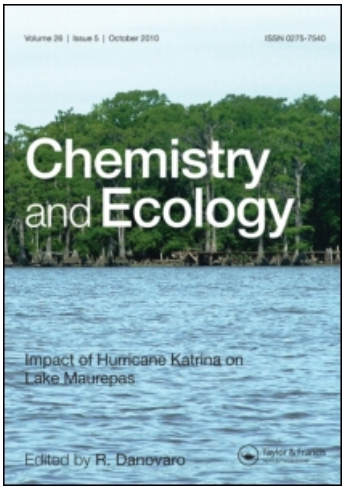

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POSSIBLE USE OF RNA:DNA RATIO FOR DETECTING OIL INDUCED DISTURBANCE: A FIELD REPORT

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The effects of oil contamination on the metabolic state of suspended particulate and sedimented organic matter were investigated through the analysis of the RNA:DNA ratio on samples collected from January 1991 to January 1992 in the Ligurian Sea (northwestern Mediterranean). The ratio of RNA to DNA appeared to be highly sensitive to hydrocarbon stress since the oil disturbance resulted in an evident decline in the RNA:DNA ratio relative to the pre-pollution conditions. Such a pattern was unusual since it occurred in a period when we would expect a seasonal increase in bacterial activity. Differences are seen when comparing the temporal trend of DNA in suspended particulates and in sediments. However, the effects of oil on the RNA:DNA ratio were similar. Oiled samples showed a RNA:DNA ratio lower than 1 whereas RNA concentrations exceeded DNA content in undisturbed samples. Bacterial DNA accounted for a significant fraction (average 26%) of total DNA in sediments. It is likely that changes in RNA:DNA ratio in oiled sediments are due to the disturbance of bacterial communities. Consistent with the meiofaunal response to oil contamination, the hydrocarbons had only temporary and limited effects and RNA:DNA values recovered to pre-pollution conditions after a few weeks. The possible use of the RNA:DNA ratio in pollution monitoring is discussed.

KEY WORDS: Nucleic acids, oil contamination, monitoring, sediments, particulate organic matter.

INTRODUCTION

DNA:RNA ratios have been used as biochemical indicators of growth in a variety of marine organisms, since RNA content changes in relation to the physiological state of the cell (Dortch *et al.*, 1983; Bamsted and Skjoldal, 1989; Mordy and Carlson, 1991). Holm-Hansen *et al.* (1968) utilized DNA concentrations as a possible measure of the living biomass in the oceans but found concentrations unreasonably high and hypothesized that DNA may not be immediately degraded. These authors concluded that to understand the significance of such DNA concentrations, it would also be necessary to determine how they are incorporated into particulate matter. Fabiano *et al.* (1993) and Fabiano *et al.* (1994) used the RNA:DNA ratios to analyse the metabolic state of particulate organic matter (POM) in different ecological processes and Danovaro *et al.*

(1993) used the RNA:DNA ratios of the sedimentary organic matter to discriminate between newly generated organic matter versus older detritus. However, to our knowledge, the RNA:DNA ratio has never been used in monitoring studies for detecting environmental disturbance.

A monitoring study in the Golfo Marconi aimed at characterizing the detrital pathways and carbon flow in a subtidal station of the Ligurian Sea started in January 1991. On 10 April 1991 several tons of crude oil were released at the mouth of the Livorno harbour (Ligurian Sea) as a consequence of a collision between the large oil tanker *Agip Abruzzo* and the ferry-boat *Moby Prince*.The oil slicks drifted north-west and arrived ashore in the Golfo Marconi 10 days later on 20 April 1991 (see Figure 1 for the location of the oil spill and the movement of the oil slicks). The oil spill caused significant changes in the structure of meiofaunal communities (Danovaro, 1993).

This paper deals with the effects of increasing oil concentrations on the metabolic state of suspended particulate and sedimented organic matter. The RNA:DNA ratio was considered as a parameter potentially sensitive to detect environmental stress. A discussion on the possible use of RNA:DNA ratio on pollution monitoring is presented.

MATERIALS AND METHODS

Study area

The study site (Zoagli) is located at 10 m depth in the Golfo Marconi, Ligurian Sea (northwestern Mediterranean Sea, Figure 1). The sampling station is exposed to wave action. This area has been intensively investigated over the past 10 years for the dynamics of benthic bacteria, macrofauna, phytoplankton, particulate matter compo-

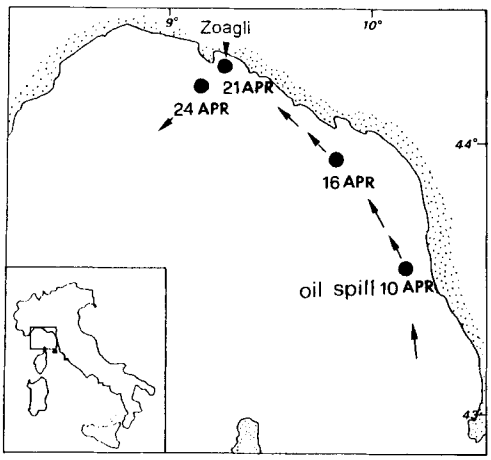

Figure 1 The sampling station and the location of the *Agip Abruzzo* accident. Illustrated are the position of the oil slicks in the two weeks following the spill and the water circulation in April 1991 (arrows).

sition, seasonal cycles of sedimentation and primary production (Fabiano *et al.*, 1984; Albertelli and Fabiano, 1990; Bavestrello *et al.*, 1991; Danovaro, 1993).

Sampling

Sediment samples were collected from January 1991 to January 1992, at fortnightly intervals from April to June and on a monthly basis for the remainder of the year. For sediment analyses three sediment cores were collected. The uppermost 2 cm, used for chemical determinations, was immediately frozen and held at −20°C until laboratory analysis. For suspended particulate matter analyses, two 5 l Niskin bottles were taken by SCUBA at the water-sediment interface.

Environmental parameters

Temperature and salinity were measured *in situ* using an AANDERAA instrument (mod. 2975). Grain size analysis was carried out using a dry sieve technique. The depth of the Redox Potential Discontinuity layer (RPD) was measured on three replicates as the depth at which the colour of the sediment turns from grey-brown to black. Porosity (as water content) was measured on four replicates, as the difference between wet and dry weight of the sediments (dried at 60°C, 24 h).

Hydrocarbon analysis

Hydrocarbon analysis was carried out on 2–3 g of sediment (on two replicates) taken from the upper 0–2 cm layer using the method of Gruenfeld (1973). Oil was extracted with 10 ml carbontetrachloride (CCl_4) solvent. The supernatant was filtered on sodium sulphate (Na_2SO_4) and Fluorisil and scanned at infra-red frequencies using a spectrophotometer (PERKIN-ELMER, mod. 983). Solutions of Bunker-C were used as a standard reference material.

Nucleic acid analysis

One litre of 200 μm prefiltered sea water was filtered through precombusted (450°C) glass fibre filters (Whatman GF/F) on two replicates. Nucleic acid extraction and measurement were performed according to Lukavsky *et al.*, 1973 and Zachleder (1984) for particulate matter. This method was modified and applied to the sediments by Fontenvieille and Fevotte (1981). Nucleic acid analysis in sediments was carried out using 1–2 g of sediment in three replicates. Details of the methods are reported in Danovaro *et al.* (1993). Briefly, the sample was treated with 2.0 ml of 0.5 N perchloric acid (PCA), stirred and sonicated three times (for 1 min with an interval of 30 s between each sonication). Hydrolysis and extraction of nucleic acid were carried out at 70°C for 15 min. The supernatant was measured at 260 nm for total nucleic acid (TNA) determination. When the TNA analysis was completed, the supernatant was used for the determination of DNA content with diphenylamine reagent. RNA content was calculated from the difference of TNA and DNA, viz:

$$\text{RNA } (\mu\text{g ml}^{-1}) = \text{TNA } (\mu\text{g ml}^{-1}) - \text{DNA } (\mu\text{g ml}^{-1})$$

Accuracy of the method had been tested previously and expressed as the coefficient of variation (CV = 8.6%). Sensitivity ($\pm$ 1μg) appears to be adequate for field investigations. Other methods are available for nucleic acid measurements (Berdalet and Dortch, 1991; Mordy and Carlson, 1991) but this method was selected for its ease and because it had already been applied to sediments (Danovaro *et al.*, 1993; 1994). In contrast, none of the fluorimetric methods reported above has, so far, been applied to the sedimentary environment.

Data analysis

Differences over time in environmental factors and nucleic acids were tested using one-way analysis of variance (ANOVA). Data were 4th-root transformed when homogeneity of variance was rejected by an F max test. Pearson's product moment correlation analysis was carried out to examine the relationships between nucleic acids and environmental factors.

RESULTS

Environmental factors

Table I shows data for temperature, salinity, average grain size, porosity and RPD depth throughout the sampling period. Temperatures showed a clear seasonality ranging from 12.6 (February) to 24.4°C (September). Salinity values, at the water-sediment interface, ranged between 37.07 (June) and 38.35‰ (December). Median grain size did not show significant changes during the sampling period ranging from 0.079 (September) to 0.202 mm (November). Porosity in the top 4 cm was almost constant during the year, ranging between 27.0 and 33.3%. The depth of the RPD exceeded 12.0 cm throughout the year.

Table I Environmental factors. Reported are: Temperature (TEM, °C); Salinity (SAL, ‰); Average grain size (AGS, mm); porosity (POR, % of sediment dry weight); redox potential discontinuity (RPD, cm).

Date	*TEM* (°C)	*SAL* ‰	*AGS* (mm)	*RPD* (cm)	*POR* (% DW)
17.01.91	13.8	38.31	0.164	20	31.0
20.02.91	12.6	38.13	0.117	20	31.2
21.03.91	14.9	38.04	0.159	20	30.1
08.04.91	14.6	38.22	0.130	20	33.3
22.04.91	14.7	38.13	0.130	20	32.1
08.05.91	15.8	37.64		20	29.1
29.05.91	19.0	37.16	0.133	20	30.4
28.06.91	22.6	37.07	0.137	19	31.4
18.07.91	22.9	37.82	0.191	20	29.1
01.08.91	23.8	38.04	0.140	20	32.1
05.09.91	24.4	38.13	0.079	20	29.5
31.10.91	18.7	38.04	0.133	16	27.0
30.11.91	16.6	38.22	0.202	20	28.1
23.12.91	14.5	38.35	0.117	20	29.9
07.01.92	13.5	38.26	0.142	12	31.5

Hydrocarbons

Hydrocarbon concentrations ranged from 44.7 to 104.3 $\mu g\, g^{-1}$ before 22 April. Immediately after the collision, oil content in sediments increased up to $214.3 \pm 13.2\, \mu g\, g^{-1}$ of sediment dry weight, decreasing to pre-pollution levels after a month (Figure 2). The sample collected on 22 April corresponded to the maximal visible pollution in the Golfo Marconi. Sediments screened through 37 μm mesh net for meiofaunal extraction showed the presence of tar particles ranging from 30 to 500 μm in size (Danovaro *et al.*, in press).

Nucleic acids in POM

Seasonal changes of DNA and RNA concentrations in particulate matter are shown in Figure 3a and b. DNA concentrations, mean 13.77 $\mu g\, l^{-1}$, remained quite constant during the year; lowest concentration was reported in March (9.12 $\mu g\, l^{-1}$), while the highest values were observed in late April-early May (19.98 and 19.96 $\mu g\, l^{-1}$). RNA was correlated to temperature ($r = 0.634$, $p < 0.05$). The RNA content of POM ranged from 4.41 (22 April) to 59.49 $\mu g\, l^{-1}$ (July), significantly lower on 22 April than at other periods of the year (ANOVA, $p = 0.014$). Changes in RNA:DNA ratio (Figure 3c) ranged between 0.22 (22 April) and 4.21 (November), with an average value of 2.59.

Nucleic acids in surface sediments

Temporal trends of DNA, RNA and RNA:DNA ratios are shown in Figure 4. DNA content showed a single peak in the oiled sample (22 April) but overall, did not change significantly during the year. RNA was correlated to temperature ($r = 0.648$, $p < 0.05$). In general, RNA concentrations exceeded DNA concentrations and ranged from 5.06 ± 1.06 in November to $13.83 \pm 0.52\, \mu g\, g^{-1}$ in July. RNA:DNA ratio showed a clear seasonality and was significantly higher in summer than in the colder months (ANOVA, $n = 3$, $p < 0.05$). The lowest value (0.80) was recorded on 22 April 1991.

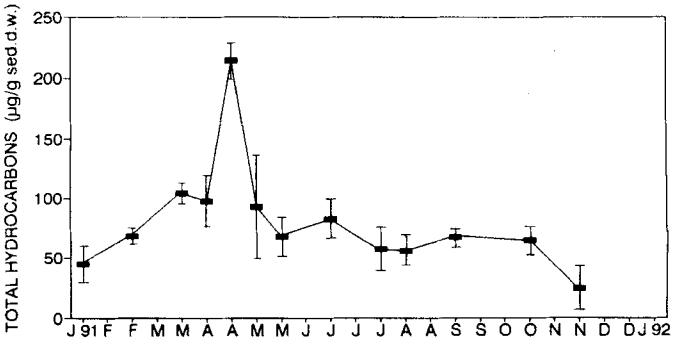

Figure 2 Seasonal variations of hydrocarbon concentrations in the study area (uppermost 1 cm sediment layer, bars represent $\pm$ 1SD).

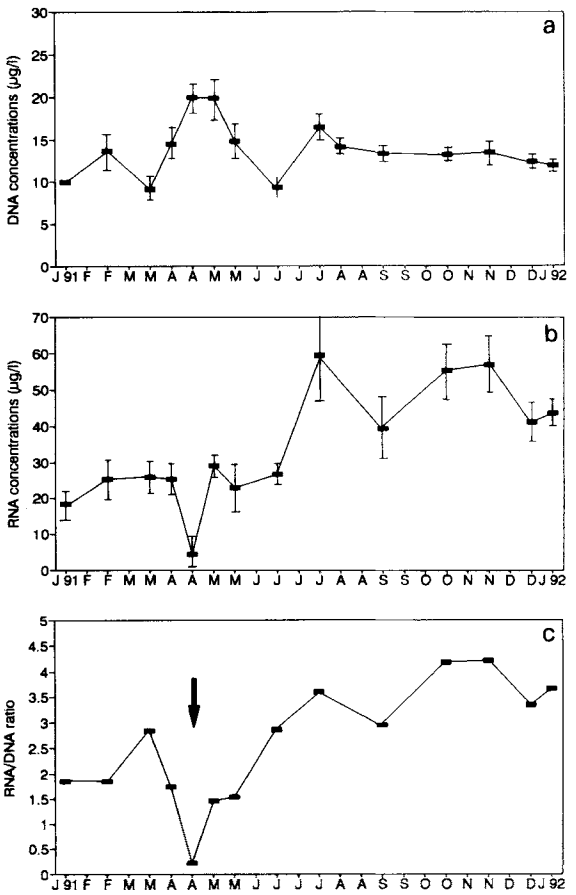

Figure 3 Changes in nucleic acid concentrations in suspended particulate matter (POM). Illustrated are: a) DNA concentrations; b) RNA concentrations and c) the RNA:DNA ratios (bars represent ± 1SD).

DISCUSSION

Hydrocarbon concentrations on the sea bed of the Golfo Marconi on 22 April were comparable to those observed by Boucher (1980) in the Bay of Morlaix and Lannion about 40 days after the *Amoco Cadiz* oil spill. As indicated by redox values, increased oil levels did not lead to any significant increase in oxygen demand (increased level of organic matter and temporary hypoxia or anoxia). At the same time, oil concentrations before collision were relatively high probably because of the intense boat activity. Hydrocarbon content decreased to pre-pollution levels after few weeks.

The use of nucleic acid concentrations as a measure of living biomass has not been universally endorsed since a large amount of DNA may still be present in newly generated detritus and this could affect the ratio of RNA to DNA (Fontenvieille and Fevotte, 1981; Fontenvieille and Cazelles, 1985; Holm-Hansen *et al.*, 1968). On the other hand, RNA is much more readily mineralized than DNA. In a study

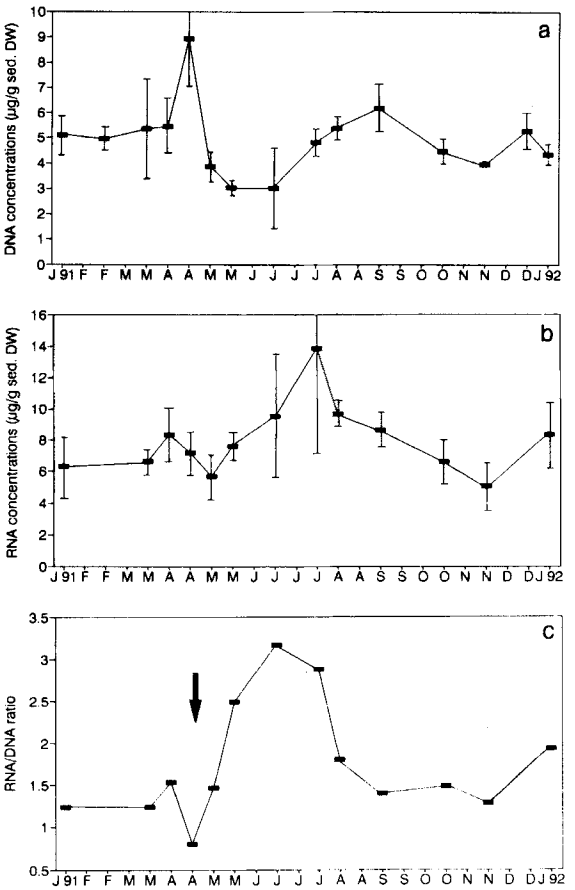

Figure 4 Changes in nucleic acid concentrations in surface sediments. Illustrated are: a) DNA concentrations; b) RNA concentrations and c) the RNA:DNA ratios (bars represent ± 1SD).

on the degradation of bacterial biomass in marine sediments, Novitsky (1986) found that, after the death of the bacterial cell, RNA mineralization was almost complete within 3 days.

Dortch *et al.* (1983) found the highest RNA:DNA ratios corresponded to the highest phytoplanktonic growth rates and protein synthesis. Pusceddu *et al.* (1993) demonstrated that RNA:DNA ratio in cultures of *Tetraselmis suecica* decreased drastically when the culture, in log-growth phase, was exposed to UV irradiation (utilized to kill the culture). Therefore, RNA content and RNA:DNA ratio seems to be a parameter sufficiently sensitive to identify an environmental stress.

Data reported in this study indicate that the ratio of RNA to DNA is highly sensitive to hydrocarbon disturbance since the oiled samples were characterized by an evident and significant decline in RNA:DNA values relative to pre-pollution values. Such values are unusual since they are reported in a period when we would expect a seasonal increase in phytoplankton and bacterial activity (Fabiano *et al.*, 1984; Danovaro *et al.*,

1994), and consequently an increase of the RNA:DNA ratio (Pusceddu *et al.*, 1993; Dortch *et al.*, 1983). This is confirmed by the significant correlation between RNA and temperature which provides further insight into the possibility of using the RNA:DNA ratio as an indicator of the metabolic state of the organic matter. RNA concentrations were higher than DNA concentrations in both particulate organic matter (POM) and in sediments. The only expection, where RNA:DNA ratios were lower than 1, was found in the oiled samples (22 April), both at the water-sediment interface and in the sediments. Similarly, in the Ross Sea, Fabiano *et al.* (1993) found RNA:DNA average values > 1 in the photic layer and < 1 in the deep (aphotic) layer.

Differences arise when comparing the changes of nucleic acid concentrations in POM and in sediments. Suspended particulate DNA concentrations did not fluctuate significantly over the year. In sediments, in contrast, DNA concentrations showed a peak in the 22 April sample. This discrepancy could be due to the accumulation by sedimentation of detrital DNA on the bottom. An alternative explanation of the increased DNA levels is that RNA degradation was being recycled into DNA (Novitsky, 1986).

In agreement with results observed for meiofaunal assemblages (Danovaro *et al.*, in press), oil induced disturbance had apparently only temporary and limited effects and RNA:DNA values recovered to pre-pollution conditions after few weeks.

It would be interesting to investigate the origin of the nucleic acids in order to understand which ecological component is responsible for the observed RNA:DNA response to increased hydrocarbon levels. For instance, in sediments collected simulataneously, Danovaro (1993) reported benthic bacterial densities ranging from 0.1 to $2.67 \pm 0.86 \times 10^9$ cells g^{-1} DW. Using the conversion factor reported by Simon and Azam (1989), 5×10^{-15} g DNA $cell^{-1}$, we calculated an annual average bacterial contribution to DNA pool of 26%. Bacterial contribution to DNA on 22 April was 15%. It is likely that changes in RNA:DNA ratio in oiled sediments are due to the disturbance of bacterial communities. These values reflect simplifying assumptions and probably overestimate the bacterial contribution to total DNA, since the conversion factor utilized is the maximum value reported by Simon and Azam (1989). Nevertheless these data indicate that bacteria represent a significant fraction of the sedimentary DNA. If we assume that a large fraction of the nucleic acid is of bacterial origin, the ratio gives a rapid response to environmental disturbance and the rapid decline and recovery of the RNA:DNA ratio would be explained.

These results must be considered with caution since little is still known about the seasonal variability of the nucleic acid concentrations in natural samples. Moreover, the reported RNA:DNA values may be site, habitat or pollutant specific. Therefore further observations are needed in order to explore the possibility of using this ratio as a tool for pollution monitoring. If these data are confirmed, the RNA:DNA ratio appears to be a sensitive, rapid and easy measure for monitoring oil induced disturbance which can be carried out routinely at a low cost.

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