hydrocarbon Residues on Natural Phytoplankton Biomass

ABSTRACT

The effect of dissolved petroleum hydrocarbons in the environment on phytoplankton biomass measured as chlorophyll a was studied near the oil tanker route in the southern Bay of Bengal. In the transect from 5°N, 77°E to 5°N, 87°E the concentrations of dissolved petroleum hydrocarbons were negatively correlated with phytoplankton biomass, whereas in the 0°N, 87°E to 1°N, 79°E transect they were positively correlated with phytoplankton biomass. The mean petroleum hydrocarbon concentrations in the two transects were $12.12 \pm 4.67 \mu\text{g litre}^{-1}$ and $11.23 \pm 4.5 \mu\text{g litre}^{-1}$, respectively.

*It is surmised that the effect of dissolved petroleum hydrocarbons on phytoplankton biomass varies depending on the nature rather than the quantity of petroleum hydrocarbons present. Culture studies with unialgal *Nitzschia sp.* in seawater collected from selected stations in the study area as well as in artificial seawater spiked with the water-soluble petroleum hydrocarbon fraction of light Arabian Crude support this.*

INTRODUCTION

Surveys done in the Northern Indian Ocean have shown the area to be contaminated with petroleum hydrocarbons, significantly in the Northern Arabian Sea across which lie the oil tanker routes to the Western hemisphere as well as to Japan and the Far East (Sen Gupta *et al.*, 1978, 1980; Sen Gupta & Kureishy, 1981). However, the biological implications of the petroleum

hydrocarbon residues in the marine environment remain to be ascertained, especially with regard to their effect on phytoplankton growth and ecology. Laboratory experiments have shown that petroleum hydrocarbons, low molecular weight aromatic hydrocarbons in particular, are stimulatory to phytoplankton growth in low concentrations, turning inhibitory at high concentrations (Gordon & Prouse, 1973; Pulich *et al.*, 1974; Dunstan *et al.*, 1975). Biodegradation products of petroleum have also been reported to have a similar effect on phytoplankton (Goutx *et al.*, 1987). Further, the inhibition is species-specific (Parsons *et al.*, 1976; Ostgaard *et al.*, 1984). However, since the petroleum hydrocarbon residues change their character rapidly in the environment (Prouse *et al.*, 1976), it is not always possible to extrapolate laboratory results to the environment.

Hence, with a view to studying the manner in which dissolved petroleum hydrocarbon residues present in the environment influence phytoplankton biomass in general, some studies were conducted in the southern Bay of Bengal in an area expected to be subject to chronic oil pollution on account of its proximity to the oil tanker route. These studies were undertaken on a cruise on RV *Gaveshani* (February–March 1986) and were restricted to the upper euphotic zone in two transects: one along 5°N from 77°E to 87°E and the other close to the equator from 0°N, 87°E to 1°N, 79°E. The results have been compared with those obtained in growth experiments with a pure culture of *Nitzschia* sp. in filtered surface water from selected stations in the study area as well as in artificial seawater spiked with the water-soluble petroleum hydrocarbon fraction of light Arabian crude oil and are reported here.

MATERIALS AND METHODS

The water samples were collected from three depths viz. 1 m, 10 m and 30 m at six stations in each transect (Fig. 1). One metre samples were collected immediately after the ship came to a halt at a station, using hexane-cleaned stainless steel buckets while samples from other depths were collected with all-PVC Niskin samplers. The samples were pre-filtered through a 150 μ m screen before proceeding with the various analyses.

Hydrocarbon analysis was done on 400 ml samples of glass fibre (GF/F)-filtered water essentially following the fluorimetric method of Keizer & Gordon (1973). The hexane extracts were analysed on a Turner Model 430 spectrofluorometer employing an excitation wavelength of 310 nm and an emission wavelength of 360 nm. Light Arabian crude was used as an arbitrary standard.

Chlorophyll *a* measurements were made, in duplicate, on 1-litre samples

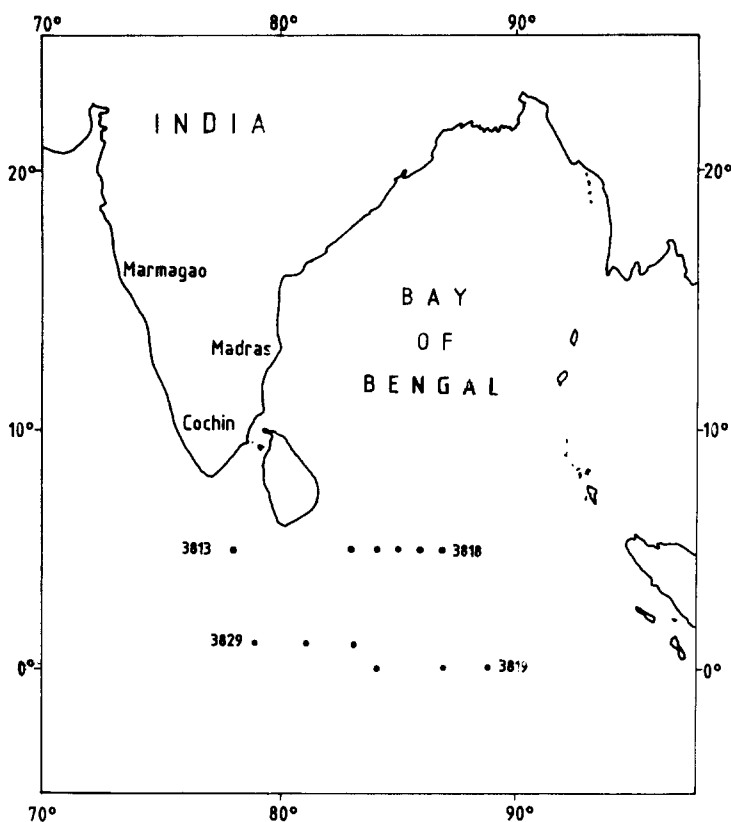


Fig. 1. Positions of sampling stations.

by the method recommended by Strickland & Parsons (1978), using a Turner Designs fluorometer.

Nutrients were determined using standard analytical techniques (Grasshoff, 1976).

Culture studies: Non-axenic unialgal *Nitzschia* sp. from the laboratory collection was batch cultured in: (i) GF/F-filtered water samples from 1 m depth and (ii) artificial sea water spiked with varying amounts of (a) freshly prepared and (b) stored (1 month in the refrigerator) water-soluble fraction (WSF) of a 10% light Arabian crude oil-in-artificial seawater solution, prepared according to Anderson *et al.* (1974).

The experiments were conducted in 1-litre Erlenmeyer flasks each containing 300 ml of growth medium (nutrients and vitamins—biotin, thiamine and B₆ enriched). The growth was measured as a change in the chlorophyll *a* content during 96 h under cool white fluorescent illumination (*ca.* 5500 lux) with a 12 h light/dark cycle.

RESULTS

Table 1 gives the mean values of chlorophyll *a*, water-accommodated petroleum hydrocarbon and nutrients in the 5°N and 0° transects. From the scatter diagrams (Fig. 2(a) and (b)) it is seen that chlorophyll *a* bears a negative linear relationship to hydrocarbon concentration in the transect along 5°N ($r = -0.58$, significant at the 0.001 level) and a positive relationship in the 0° transect ($r = 0.54$, significant at the 0.001 level). Unialgal culture studies with *Nitzschia* sp. grown in water sampled from selected stations in the two transects also showed similar relationship between chlorophyll *a* and petroleum hydrocarbon (Fig. 3(a) and (b)). In growth experiments conducted with *Nitzschia* sp. in artificial seawater incorporating WSF of light Arabian Crude oil it was observed that in the presence of freshly prepared WSF the change in chlorophyll *a* with petroleum hydrocarbon concentration was similar to that seen when the culture was grown in water samples obtained from the 5°N transect (Fig. 4(a)) whereas in artificial seawater spiked with aged WSF (Fig. 4(b)) the pattern resembled that seen in waters from the equatorial transect. However, in very low concentrations, fresh extracts could also promote phytoplankton growth; the enhancement being greater in this case than in the case of the aged fraction used at a similar concentration.

TABLE 1

Values of Some of the Parameters Existing at the Time of Study in the 5°N, 77°E to 5°N, 87°E and 0°N, 87°E to 1°N, 79°E Transects

Transect	Nutrients ($\mu\text{g-at litre}^{-1}$)			Water-soluble Chlorophyll <i>a</i> ^b hydrocarbons ^a ($\mu\text{g litre}^{-1} \times 10^2$)	
	<i>PO</i> ₄ - <i>P</i>	<i>NO</i> ₃ - <i>N</i>	<i>SiO</i> ₄ - <i>S</i>	($\mu\text{g litre}^{-1}$)	
(Mean values for the 0 m–30 m (upper euphotic zone))					
5°N	0.27 (± 0.12)	0.34 (± 0.08)	9.05 (± 4.50)	12.12 (± 4.67)	3.27 (± 0.68)
0°N	0.29 (± 0.05)	0.34 (± 0.10)	9.40 (± 4.50)	11.23 (± 4.50)	3.72 (± 1.41)

^a Measured in glass fibre-filtered sea water.

^b Relative mean deviation of replicate measurements was 3.59%.

DISCUSSION

In highly productive but unpolluted waters a non-linear relationship has been observed between chlorophyll *a* and (biogenic) hydrocarbon concentrations under natural conditions (Zsolnay, 1977). However, in the

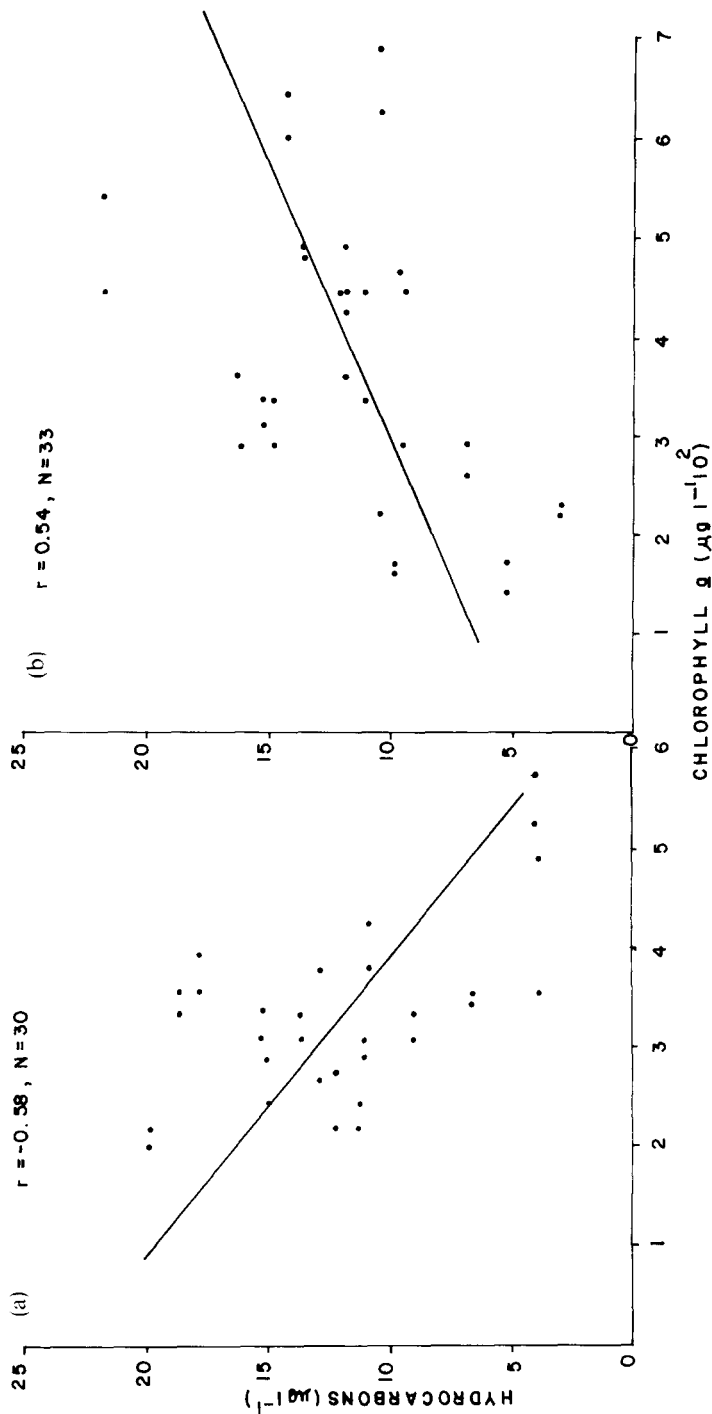


Fig. 2. Relationship between chlorophyll *a* and dissolved petroleum hydrocarbon in (a) 5°N transect, and (b) 0°-1°N transect.

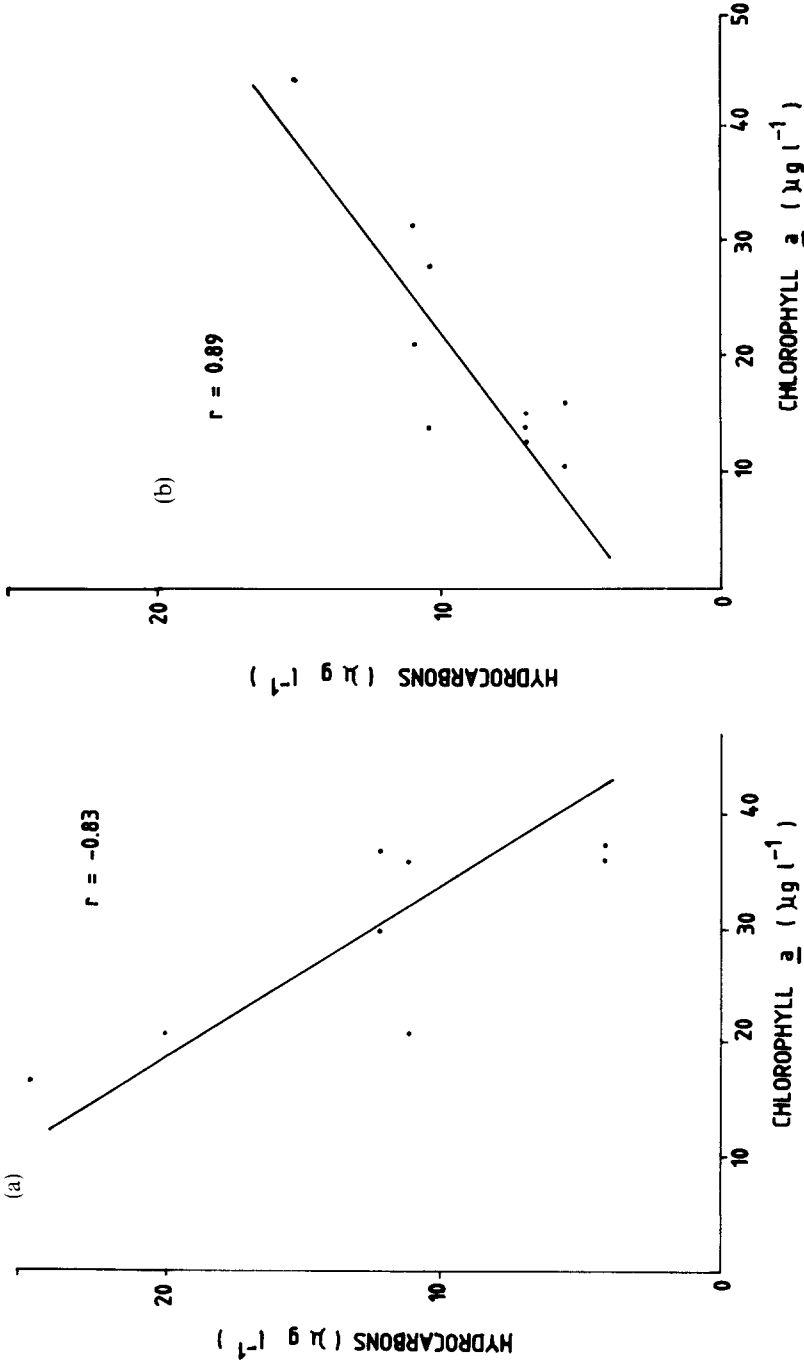


Fig. 3. Growth pattern of unialgal *Nitzschia* sp. in water samples from (a) 5-N transect, and (b) 0-1-N transect.

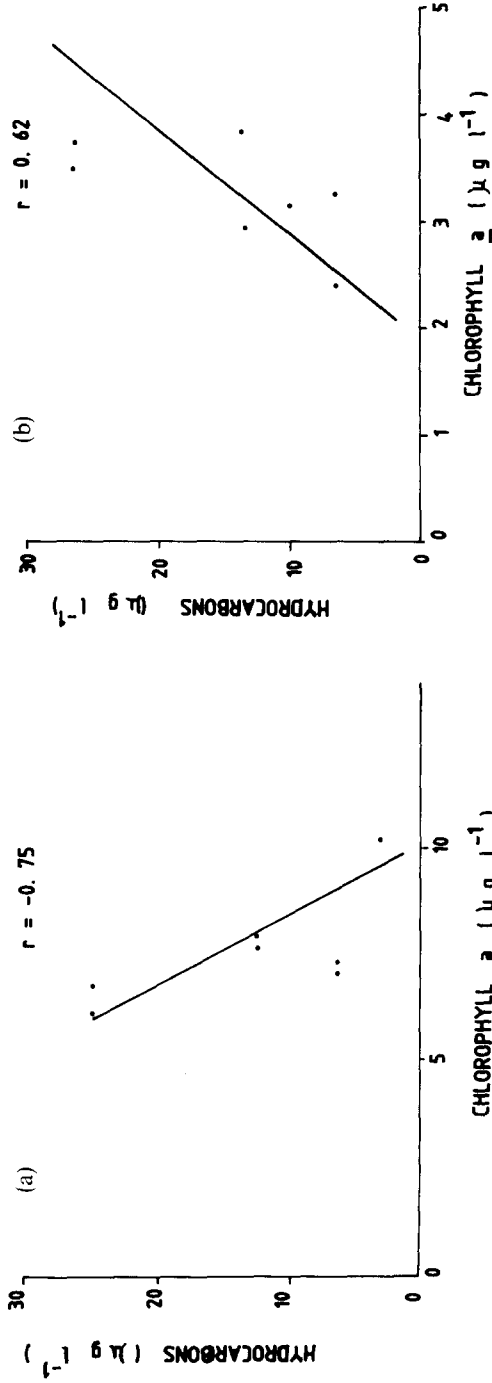


Fig. 4. Growth pattern of unialgal *Nitzschia* sp. in artificial sea water incorporating (a) freshly prepared, and (b) aged water-soluble fraction of light Arabian crude oil.

presence of petroleum hydrocarbon residues the pattern changes to a linear relationship, either positive or negative, as seen in the 5°N and equatorial transects of this study (Figs 2(a) and (b)). This and the chlorophyll *a*-petroleum hydrocarbon relationship obtained in *Nitzschia* sp. culture studies conducted in filtered surface water from selected stations in the two transects (Figs 3(a) and (b)) when considered in the light of the facts that the mean dissolved hydrocarbon concentrations were not dissimilar in the two transects ($Z = 0.77$; $p = 0.002$) and the nutrient values were also nearly similar (Table 1) lead one to conclude that qualitative rather than quantitative differences in petroleum hydrocarbon compounds were responsible for the observed difference in the chlorophyll *a*-petroleum hydrocarbon relationship along the two transects. This is supported by growth experiments conducted with *Nitzschia* sp. in artificial seawater spiked with freshly-prepared and aged WSF of light Arabian Crude oil. The culture studies indicated, in addition, that the petroleum hydrocarbon components present in the waters around the 5°N transect may have been similar to those present in the freshly prepared WSF of crude oil (Fig. 4(a)), both being toxic to phytoplankton. On the other hand, around the equator certain fractions of the petroleum hydrocarbon residue may have been removed or transformed in a manner beneficial to phytoplankton growth thus resembling the petroleum hydrocarbon components of the aged WSF in their biological effect (Fig. 4(b)). Detailed chemical analyses of seawater samples are required to substantiate this hypothesis.

Microbial biodegradation and processes such as evaporation, photo-oxidation, etc., are known to bring about changes in the quality of petroleum hydrocarbons present in water. In the present study, however, it is likely that the current pattern existing in the area, particularly the North Equatorial current separating the two transects, plays a role as well in the transformation of the petroleum residues present in the water in the equatorial transect, rendering it less toxic to phytoplankton as compared to the 5°N transect. Incidentally, we have obtained comparable results in studies conducted near another segment of the oil tanker route in the Northern Arabian Sea (unpublished data) which corroborate this view.

Thus, this study suggests that, depending on the current pattern, the waters in the vicinity of an oil tanker route may be either chronically toxic to phytoplankton or growth promoting as a result of modification of hydrocarbon components under the action of the currents. Additionally, this study suggests that irrespective of the variations noted in individual responses of different phytoplankton species to different petroleum hydrocarbon components in the laboratory (Dunstan *et al.*, 1975; Parsons *et al.*, 1976; Winters *et al.*, 1976), the overall effect of such hydrocarbon residues on natural phytoplankton communities may be either inhibition or

stimulation of growth. This would be due in all probability to alterations brought about by petroleum hydrocarbon compounds in the structure of a natural phytoplankton community through selective growth stimulation of certain phytoplankton species (Dunstan *et al.*, 1975; Parsons *et al.*, 1976). We do not have the cell count data to prove this, though.

In conclusion, it is clear that a mere quantitative survey of total petroleum hydrocarbons in seawater is not enough to determine the potential toxicity of the water to natural phytoplankton populations. This view is also held by Winters *et al.* (1976) who found in their laboratory experiments that it is not necessarily the total amount of water solubles that is critical but rather specific compounds, possibly present in small amounts, which are the important toxic agents.

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REFERENCES

- Anderson, J. W., Neff, J. M., Cox, B. A., Tatem, H. E. & Hightower, G. M. (1974). Characteristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.*, **27**, 75–88.
- Dunstan, W. M., Atkinson, L. P. & Natoli, J. (1975). Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. *Mar. Biol.*, **31**, 305–10.
- Gordon, D. C. & Prouse, N. J. (1973). The effects of three oils on marine phytoplankton photosynthesis. *Mar. Biol.*, **22**, 329–33.
- Goutx, M. M., Al-Mallah, M. & Bertrand, J. C. (1987). Effects of 9–10 dihydroanthracene and its biodegradation products on the marine diatom *Phaeodactylum tricornutum*. *Mar. Biol.*, **94**, 111–15.
- Grasshoff, K. (1976). *Methods of Sea Water Analysis*. Verlag Chemie, Weinheim, 317 pp.
- Keizer, P. D. & Gordon, D. C. Jr (1973). Detection of trace amounts of oil in sea water by fluorescence spectroscopy. *J. Fish. Res. Bd. Can.*, **30**, 1039–46.
- Ostgaard, Kjetill, Eide, Ingvar & Jensen, Arne (1984). Exposure of phytoplankton to Ekofisk crude oil. *Mar. Env. Res.*, **11**, 183–200.

- Parsons, T. R., Li, W. K. W. & Waters, R. (1976). Some preliminary observations on the enhancement of phytoplankton growth by low levels of mineral hydrocarbons. *Hydrobiologia*, **51**, 85–89.
- Prouse, N. J., Gordon, D. C. Jr & Keizer, P. D. (1976). Effects of low concentrations of oil accommodated in sea water on the growth of unialgal marine phytoplankton cultures. *J. Fish. Res. Bd. Can.*, **33**, 810–18.
- Pulich, W. M. Jr, Winters, K. & Van Baalen, C. (1974). The effects of a No. 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. *Mar. Biol.*, **28**, 87–94.
- Sen Gupta, R. & Kureishy, T. W. (1981). Present state of oil pollution in the Northern Indian Ocean. *Mar. Poll. Bull.*, **12**, 295–301.
- Sen Gupta, R., Fondekar, S. P. & Topgi, R. S. (1978). A survey of petroleum hydrocarbon concentrations in the shelf regions and adjacent waters of the West Coast of India. *Indian J. Mar. Sci.*, **7**, 12–14.
- Sen Gupta, R., Qasim, S. Z., Fondekar, S. P. & Topgi, R. S. (1980). Dissolved petroleum hydrocarbons in some regions of the Northern Indian Ocean. *Mar. Poll. Bull.*, **11**, 65–68.
- Strickland, J. D. H. & Parsons, J. R. (1968). *A Practical Handbook of Seawater Analysis*. Bulletin No. 167, Fish. Res. Bd. Can., Ottawa, p. 201.
- Winters, K., Donnell, R. O., Batterton, J. C. & Van Baalen, C. (1976). Water-soluble components of four fuel oils: Chemical characterization and effects on growth of microalgae. *Mar. Biol.*, **36**, 269–76.
- Zsolnay, A. (1977). Hydrocarbon content and chlorophyll correlation in the waters between Nova Scotia and the Gulf stream. *Deep-Sea Res.*, **24**, 199–207.

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