

Early diagenesis and trophic role of extracellular DNA in different benthic ecosystems

Cinzia Corinaldesi, Antonio Dell'Anno, and Roberto Danovaro¹

Department of Marine Sciences, Faculty of Science, Polytechnic University of Marche, Via Breccie Bianche, 60131 Ancona, Italy

Abstract

To provide new insights into the early diagenesis of extracellular deoxyribonucleic acid (DNA) and its relevance in trophodynamic processes, we collected sediment samples from a variety of coastal and deep-sea sites. Turnover times of extracellular DNA were approximately threefold shorter in coastal benthic systems than in deep-sea sediments (0.35 yr and 1.2 yr, respectively). This was due to the higher concentrations of bioavailable extracellular DNA in deep-sea over coastal sediments (19.8 ± 0.6 and $9.8 \pm 2.6 \mu\text{g DNA g}^{-1}$, respectively), since deoxyribonuclease (DNase) activities did not show significant differences between sampling sites (135.8 ± 72.7 and $62.8 \pm 19.0 \text{ ng DNA g}^{-1} \text{ d}^{-1}$, in coastal and deep-sea sediments, respectively). The coastal benthic systems were characterized by DNase activities per prokaryotic cell significantly lower than those of their deep-sea counterparts (0.12 ± 0.05 and $0.27 \pm 0.06 \text{ fg DNA degraded cell}^{-1} \text{ d}^{-1}$, respectively), although carbon production per cell was not significantly different (6.9 ± 4.4 and $2.6 \pm 0.9 \text{ fg C cell}^{-1} \text{ d}^{-1}$, respectively). These results suggest that DNase activity per cell may be more dependent on the amount of bioavailable substrate rather than on prokaryotic metabolism. Extracellular DNA may supply 20% and 47% of the daily prokaryote anabolic requirements for organic phosphorous in both coastal and deep-sea sediments, respectively. Overall, our data suggest that bioavailable extracellular DNA might represent an important nutrient source for benthic heterotrophic metabolism and open new perspectives for a better understanding of the factors that influence the functioning of benthic systems.

Bacteria-mediated degradation of organic matter plays a key role in carbon cycling and nutrient regeneration in the oceans (Azam 1998). This process is largely mediated by extracellular enzymatic hydrolysis, which allows the breakdown of high-molecular-weight compounds into bioavailable monomers (Hoppe 1991; Keith and Arnosti 2001).

Extracellular deoxyribonucleic acid (DNA) is a minor component of the organic-matter pool in the oceans (Jiang and Paul 1995; Danovaro et al. 2006), although, due to its high lability and high nitrogen and phosphorous contents, it might represent an important resource for bacterial metabolism (Jørgensen and Jacobsen 1996; Danovaro et al. 1999). Moreover, previous studies have indicated that extracellular DNA might also be a source of exogenous nucleotides for the de novo synthesis of bacterial DNA (Paul et al. 1988, 1989).

The two main pathways of extracellular DNA removal in marine ecosystems are: (1) internalization of DNA fragments through uptake by competent bacterial cells (Dubnau 1999), and (2) assimilation and mineralization of

nucleosides and nucleobases after DNA degradation by cell-associated and free deoxyribonuclease (DNases; Paul et al. 1988; Ammerman and Azam 1991; Dell'Anno and Danovaro 2005). Since deoxyribonuclease (DNase) is widespread in all aquatic environments, the latter process is expected to be the main route for extracellular DNA cycling (Lorenz and Wackernagel 1994; Dell'Anno and Corinaldesi 2004).

Experimental studies in pelagic environments have reported that extracellular DNA degradation supplies a relevant fraction of the daily bacterial N and P requirements (Jørgensen et al. 1993; Jørgensen and Jacobsen 1996), and recent studies in marine sediments have provided evidence that extracellular DNA degradation rates are much higher in sediments than in the water column (Dell'Anno and Corinaldesi 2004). This has allowed us to hypothesize an important trophic role for extracellular DNA in benthic ecosystems (Danovaro et al. 1999; Dell'Anno et al. 2002a). This role could be even more important in deep-sea systems, where the highly oligotrophic conditions can severely limit the abundance, biomass, and metabolic activity of the benthic prokaryotes and higher trophic levels (Turley 2000; Dell'Anno and Danovaro 2005).

Although bacteria are recognized as having an active role in mediating extracellular DNA degradation processes in marine environments, ecological factors controlling the rates at which extracellular DNA is recycled are still unknown. Paul et al. (1989) reported that the turnover time of extracellular DNA in pelagic ecosystems was quite constant and independent of the trophic state and bacteria standing stock. Conversely, recent studies carried out in benthic systems have shown that extracellular DNA is recycled at much faster rates in sediments characterized by a high organic-matter content and by bacterial abundance (Dell'Anno et al. 2005).

¹ Corresponding author (r.danovaro@univpm.it).

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Table 1. Station location, grain size, protein, carbohydrate, and lipid concentrations, and biopolymeric carbon content (BPC) in coastal and deep-sea sediments. For each variable, standard deviations (\pm SD; $n = 3$) are given, whereas for the mean values, standard errors are given; na = not available.

Location	Latitude	Longitude	Depth (m)	Silt-clay (%)	Proteins (mg g ⁻¹)	Carbohydrates (mg g ⁻¹)	Lipids (mg g ⁻¹)	BPC (mg C g ⁻¹)
Coastal stations								
N. Adriatic Sta.1	44°49.2N	12°19.3E	1.9	80.2	13.4 \pm 1.8	7.6 \pm 0.7	2.7 \pm 0.2	11.6
N. Adriatic Sta.2	44°48.0N	12°19.6E	2.5	65.4	9.1 \pm 1.8	4.8 \pm 0.9	2.4 \pm 0.4	8.2
N. Adriatic Sta.3	44°47.9N	12°21.3E	3.0	75.6	26.3 \pm 3.6	6.6 \pm 1.0	3.9 \pm 0.6	18.5
C. Adriatic Sta.1	43°37.3N	13°29.9E	12.4	79.9	4.7 \pm 0.6	2.0 \pm 0.8	1.1 \pm 0.2	3.9
C. Adriatic Sta.2	43°37.3N	13°29.0E	9.0	16.3	na	na	na	na
N.W. Mediterranean	37°10.0N	01°15.0E	35.0	15.3	2.0 \pm 0.4	7.2 \pm 1.5	0.7 \pm 0.2	4.4
<i>Means</i>					11.1 \pm 4.3	5.6 \pm 1.0	2.2 \pm 0.6	9.3 \pm 2.7
Deep-sea stations								
W. Mediterranean	40°33.9N	04°57.1E	2,755	43.7	0.9 \pm 0.0	4.4 \pm 1.7	0.3 \pm 0.1	2.4
S. Pacific Ocean	33°18.5S	76°55.4W	3,060	83.2	0.6 \pm 0.1	1.6 \pm 0.3	0.1 \pm 0.0	1.0
E. Mediterranean	35°42.4N	20°08.8E	3,200	74.2	0.4 \pm 0.0	8.0 \pm 1.6	0.3 \pm 0.1	3.6
N.E. Atlantic Ocean	48°50.2N	16°29.9W	4,850	84.5	0.9 \pm 0.2	1.3 \pm 0.1	0.8 \pm 0.0	1.5
<i>Means</i>					0.7 \pm 0.1	3.8 \pm 1.5	0.4 \pm 0.2	2.1 \pm 0.6

In the present study, we investigated the degradation and turnover rates of extracellular DNA in a variety of benthic systems that are characterized by different trophic conditions to provide new insights into the factors controlling early diagenesis of extracellular DNA and into the relevance of DNA degradation in benthic trophodynamic processes.

Materials and methods

Study area and sampling—Sediment samples were collected at six coastal stations and four deep-sea stations. Coastal stations were located in the Mediterranean Sea: three stations in the north Adriatic Sea (within the Goro Lagoon), two stations in the central Adriatic Sea, and one station in the northwestern Mediterranean Sea (off Alicante, Spain; Table 1). The Goro Lagoon is eutrophic due to high continental inputs from the Po River, high values of micro- and macroalgae primary production, and a large accumulation of organic matter (Manini et al. 2003). Stations located in the central Adriatic Sea are mesotrophic, with pelagic primary production values exceeding 100 g C m⁻² yr⁻¹ (Pugnetti et al. 2005). The coastal station located in the northwestern Mediterranean is characterized by sea grass beds (*Posidonia oceanica*), and benthic primary production values exceed 300 g C m⁻² yr⁻¹ (Pergent et al. 1994). Coastal sediments were collected with manual corers by scuba divers or using a multiple-corer.

Deep-sea sediments were collected using a multiple-corer (Maxicorer; inner diameter, 9.5 cm) in the northeastern Atlantic Ocean and in the western and eastern Mediterranean Sea, and using a spade corer in the southern Pacific Ocean (Table 1). All deep-sea systems were characterized by oligotrophic conditions, with sedimentation rates of 0.003–0.023 cm yr⁻¹ in the south Pacific Ocean and in the western Mediterranean Sea, respectively.

To provide information on organic-nutrient availability in the different benthic systems, we used the biochemical composition of sedimentary organic matter (i.e., proteins, carbohydrates, and lipids) as a proxy for the trophic conditions (sensu Dell'Anno et al. 2002b). Five to nine independent cores were collected at each station, and the top 1 cm of sediment was sliced under a laminar-flow hood using sterile utensils and used for laboratory analyses.

For the determinations of grain size, biochemical composition of organic matter, and bioavailable extracellular DNA, the sediment samples were immediately frozen at -20°C . Previous studies have demonstrated that the analysis of extracellular DNA carried out on fresh and frozen sediment samples provides similar quantitative results (Dell'Anno et al. 2002a).

For the analysis of prokaryote abundance and biomass, sediment subsamples were fixed with 0.02- μm -filtered formalin (final concentration, 2%), previously buffered with sodium tetraborate and stored in the dark at 4 $^{\circ}\text{C}$ until analysis (conducted 1–2 weeks after sampling). For prokaryotic carbon production determination, additional sediment subsamples were immediately processed as described below.

All measurements were carried out on three independent sediment cores collected at each study site.

Sedimentary parameters—The grain-size analyses were carried out by dry sieving. The sediment samples were sieved through a 63- μm mesh to distinguish between the sandy and the silt-clay fractions.

Total carbohydrates were analyzed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. Protein analysis was carried out following an extraction with NaOH (0.5 mol L⁻¹; 4 h), and the concentrations were determined according to Hartree (1972), as modified by Rice (1982) to compensate for phenol interference. Concentrations are expressed as

bovine albumin equivalents. Lipids were extracted from sediments by direct elution with chloroform and methanol as described by Bligh and Dyer (1959) and Marsh and Weinstein (1966). For each biochemical analysis, blanks were made using the same sediments previously treated in muffle furnace (550°C; 4 h). Carbohydrate, protein, and lipid concentrations were converted to C equivalents using the conversion factors of 0.40, 0.49, and 0.75, respectively (Fabiano and Danovaro 1994). The sum of lipid, protein, and carbohydrate C is given as biopolymeric C (BPC; Fabiano and Danovaro 1994). All of the data were normalized to sediment dry weight after desiccation (60°C; 24 h).

Bioavailable extracellular DNA concentration—Extracellular DNA in sediment samples was extracted and quantified according to Dell'Anno et al. (2002a). This method is based on extracellular DNA hydrolysis using commercial nucleases and allows quantification of the most available degradable fraction of extracellular DNA without any contamination by DNA associated with living biomass. Frozen sediment samples were stirred at 150 rpm in 0.1 mol L⁻¹ Tris-HCl pH 7.5, 0.1 mol L⁻¹ NaCl, 1 mmol L⁻¹ CaCl₂ and MgCl₂ (buffer:sediment ratio, 2.5; v/w). Aliquots of DNase I (1.9 U mL⁻¹), P1 and S1 nucleases (4.0 and 2.3 U mL⁻¹, respectively), and esonuclease-3 (1.9 U mL⁻¹) were added to sediment slurries (*n* = 3); another set of replicates was added to an equal volume of buffer (without enzymes) and used as control. These samples were incubated at room temperature for 2 h under gentle agitation. After this incubation, all of the samples were centrifuged at 2,000 × *g* for 5 min, and the supernatants were analyzed for the amounts of DNA released from the sediment. The supernatants were filtered through 0.2- μ m-pore-size filters, dried under vacuum, and analyzed fluorometrically using diaminobenzoic acid (DABA; Karl and Bailiff 1989). The fluorescence levels of the samples were converted into concentrations using calibration curves obtained from standard solutions of calf-thymus DNA (0.05–5.0 μ g DNA mL⁻¹). The amount of extracellular DNA hydrolyzed by nucleases was obtained by the difference between the DNA concentrations determined from the supernatants of enzyme-treated samples and the supernatants of the control samples. In all of the sediment types, the control samples contained less than 5% of the extracellular DNA pool released by the nucleases. The bioavailable extracellular DNA concentrations were normalized to sediment dry weights after desiccation (60°C; constant weight). Previous studies using different sediment types have demonstrated that under the same experimental conditions to those used here, no inhibition of the added nuclease was seen (Dell'Anno et al. 2002a).

DNase activity—DNase activity was determined fluorometrically using a fluorescent DNA analogue (poly[dεA]; polydeoxyribo-1-N⁶ ethenoadenylic acid) (Dell'Anno and Corinaldesi 2004). The poly(dεA) substrate was prepared through chemical modification of poly(dA) (fragment length, 50 bases) using chloroacetaldehyde, according to

Dell'Anno and Corinaldesi (2004). Fresh sediment subsamples (~1 mL) were brought up to a final volume of 5 mL with prefiltered (0.2- μ m pore size) and autoclaved seawater containing 100 ng poly(dεA) mL⁻¹ (i.e., the concentration of poly[dεA] at which DNase activity reached V_{max}). Autoclaved sediments were used as blanks. All samples were incubated in the dark at the in situ temperature for 1 h. After incubation, the supernatants obtained from the sediment samples (following centrifugation at 2,000 × *g* for 5 min) were analyzed fluorometrically at 320-nm excitation and 410-nm emission wavelengths. The fluorescence of the samples was converted into amount of released mononucleotides using calibration curves obtained from standard solutions of 1,N⁶-ethenoadenine deoxyribose-5'-monophosphate (dεAMP, 2.5–150 ng mL⁻¹). DNase activity was expressed as ng DNA g⁻¹ dry sediment d⁻¹. A comparative analysis was carried out to test the effects of sediment treatment on the determination of DNase activities. Comparisons between sediment slurries and intact cores (where the fluorescent DNA analog was injected and incubated prior to any further processing) revealed that there were no significant differences in DNase activities between treatments incubated for 1 h (*t*-test, ns).

Since extracellular DNA concentrations in the sediments of all investigated sites were always higher than those sufficient to saturate the DNases, and substrate was never a limiting factor (as a continuous extracellular DNA supply to surface sediments is ensured by export from the water column and in situ production; Corinaldesi et al. 2007), in this study, we assumed a zero-order kinetic of degradation of extracellular DNA.

DNA turnover—The turnover time of extracellular DNA was calculated as the ratio between bioavailable extracellular DNA concentrations and DNA degradation rates (i.e., DNase activity).

Prokaryote abundance—Benthic prokaryotes were detached from sediment subsamples using pyrophosphate (final concentration, 5 mmol L⁻¹) and ultrasound treatment (three times for 1 min; Branson 2200 sonicator, 60 W), which optimizes the extraction yields (Danovaro et al. 2001). After dilution in Milli-Q water, three replicate sediment subsamples (~1 mL) were incubated for 15 min in the dark with acridine orange (final concentration, 0.01%) and then filtered under low vacuum (<13.3 kPa) through 0.2- μ m-pore-size Nucleopore filters (black-stained polycarbonate). Prokaryote counts were carried out under epifluorescence microscopy at ×1,000 magnification. For each slide, at least 10 microscope fields were examined and at least 400 cells counted per filter.

Prokaryote counts were corrected for the extraction efficiency by using a factor of 1.44 (Ellery and Scheyer 1984). Prokaryote abundance was normalized to sediment dry weight after desiccation (60°C; 24 h).

Prokaryote DNA content (i.e., intracellular DNA) was estimated using a conversion factor of 3.2 fg DNA cell⁻¹. This conversion factor was calculated using the genome size and molar GC (guanine-cytosine) content of all prokar-

yotes ($n = 197$) contained in the NCBI (National Center for Biotechnology Information) gene bank (<http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi>). Such a conversion factor has been widely used in previous studies (Danovaro et al. 1999; Dell'Anno and Danovaro 2005; Dell'Anno et al. 2005), thus allowing direct comparison with the available literature. This conversion factor is also consistent with the DNA content determined experimentally with prokaryotic cells extracted from sediment types analogous to those used in this study ($3.3\text{--}3.6$ fg DNA cell $^{-1}$; Corinaldesi et al. 2005).

Prokaryotic carbon production—The determination of prokaryote C production was carried out using [^3H]-leucine incorporation immediately after sampling, according to the standard procedure for marine sediments (Danovaro et al. 2002). An aqueous solution of [^3H]-leucine (0.1 nmol leucine and 222 kBq final concentration) was added to sediment subsamples (200 μL), which were then incubated for 1 h in the dark at the in situ temperature. Additional time-course experiments were carried out to test the linearity of [^3H]-leucine incorporation. After this incubation, all of the samples were treated with ethanol (80%) and processed according to Van Duyl and Kop (1994) before scintillation counting. Sediment blanks were made by adding ethanol immediately after [^3H]-leucine addition. Prokaryotic C production was normalized to sediment dry weight after desiccation (60°C; 24 h).

Statistical analyses—Analyses of variance (ANOVA) were carried out to test for differences of the investigated variables among the sampling sites and between coastal and deep-sea stations.

Results

Sedimentary parameters—The grain-size composition, carbohydrate, protein, and lipid concentrations, and biopolymeric C contents from the coastal and deep-sea sediments are reported in Table 1. The silt-clay fraction ranged from 15.3% to 80.2% in coastal sediments, and from 43.7% to 84.5% in deep-sea sediments. The mean carbohydrate concentrations were 5.6 ± 1.0 mg g $^{-1}$ in coastal sediments and 3.8 ± 1.5 mg g $^{-1}$ in deep-sea sediments. In the coastal sediments, the protein concentrations were more than one order of magnitude greater than those in the deep-sea sediments (means \pm standard error: 11.1 ± 4.3 vs. 0.7 ± 0.1 mg g $^{-1}$; $p < 0.001$). Finally, the mean lipid concentrations in the coastal sediments were ~ 5 -fold higher than in the deep-sea sediments (means \pm standard error: 2.2 ± 0.6 vs. 0.4 ± 0.2 mg g $^{-1}$; $p < 0.01$). The mean biopolymeric C concentrations in the coastal sediments were ~ 4 -fold higher than those in the deep-sea sediments (means \pm standard error: 9.3 ± 2.7 vs. 2.1 ± 0.6 mg g $^{-1}$; $p < 0.01$).

Extracellular DNA concentrations—The bioavailable extracellular DNA concentrations in coastal sediments showed significant differences across the stations investigated ($p < 0.01$), with the highest values at station 1 in the

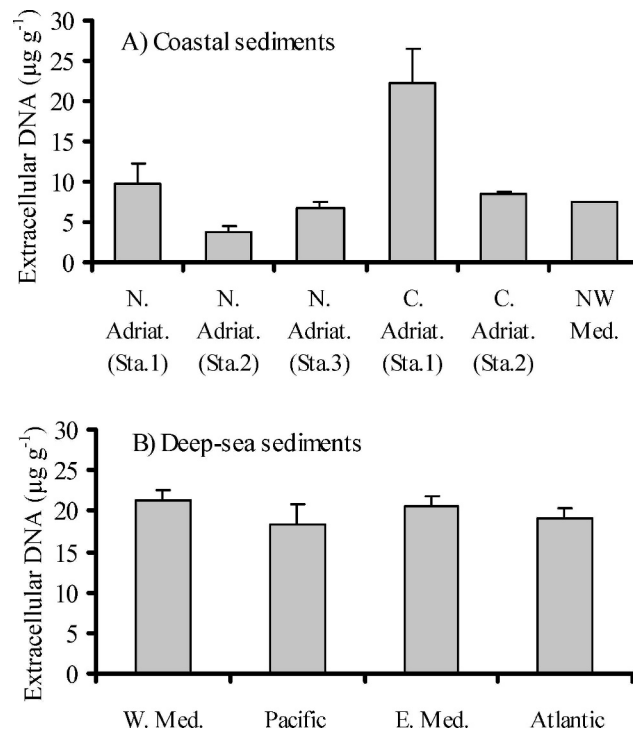


Fig. 1. (A) Bioavailable extracellular DNA concentrations in coastal sediments (northern and central Adriatic Sea, and northwestern Mediterranean Sea); and (B) in deep-sea sediments (western and eastern Mediterranean Sea, Pacific Ocean, and Atlantic Ocean). Standard deviations are given ($n = 3$).

central Adriatic Sea (22.3 ± 4.2 $\mu\text{g DNA g}^{-1}$), and the lowest values at station 2 in the north Adriatic sea (3.9 ± 0.5 $\mu\text{g DNA g}^{-1}$; Fig. 1A). The bioavailable extracellular DNA concentrations in the deep-sea sediments were approximately twice those in the coastal sediments (means \pm standard error: 19.8 ± 0.6 vs. 9.8 ± 2.6 $\mu\text{g DNA g}^{-1}$, respectively; $p < 0.001$). The bioavailable extracellular DNA concentrations in deep-sea sediments were not statistically different among the stations, ranging from 18.4 ± 2.3 to 21.2 ± 1.3 $\mu\text{g DNA g}^{-1}$ in the Pacific Ocean and the western Mediterranean Sea, respectively ($p = 0.20$; Fig. 1B).

DNase activities—The DNase activities in the coastal sediments ranged from 19.2 ± 7.5 to 492.0 ± 258.8 ng DNA g $^{-1}$ d $^{-1}$ (station 2 in the north Adriatic Sea and station 1 in the central Adriatic Sea, respectively) and from 26.3 ± 3.8 to 103.8 ± 10.0 ng DNA g $^{-1}$ d $^{-1}$ in the deep-sea sediments of the southern Pacific Ocean and the western Mediterranean, respectively (Fig. 2A,B). The DNase activities did not show significant differences between coastal and deep-sea sediment samples ($p = 0.27$).

The turnover time of extracellular DNA ranged from 45 d to 201 d in coastal sediments (station 1 in the central Adriatic, and station 2 in the north Adriatic Sea, respectively) and 204 d to 701 d in deep-sea sediments (western Mediterranean Sea and southern Pacific Ocean, respectively; Fig. 3A,B).

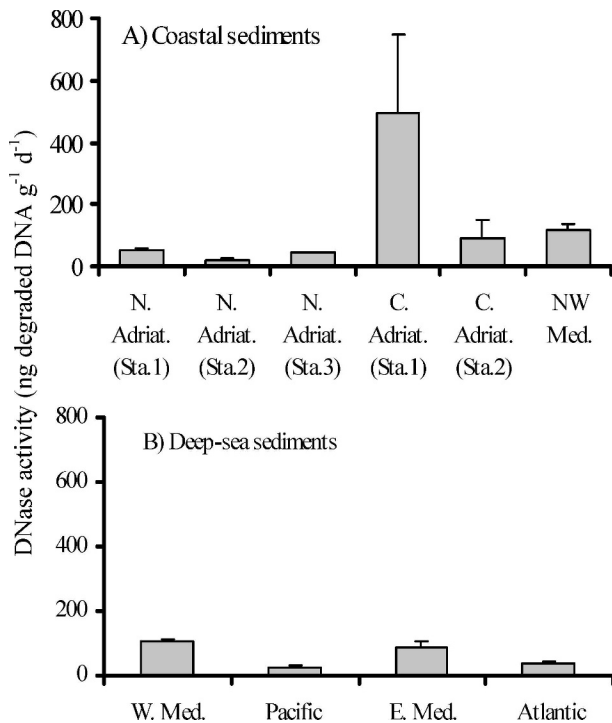


Fig. 2. (A) DNase activities in coastal sediments (northern and central Adriatic Sea, and northwestern Mediterranean Sea); and (B) in deep-sea sediments (western and eastern Mediterranean Sea, Pacific Ocean, and Atlantic Ocean). Standard deviations are given ($n = 3$).

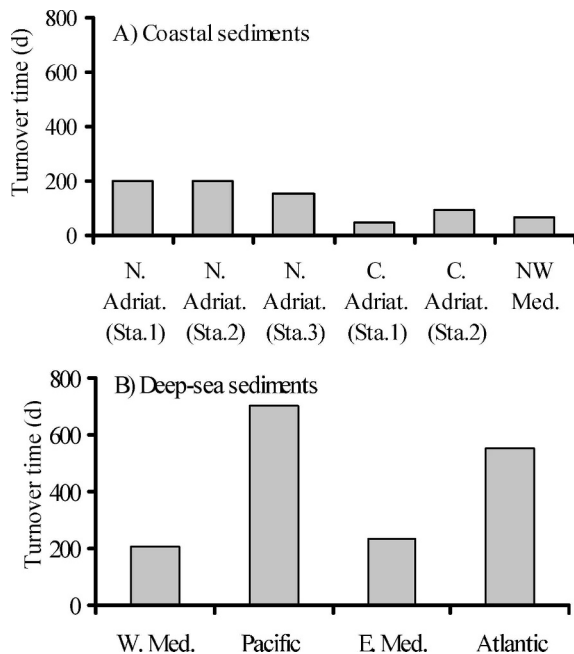


Fig. 3. (A) Turnover times in coastal sediment samples (northern and central Adriatic Sea, and northwestern Mediterranean Sea), and (B) in deep-sea sediment samples (western and eastern Mediterranean Sea, Pacific Ocean, and Atlantic Ocean). Standard deviations are given ($n = 3$).

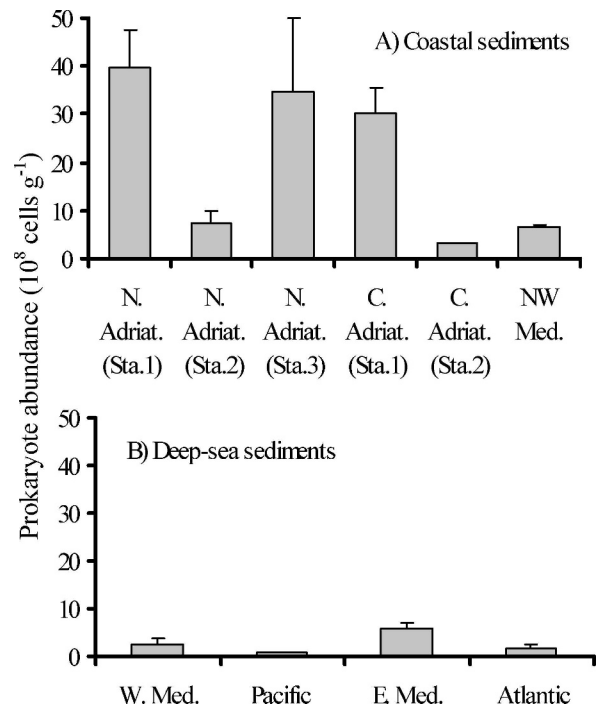


Fig. 4. Prokaryote abundance in (A) coastal and (B) deep-sea sediments. Standard deviations are given ($n = 3$).

Prokaryote abundance and carbon production—Prokaryote abundance in the coastal sediments ranged from 3.1 ± 0.1 to $39.5 \pm 8.2 \times 10^8$ cells g^{-1} (station 2 in the central Adriatic and station 1 in the north Adriatic Sea, respectively; Fig. 4A). In the deep-sea sediments, prokaryote abundance ranged from 0.84 ± 0.08 to $5.66 \pm 1.24 \times 10^8$ cells g^{-1} (in the Pacific Ocean and eastern Mediterranean Sea, respectively; Fig. 4B). Prokaryotic abundance in deep-sea sediments was ~ 7 -fold lower than in coastal sediments (means \pm standard error: 2.71 ± 1.04 vs. $20.30 \pm 6.66 \times 10^8$ cells g^{-1} , respectively; $p < 0.001$).

In the coastal sediments, prokaryotic C production was highest at coastal stations in the northwestern Mediterranean Sea ($17.5 \pm 3.6 \mu g C g^{-1} d^{-1}$) and lowest at stations 2 and 3 in the north Adriatic Sea (0.86 ± 0.29 and $0.87 \pm 0.30 \mu g C g^{-1} d^{-1}$, respectively; Fig. 5A). In deep-sea sediments, the prokaryotic C production ranged from 0.18 ± 0.03 to $1.34 \pm 0.2 \mu g C g^{-1} d^{-1}$ (in the southern Pacific Ocean and western Mediterranean Sea, respectively; Fig. 5B). Prokaryotic C production was significantly higher in the coastal benthic systems than in the deep-sea sediments (means \pm standard error: 4.70 ± 2.64 vs. $0.63 \pm 0.26 \mu g C g^{-1} d^{-1}$, respectively; $p < 0.001$).

Discussion

Extracellular DNA in benthic systems—Extracellular DNA is a constituent of both the dissolved and particulate organic-matter pools in all aquatic ecosystems, and it includes a soluble fraction (i.e., free DNA) and a nonsoluble fraction (i.e., DNA adsorbed in detrital-organic and/or inorganic particles; Jiang and Paul 1995; Danovaro et al. 2006). Previous studies have demonstrated that free DNA

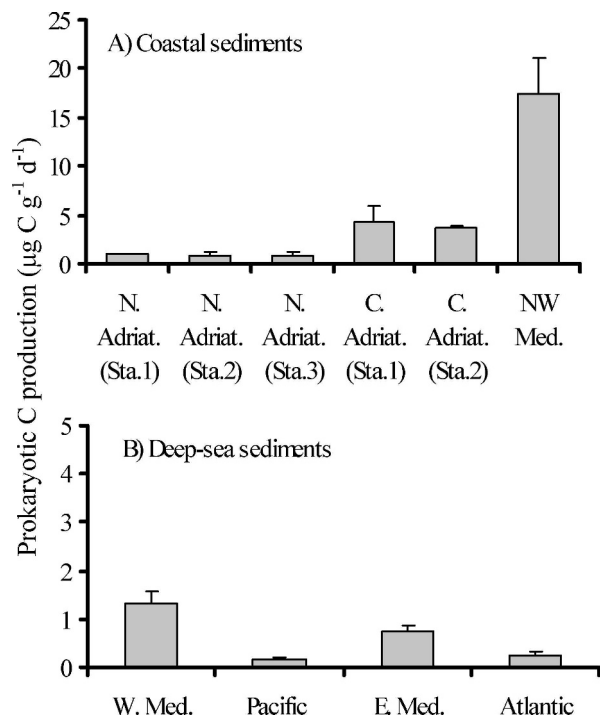


Fig. 5. Prokaryotic carbon production in (A) coastal and (B) deep-sea sediments. Standard deviations are given ($n = 3$).

concentrations in sediment are negligible when compared with concentrations of DNA bound to the sedimentary matrix (Dell'Anno et al. 2002a; Corinaldesi et al. 2005). This was confirmed in the present study. In all of the sediment samples, we saw that the free DNA fraction accounted for less than 5% of the extracellular DNA pool released after nuclease digestion. Although the nuclease-based protocol used here is the most effective method for the determination of bioavailable extracellular DNA in marine sediments (Dell'Anno et al. 2002a; Corinaldesi et al. 2005), we cannot exclude the possibility that a fraction of the DNA can remain adsorbed to mineral and/or organic particles (Dell'Anno et al. 2002a, 2005). Therefore the concentrations of bioavailable extracellular DNA present in the sediment could be larger than those quantified using the enzymatic approach. Moreover, since the fraction of hydrolysable extracellular DNA is recycled, whereas the fraction resistant to degradation can be preserved in the sediment over geological timescales (Coolen et al. 2004; Dell'Anno et al. 2005), all measurements of extracellular DNA concentrations reported here should be considered as estimates of the most readily labile fraction of the extracellular DNA pool (Dell'Anno and Corinaldesi 2004).

The quantitative relevance of the bioavailable extracellular DNA pool in marine sediments is controlled by the interaction of different processes, including: (1) input of DNA from the photic zone and/or from lateral particle advection; (2) in situ production; (3) DNA degradation rates; and (4) DNA interactions with organic matter and mineral particles (Dell'Anno et al. 2002a; Coolen et al. 2004; Dell'Anno and Danovaro 2005).

We show here that the bioavailable extracellular DNA concentrations in deep-sea sediments were significantly

higher than those determined in the benthic coastal sites. These higher concentrations in deep-sea sediments cannot be explained by an enhanced in situ production (given the relatively low prokaryotic C production) nor by a higher DNA supply from the water column (Dell'Anno et al. 2005), but they might depend on the rates at which this component is recycled. In a previous study carried out in surface deep-sea sediments worldwide, Dell'Anno and Danovaro (2005) estimated a residence time for total extracellular DNA of approximately 10 yr. We report here that the turnover time of the most promptly degradable fraction of extracellular DNA in deep-sea benthic systems may be shorter (~ 1.2 yr). These differences could also be due to the different approaches utilized. In fact, here we estimated DNA degradation rates assuming a zero-order kinetic, as the concentrations of extracellular DNA in surface sediments were always higher than those saturating the DNases. As such, the degradation velocities were independent of substrate concentration. Conversely, Dell'Anno and Danovaro (2005) estimated the degradation rates of DNA through a steady-state diagenetic model based on the vertical profiles of the extracellular DNA concentrations. In this latter case, the concentrations of bioavailable extracellular DNA tended to zero in deeper sediment horizons, and, therefore, a first-order kinetic of degradation has been assumed.

The interaction of extracellular DNA with organic macromolecules and/or mineral particles in the sediments may alter its bioavailability and degradation rates (Coolen et al. 2002; Dell'Anno et al. 2005). Findings from the present study lead us to hypothesize that, when compared with deep-sea sediments, the higher organic matter loads of coastal sediments might be accompanied by lower concentrations of bioavailable DNA.

Since we found that the DNase activities in deep-sea and coastal sediments were not statistically different, the turnover times of extracellular DNA in coastal sediments (on average 0.35 yr) were approximately threefold shorter than in deep-sea sediments.

The DNase activity per cell (assuming that all of the DNases are produced by prokaryotes) in the deep-sea sediments was approximately twice that in the coastal sediments (0.27 ± 0.1 vs. 0.12 ± 0.05 fg DNA degraded cell⁻¹ d⁻¹, respectively). Since prokaryotic C production per prokaryotic cell was not significantly different in coastal and deep-sea sediments (6.9 ± 4.4 and 2.6 ± 0.9 fg C cell⁻¹ d⁻¹, respectively), these results suggest that DNases may be more dependent on the amounts of bioavailable substrate rather than on prokaryotic metabolism.

Further studies are needed to clarify the relative importance of the biotic (e.g., heterotrophic metabolism) and abiotic factors (e.g., grain size, mineralogical, and organic-matter composition of sediments) influencing the early diagenetic processes of extracellular DNA in benthic ecosystems.

Role of extracellular DNA in benthic metabolism— Although the presence of DNA-hydrolyzing microorganisms has been known for a long time (Maeda and Taga

1973, 1974), the importance of extracellular DNA in benthic trophodynamic processes has not yet been clarified. The use of extracellular DNA is dependent upon the prokaryote ability to convert this macromolecule into its basal constituents (i.e., nucleobases and/or nucleosides) through DNase activity. Experimental studies have reported that extracellular DNA may support a large fraction of prokaryotic C production (Jørgensen et al. 1993; Jørgensen and Jacobsen 1996), and recent studies have extended these conclusions to deep-sea sediments worldwide (Dell'Anno and Danovaro 2005). To provide information on the role of extracellular DNA in coastal and deep-sea benthic trophodynamics, we have estimated the daily prokaryotic C, N, and P requirements. To do this we determined the prokaryotic C production, and assuming a prokaryotic C:N:P ratio of 40:10:1 (Kirchman 1994), we estimated N and P production. We then estimated the potential daily C, N, and P supply derived from DNA degradation rates, assuming an average C, N, and P content of 33%, 15%, and 10% DNA weight, respectively. Our results suggest that in coastal sediments, bioavailable extracellular DNA corresponded to, on average, ~2%, 4%, and 20% of the daily C, N, and P prokaryote anabolic requirements, whereas in deep-sea sediments, this compound provided 4%, 7%, and 47% of the C, N, and P prokaryote requirements. These results suggest that, depending on the size of other nutrient sources, extracellular DNA might represent an important trophic source for sustaining benthic heterotrophic metabolism not only in nutrient-limited ecosystems (such as the deep sea), but also in systems characterized by high nutrient availability, such as coastal benthic environments.

Once converted into nucleotides and/or nucleosides through DNases, extracellular DNA can also be recycled for the synthesis of new prokaryotic DNA (Paul et al. 1988, 1989). In the present study, we calculated the ratio between DNA synthesis (calculated as daily prokaryotic cell production \times DNA content per cell) and the concentration of bioavailable extracellular DNA mobilized by DNases. Our results suggest that in all of the investigated sediments, the quantity of DNA mobilized exceeded the amount potentially taken up by prokaryotes for the synthesis of new DNA. Therefore extracellular DNA could represent a reservoir of bioavailable organic C, N, and P that is useful for coping with periods of reduced nutrient supply.

Although our results do not allow us to elucidate the metabolic pathways through which extracellular DNA is used, this study opens new perspectives for a better understanding of the factors that influence microbial-loop functioning and trophodynamic processes in benthic systems.

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