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Differences in the optical and biological reactivity of the humic and nonhumic dissolved organic carbon component in two contrasting coastal marine environments

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Abstract

The effect of surface solar radiation on the optical properties and the biological availability of dissolved organic carbon (DOC) and its humic and nonhumic component was investigated in the northern Adriatic Sea (44°52′N 13°52′E) and the coastal North Sea (53°00′N 4°45′E). In the northern Adriatic Sea, humic substances of primarily autochthonous origin contributed, on average, 15% to the bulk DOC, whereas in the coastal North Sea the mean contribution of the mainly terrestrially derived humic substances to the bulk DOC was about 43%. DOC-normalized absorbance and fluorescence were both ~1.5-fold higher, and the absorbance ratio 250 : 365 nm was twofold higher for the humic DOC of the northern Adriatic Sea as compared to the coastal North Sea. Bioassay experiments with indigenous bacterioplankton indicated a ~5 times higher DOC-normalized bacterial growth on the humic and nonhumic DOC from the northern Adriatic Sea as compared to the corresponding fractions from the coastal North Sea. Exposure of the different DOC fractions to surface solar radiation for 5–8 h and subsequent inoculation with a natural bacterial community resulted in stimulated bacterial growth on the preexposed humic and nonhumic DOC by 35 and 45%, respectively, as compared to the corresponding dark controls for the northern Adriatic Sea, whereas in the coastal North Sea stimulated bacterial growth (by 50%) was found only for the humic DOC. The increase in the DOC bioavailability upon irradiation was linearly related to a decrease in the absorbance and an increase in the absorbance ratio 250 : 365 nm. Both DOC-normalized photochemical and bacterial oxygen demand of surface water were, on average, about tenfold higher in the northern Adriatic Sea as compared to the coastal North Sea.

Photochemical processes play an important role in the degradation of dissolved organic matter (DOM), either directly, via the production of carbon gases (Mopper et al. 1991; Valentine and Zepp 1993; Salonen and Vähätalo 1994; Graneli et al. 1996), or indirectly by cleaving complex molecules into smaller, more labile organic (Mopper and Stahovec 1986; Kieber et al. 1989; Wetzel et al. 1995) and inorganic moieties (Francko and Heath 1982; Bushaw et al. 1996), which are efficiently taken up by bacterioplankton (Kieber et al. 1989; Bertilsson and Tranvik 1998; Obernosterer et al. 1999*a*). However, recent findings indicate that photochemically induced transformations can also counteract DOM degradation by rendering originally labile DOM compounds more refractory (Kieber et al. 1997; Benner and Biddanda 1998; Obernosterer et al. 1999*b*; Pausz and Herndl 1999). Direct physicochemical interactions between chromophoric organic matter and nonabsorbing compounds are probably important in these transformation processes (Tranvik and Kokalj 1998).

Humic substances are a characteristic component of the dissolved organic carbon (DOC) pool in freshwater and marine systems; however, their concentration varies greatly among aquatic ecosystems. In marine environments, humic substances generally make up a smaller percentage of the bulk DOC (5 to 25%) than in freshwater environments (40 to 80%; Thurman 1985). Humic substances are considered as heterogeneous biomolecules of varying but frequently high molecular weight and as biologically recalcitrant (Ishiwatari 1992; McKnight and Aiken 1998). Despite their complex structure, humic substances have also been reported to be an important carbon pool for bacterioplankton, particularly in freshwater systems (Tranvik 1990; Reitner et al. 1997). The high aromaticity of humic substances (Thurman 1985) renders them particularly susceptible to absorption of UV light and makes them photochemically more reactive than other fractions of the DOC. Humic substances, therefore, play an important role in the photochemistry of surface waters, acting either directly or indirectly as photosensitizers.

Most studies on humic substances have been performed in freshwater systems, and only little is known on the origin and distribution of marine humic substances (Harvey et al. 1983; Lara et al. 1993; Moran and Hodson 1994). In coastal areas, terrestrial humic material, derived from the decay of lignin-containing plants, accounts for most of the humic substances present (Ehrhardt 1984). Marine humic substances, however, are also formed autochthonously and are structurally different from their terrestrial counterparts (Nissenbaum and Kaplan 1972; Malcolm 1990). Autochthonously produced marine humic substances are mainly fulvic and therefore characterized by comparatively low molecular weight (<2,000 Da). They further differ from terrestrially derived

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humic substances by their lower aromaticity and phenolic content, whereas the nitrogen and sulfur content of marine humic substances has been found to be relatively high (Nissenbaum and Kaplan 1972; Malcolm 1990).

There were three aims to this study. The primary aim was to determine the distribution of the humic and the nonhumic DOC in two coastal marine environments of contrasting trophic status and input from land, i.e., the northern Adriatic Sea and the coastal North Sea. Although the main source of humic DOC in the coastal North Sea is the terrestrial environment, the terrestrial input is negligible in the northern Adriatic Sea; thus the humic DOC present there is primarily autochthonously produced. The second aim was to compare the optical properties of the humic and the nonhumic DOC and their biological reactivity upon exposure to surface solar irradiation. The third was to measure the photochemical oxygen demand of the bulk DOC and the bacterial respiration in order to evaluate the importance of photooxidation versus biological oxidation in these two contrasting coastal marine environments. Thus, our goal was to determine the differences in photoreactivity and subsequent bacterial use between autochthonously produced and primarily terrestrially derived humic DOC in coastal marine environments.

Material and methods

Characteristics of the study sites—Two contrasting study sites were sampled: the mesotrophic to oligotrophic eastern part of the northern Adriatic Sea (44°52′N 13°52′E) and the highly eutrophic coastal North Sea (53°00′N 4°45′E). The northern Adriatic Sea is a shallow (mean depth 30 m) marginal sea, with the river Po as an important source of nutrients, contributing approximately 50% to the overall nitrogen input (Degobbis et al. 1986). Previous studies have described the trophic gradient from the eutrophic western part, close to the mouth of the river Po, to the mesotrophic to oligotrophic eastern part of the northern Adriatic Sea (Karner et al. 1992; Weinbauer et al. 1993). For the present study, seawater was collected from the oligotrophic region off Rovinj (Croatia), where mean annual concentrations of inorganic nutrients are ~4 μM ΣN_{inorg} and ~0.1 μM PO_4 (Ivancic and Degobbis 1987; Kaltenböck and Herndl 1992) and chlorophyll *a* concentrations range between 0.02 and 0.31 μg L^{-1} from May to September (Kaltenböck and Herndl 1992). The other sampling site, the Marsdiep Tidal Inlet, is the entrance of the North Sea to the western Dutch Wadden Sea. At high tide, coastal North Sea water is transported along the Marsdiep into the Wadden Sea. The discharge of the river Rhine has a significant impact on the concentrations of inorganic nutrients and chlorophyll *a* in this area (Cadee 1992). During the sampling period (July and August 1997), inorganic nutrients averaged ~6 μM ΣN_{inorg} and ~1 μM PO_4, and concentrations of chlorophyll *a* were ~10 μg L^{-1} (Cadee pers. comm.).

Sampling—In the northern Adriatic Sea, water was collected from a boat ~3 km off Rovinj at 10-m depth using a 10-liter Niskin bottle. Water samples were transferred to acid-rinsed polycarbonate bottles and brought back to the lab within 30 min. At the Marsdiep, sampling was done from the NIOZ pier at high tide to collect incoming North Sea water; water was sampled from 0.5-m depth using an acid-rinsed bucket. At both study sites, the freshly collected seawater was immediately filtered either through 0.8-μm polycarbonate filters to prepare the bacterioplankton inocula (Poretics, 47-mm filter diameter) or through 0.2-μm polycarbonate filters (Gelman, 42-cm filter diameter) for fractionation of the DOC using combusted glassware (450°C for 4 h). The filters were rinsed with 500 ml of Milli-Q water and 0.1 and 1 liter of seawater for the 0.8- and 0.2-μm filters, respectively, before use.

Experimental setup to determine the effect of solar radiation on the optical properties and the biological reactivity—At both study sites, ~2–3 liters of the 0.2-μm filtrate were fractionated into humic and nonhumic DOC using a macroporous Amberlite XAD-8 resin as described below. Two liters of each, the unfractionated 0.2-μm filtrate (referred to as bulk DOC), the humic and the nonhumic DOC were split, and one part was exposed to surface solar radiation for 5–8 h, and the other part was kept in the dark. The incubations were performed in 1-liter quartz tubes sealed at both ends with Teflon foil (200-μm thick, Fluorplast, NL). The dark controls were wrapped in aluminum foil. Both the quartz tubes and the Teflon foil were soaked in 1 N HCl overnight and extensively rinsed with Milli-Q water before use. All incubations were carried out in a flow-through water bath providing surface water temperature. During the incubation period, the water temperature was kept constant (24 ± 1°C for the northern Adriatic Sea and 19 ± 1°C for the North Sea). For the optical characterization of the different DOC fractions, 50-ml samples were withdrawn from each treatment before and after the exposure period, and the DOC absorbance and fluorescence were measured within 1 h (*see below*). In order to determine the biological reactivity of the different DOC fractions, the irradiated and dark treatments (900-ml volume) were inoculated with a natural bacterial assemblage (0.8-μm filtered seawater; 100 ml), and the increase in bacterial abundance followed over time. The bacterial batch cultures were kept in the dark at ~25°C and subsampled for bacterial abundance at 8-h intervals. The bacterial growth was calculated from the net increase in bacterial abundance during the exponential growth phase.

In order to determine the effect of irradiation on the bioavailability of a labile organic compound in the presence of DOC of different biological reactivity, an additional set of experiments was performed in which the model protein bovine serum albumin (BSA) was added to the humic, the nonhumic, and the bulk DOC collected from the North Sea. BSA was also added to artificial seawater before exposure to surface solar radiation (final concentration 916 μM C). Preparation of artificial seawater was done according to Parsons et al. (1984). Exposure to surface solar radiation of the BSA-amended treatments and the subsequent preparation of the bacterial batch cultures followed the protocol described above. Experiments with water from the northern Adriatic Sea were performed in September and November 1994; in May, August, and September 1995; and in June and September 1996. Experiments with North Sea water were carried out in July and August 1997.

Determination of the photochemical and bacterial oxygen demand of DOC—To measure the photochemical O_2 demand, 0.2-μm filtered seawater from the northern Adriatic and the North Sea was exposed to surface solar radiation for 5 and 8 h, respectively. At both study sites, the incubations started at ~1,000 h. Incubations were done in triplicate in quartz-glass BOD (biological oxygen demand) bottles (~120 ml) with corresponding dark controls wrapped in aluminum foil. In order to exclude ultraviolet B (UVB, 280–320 nm) radiation, an additional set of quartz-glass BOD bottles (also in triplicate) was wrapped in Mylar-D foil. All the BOD bottles were placed in a flow-through water bath as described above. To determine the bacterial O_2 demand (also referred to as bacterial respiration), borosilicate BOD bottles (~120 ml) were filled with 0.8-μm filtered seawater, wrapped in aluminum foil, and incubated under the same conditions as for the determination of the photochemical O_2 demand. Concentrations of dissolved O_2 were determined by Winkler titration (Parsons et al. 1984) for the northern Adriatic Sea samples and by the spectrophotometric determination of total iodine as described in Pai et al. (1993) for North Sea water (*see below*). The photochemical O_2 demand was calculated from the difference in the dissolved O_2 concentration in the 0.2-μm filtrate between the irradiation-exposed and the dark treatment. Bacterial respiration was calculated from the difference in the dissolved O_2 concentration in the 0.8-μm filtrate before and after the incubation. The experiments with water from the northern Adriatic Sea were carried out in August 1995 and June 1996; the experiments with North Sea water were performed during July and August 1997.

Bacterial enumeration—Ten-milliliter subsamples were fixed with 37% formaldehyde (4% final concentration), and the bacterial abundance was subsequently determined by acridine orange staining and epifluorescence microscopy (Hobbie et al. 1977).

Fractionation of bulk DOC into a humic and a nonhumic component—The extraction of humic substances was performed using an Amberlite XAD-8 resin (nonionic polymeric adsorbent, Sigma; Thurman and Malcolm 1981; Aiken et al. 1992). We defined humic substances as the fraction of the 0.2-μm filtrate that adsorbed to the XAD-8 resin at pH 2; the DOC fraction not retained was considered as nonhumic (Thurman and Malcolm 1981). The resin was cleaned by sequential extractions in a Soxleth extractor for 24 h with diethyl ether, acetonitril, methanol, and diethyl ether and kept thereafter in Milli-Q water for 24 h. The cleaned resin was stored in methanol until used (Thurman and Malcolm 1981). A glass column was packed with the resin (30 ml resin bed volume) and rinsed with Milli-Q water to remove the methanol. Subsequently, the resin was rinsed with 200 ml of 0.1 N NaOH and 200 ml of 0.01 N HCl. Two to three liters of the 0.2-μm filtered seawater were adjusted to pH 2 with 6 N HCl and subsequently poured through the column at a flow rate of 5 ml min^{-1}. The adsorbed substances were eluted with 200 ml of 0.1 N NaOH and 100 ml Milli-Q water at a flow rate of 1 ml min^{-1} and subsequently brought back to the original volume with artificial seawater and the original pH adjusted with 1 N HCl. The fraction not retained by the resin (considered as nonhumic DOC) was neutralized with 1 N NaOH. For DOC analyses, 10-ml subsamples were taken from the bulk 0.2-μm filtered seawater, the humic and the nonhumic DOC and stored frozen (−20°C) in combusted and sealed glass ampoules until analysis. For the characterization of the optical properties of the different DOC fractions, 50-ml subsamples were collected from the 0.2-μm filtrate and the humic and nonhumic DOC, and the absorbance and fluorescence were measured as described below within 1 h.

Dissolved organic carbon—DOC concentrations were determined by the high-temperature combustion method using a Shimadzu TOC-5000A (Benner and Strom 1993). Ten-milliliter samples were acidified with three drops of concentrated phosphoric acid (45% w/v) and 50-μl subsamples were subsequently injected into the TOC analyzer. The DOC content was determined after sparging the samples with CO_2-free air. Standards were prepared with potassium hydrogen biphtalate (Kanto Chemical). Both water blank (i.e., Milli-Q water redistilled with 10 mM potassium peroxide sulfate and 20 mM *o*-phosphoric acid; both obtained from Sigma Chemical) and instrument blank were assessed before and after sample analysis. Blanks typically ranged between 3.3 and 4.2 μM (water) and between 1.7 and 2.5 μM (instrument), respectively. The overall analytical precision was always better than 3%.

DOC absorbance and fluorescence—DOC absorbance was measured with a Beckman DU spectrophotometer (for northern Adriatic Sea samples) and a Spectronic Genesys 2PC spectrophotometer (for North Sea samples) using a 5-cm quartz cuvette and zeroed against Milli-Q water. In addition to absorbance spectra (250–500 nm wavelength), absorbance at 250 and 365 nm was measured. DOC fluorescence was measured at an excitation wavelength of 350 nm and an emission wavelength of 450 nm using a 1-cm quartz cuvette. The DOC fluorescence was measured with a Jasco 820 (for northern Adriatic Sea samples) and a Hitachi F-2000 (for North Sea samples) spectrofluorometer, respectively, and is expressed in quinine sulfate units (1 QSU = 1 ppb quinine sulfate in 0.05 M H_2SO_4).

Determination of dissolved oxygen—At both study sites, sample treatment principally followed the standard protocol for the determination of O_2 by Winkler titration (Parsons et al. 1984). The amount of total iodine was determined by titration with 0.0048 N thiosulfate (Pomeroy et al. 1994) for the samples from the northern Adriatic Sea. For the North Sea, the amount of total iodine was determined spectrophotometrically at 465 nm wavelength (Pai et al. 1993; Roland et al. 1999). Samples were withdrawn from the BOD bottles by a sipper system. The end of the narrow inlet tube was placed near the bottom of the BOD bottle to avoid possible loss of volatile iodine. The instrument was zeroed against Milli-Q water. Measurements were done on a Hitachi U-1000 spectrophotometer using a 1-cm flow-through cuvette. Calibration was performed by standard additions of iodate to distilled water resulting in an empirical coefficient of 0.54455 nM cm^{-1} at 456 nm wavelength (G. Kraay, pers.

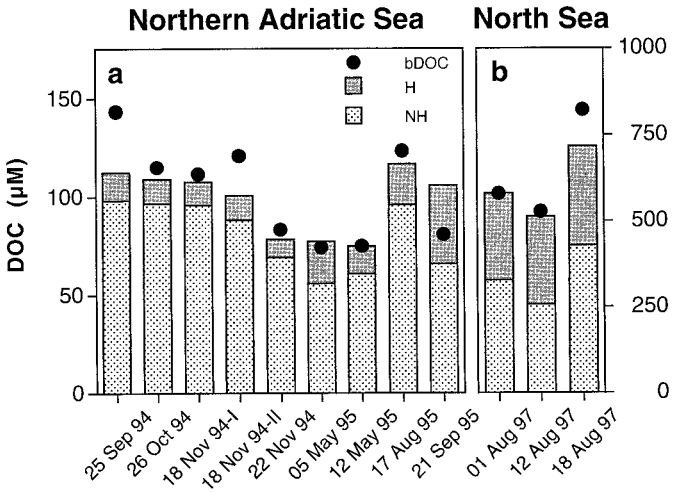

Fig. 1. Concentration of the bulk, humic, and nonhumic DOC during the sampling period in (a) the northern Adriatic Sea and (b) the coastal North Sea. On 18 November 1994, samples were collected at two stations during a cruise in the northern Adriatic Sea: Sta. I is located in the mesotrophic eastern part of the northern Adriatic Sea, and Sta. II is about 36 km off the river Po. Note different scales. bDOC—bulk DOC. H—humic DOC. NH—nonhumic DOC.

comm.). A four-digit voltmeter (Metex M4650) was connected to the spectrophotometer to increase the sensitivity of the absorption readings. All incubations were done in triplicate, the analytical standard deviation (i.e., difference among the three replicates) was <0.5% ($n = 60$).

Irradiance measurements—In the northern Adriatic Sea, surface irradiance was measured at 305, 320, 340, and 380 nm wavelength and in the photosynthetic active radiation (PAR, 400–700 nm) range with a biospherical PUV-510 radiometer using the correction factor for the 305 nm channel as suggested by Kirk (1994). Surface irradiance was recorded at 10-min intervals and integrated over the incubation period.

In the North Sea, a radiometer (International Light) with sensors for ultraviolet A (UVA, 320–380 nm) and PAR (400–700 nm) was used to measure surface solar radiation. Irradiation measurements were recorded every 30 min and integrated over the 8 h incubation period.

Statistical analysis—Comparisons between sites and among different treatments were made by the analysis of variance (ANOVA) and the Bonferroni-test for multiple comparison. The Wilcoxon test for matched pairs was used to check for treatment effects (irradiation vs. dark) on variables. Statistics were performed with SYSTAT 5.2 (Wilkinson 1990).

Results

Concentration of dissolved organic carbon (DOC) and the contribution of humic substances—In the northern Adriatic Sea, the concentrations of the bulk DOC varied between 74 and 151 μM ($n = 15$), whereas DOC concentrations in the coastal North Sea were substantially higher, ranging from 366–823 μM C ($n = 11$). In the northern Adriatic Sea, humic substances contributed, on average, ~15 ± 7% (range 10–38%, $n = 9$) to the bulk DOC (Fig. 1a); in the coastal North Sea, the mean contribution of humic substances to the bulk DOC was ~43 ± 7% (range 35–49%, $n = 3$; Fig. 1b). The recover efficiency, i.e., the concentration of the humic plus the nonhumic DOC, was 97 ± 13% of the bulk DOC prior to extraction (range 78–131%, $n = 12$, both study sites pooled).

Optical characteristics of the bulk DOC, its humic and nonhumic component—At both study sites, a distinct pattern in absorbance and fluorescence of the different DOC fractions was detectable (Table 1). Generally, $Abs_{365\,nm}$ m^{-1} and fluorescence were found to be ~5- and 8.5-fold, respectively, higher in the coastal North Sea as compared to the northern Adriatic Sea. This is mainly due to the higher DOC concentration in the coastal North Sea because DOC-normalized $Abs_{365\,nm}$ m^{-1} of the bulk DOC was similar at both study sites and DOC-normalized fluorescence was only one-third higher in the coastal North sea as compared to the northern Adriatic Sea. DOC-normalized $Abs_{365\,nm}$ m^{-1} and

Table 1. Absorbance and fluorescence properties of the bulk DOC and the humic and nonhumic DOC from the northern Adriatic Sea and the coastal North Sea. DOC fluorescence is given in quinine sulfate units (QSU). bDOC—bulk DOC. H—humic DOC. NH—nonhumic DOC Mean values ±SD are given.

	Abs_{365nm} m^{-1}	Abs_{365nm} (μM DOC)$^{-1}$ m^{-1} ($\times 10^{-3}$)	Absorbance ratio$_{250/365nm}$	DOC fluorescence	
				QSU	QSU (μM DOC)$^{-1}$
Northern Adriatic Sea					
bDOC	0.124 ± 0.05	1.40 ± 0.7	11.50 ± 5.5	0.84 ± 0.3	0.010 ± 0.002
H	0.043 ± 0.02	2.24 ± 0.9	9.74 ± 8.1	0.39 ± 0.2	0.024 ± 0.009
NH	0.061 ± 0.04	0.70 ± 0.4	22.53 ± 14.4	0.63 ± 0.3	0.009 ± 0.003
	($n = 10$)	($n = 8$)	($n = 10$)	($n = 6$)	($n = 4$)
North Sea					
bDOC	0.640 ± 0.14	1.20 ± 0.5	7.70 ± 0.7	8.77 ± 1.9	0.015 ± 0.006
H	0.405 ± 0.18	1.36 ± 0.7	5.23 ± 0.5	4.79 ± 1.8	0.016 ± 0.004
NH	0.135 ± 0.06	0.39 ± 0.2	14.48 ± 3.2	3.65 ± 0.9	0.012 ± 0.005
	($n = 4$)	($n = 3$)	($n = 4$)	($n = 4$)	($n = 3$)

Table 2. Optical characteristics of the bulk DOC of the coastal North Sea, the humic and nonhumic DOC fraction after exposure to surface solar irradiation for 8 h as percentage of the corresponding values in the dark control. Optical properties of the treatments kept in the dark did not differ significantly from the initial values. +BSA—916 μM C (final concentration) in the form of BSA added to the respective DOC fraction or to the bulk DOC. BSA—bovine serum albumin. bDOC—bulk DOC. H—humic DOC. NH—nonhumic DOC. Mean values ±SD are given.

	Abs_{365nm} m^{-1}	+BSA	$Abs_{250/365nm}$	+BSA	DOC fluorescence	+BSA
bDOC	85 ± 7 (n = 9)	91 ± 6 (n = 4)	114 ± 7 (n = 9)	113 ± 18 (n = 4)	83 ± 7 (n = 4)	96 ± 6* (n = 4)
H	83 ± 2 (n = 4)	85 ± 5 (n = 3)	117 ± 4 (n = 4)	114 ± 4 (n = 3)	79 ± 13 (n = 4)	73 ± 19 (n = 3)
NH	97 ± 6* (n = 4)	160 ± 42 (n = 3)	98 ± 11* (n = 4)	72 ± 21 (n = 3)	79 ± 13 (n = 4)	89 ± 7 (n = 3)

* No significant difference between the irradiated and dark treatment ($P > 0.05$).

fluorescence of the humic DOC, however, were ~1.5-fold higher in the northern Adriatic Sea as compared to the coastal North Sea. For humic substances, both DOC-normalized $Abs_{365\,nm}$ m^{-1} and fluorescence were 1.3–3 times higher than the corresponding value for the nonhumic DOC fraction at both study sites (Table 1).

Generally, highest absorbance ratios ($Abs_{250:365\,nm}$) were detected for the nonhumic DOC at both study sites, which indicates a relatively high contribution of low molecular weight compounds in the nonhumic DOC fraction (Table 1). Humic DOC exhibited the lowest $Abs_{250:365\,nm}$. Comparison of the two study sites indicated a 1.9- and 1.5-fold higher $Abs_{250:365\,nm}$ of the humic and nonhumic DOC, respectively, for the northern Adriatic Sea than for the coastal North Sea.

Effect of irradiation on the optical properties and the bioavailability of the bulk DOC and its humic and nonhumic component to bacterioplankton—Significant changes in the absorbance characteristics of the humic and the nonhumic DOC were detected only in the samples from the coastal North Sea (Table 2). $Abs_{365\,nm}$ m^{-1} decreased over the incubation period (5–8 h) for the bulk DOC and the humic DOC (by ~15% for coastal North Sea water; Table 2), whereas no such changes were observed for the nonhumic DOC fraction. In contrast to that, the $Abs_{250:365\,nm}$ increased for bulk DOC and humic DOC (for each fraction by ~15%); however, no increase in the $Abs_{250:365\,nm}$ was detectable for the nonhumic DOC fraction. In the northern Adriatic Sea, experiments have been performed at different times of the year, resulting in large variations in incident solar radiation. A clear relation was detectable between the loss in $Abs_{365\,nm}$ m^{-1} and the increase in the absorbance ratio $Abs_{250:365\,nm}$, respectively, and the dose of UVB radiation received (Fig. 2). As was true for the absorbance, no distinct changes were observed for DOC fluorescence for northern Adriatic Sea samples when exposed to solar irradiation. The DOC fluorescence, however, decreased during the exposure to solar radiation in samples from the coastal North Sea, for bulk, the humic and nonhumic DOC (by ~20% for each DOC fraction; Table 2).

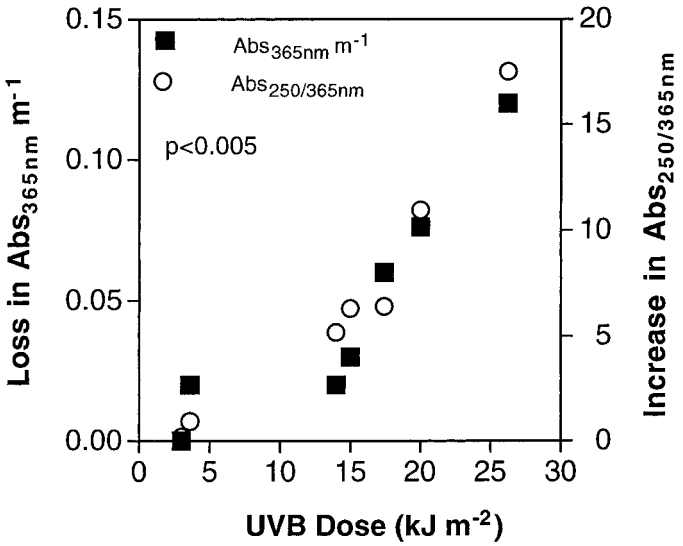

Fig. 2. Relationship between the change in the optical properties of the bulk DOC and the UVB dose received during the experiments performed in the northern Adriatic Sea over the course of the exposure to surface solar irradiation; a similar relationship has been calculated also for the UVA dose. $Abs_{365\,nm}$ m^{-1}—absorbance at 365 nm. $Abs_{250:365\,nm}$—absorbance ratio 250 : 365 nm.

When BSA was added, the $Abs_{365\,nm}$ m^{-1} decreased by ~10 and 15% in the bulk DOC and the humic DOC, respectively, whereas nonhumic DOC with BSA added revealed a significant increase in $Abs_{365\,nm}$ m^{-1} and a decrease in the $Abs_{250:365\,nm}$ as compared to the corresponding dark controls (Table 2). DOC fluorescence in the BSA-amended samples decreased by ~30 and 20% in the humic and nonhumic fraction, respectively, whereas no significant loss in fluorescence was observed for the bulk DOC (Table 2). In a set of experiments with North Sea water, UVB radiation was shielded off using Mylar-D foil; no effects of UVB radiation on the optical characteristics of the different DOC fractions were detectable (data not shown).

The bacterial growth in the batch cultures established with the different DOC fractions kept in the dark (not exposed to solar irradiation prior to inoculating bacteria) was significantly higher for the nonhumic DOC as compared to the humic DOC (two-way ANOVA, Bonferroni, $p = 0.029$, $n = 12$, data from both study sites pooled; Table 3). Mean bacterial growth on bulk DOC and on the humic DOC was not significantly different. DOC-normalized bacterial growth was, at both study sites, ~1.5-fold higher for the nonhumic DOC than for the humic DOC component; however, no sig-

Table 3. Bacterial growth on bulk DOC and its humic and nonhumic fraction and DOC-normalized bacterial growth on DOC of the northern Adriatic and the coastal North Sea. The different DOC fractions were held in the dark prior to inoculating bacterioplankton. Bacterial growth was calculated form the increase in bacterial abundance during the exponential growth phase. BSA—bovine serum albumin (916 μM C final concentration); mean values ±SD are given.

	$N \times 10^8$ L^{-1} h^{-1}			$N \times 10^5$ (μmol DOC)$^{-1}$ h^{-1}		
	Bulk DOC	Humic	Nonhumic	Bulk DOC	Humic	Nonhumic
Northern Adriatic Sea	0.293 ± 0.30 (n = 8)	0.126 ± 0.09 (n = 8)	0.374 ± 0.35 (n = 8)	2.44 ± 2.5 (n = 6)	2.39 ± 1.6 (n = 6)	3.73 ± 3.6 (n = 6)
North Sea	0.263 ± 0.13 (n = 3)	0.219 ± 0.12 (n = 4)	0.250 ± 0.08 (n = 4)	0.54 ± 0.4 (n = 3)	0.53 ± 0.3 (n = 4)	0.77 ± 0.4 (n = 4)
+ BSA	1.259 (n = 1)	0.475 ± 0.01 (n = 3)	1.060 ± 0.28 (n = 3)	0.96 (n = 1)	0.35 ± 0.03 (n = 3)	0.86 ± 0.3 (n = 3)

nificant differences were detectable among treatments (two-way ANOVA, p = 0.4, n = 10, data from both study sites pooled; Table 3). Comparison of the two study sites revealed an approximately fivefold higher DOC-normalized bacterial growth for the humic and the nonhumic DOC in the northern Adriatic Sea as compared to the North Sea. Bacterial growth in the dark treatments of the humic and the nonhumic DOC was stimulated by factors of 2 and 4, respectively, if BSA was added (Table 3).

Exposure of the different DOC fractions from the northern Adriatic Sea to surface solar irradiation resulted in stimulated bacterial growth on the bulk DOC [mean enhancement factor (EF) ~1.7; Wilcoxon, p = 0.009, n = 8], the humic (mean EF ~1.4; Wilcoxon, p = 0.035, n = 8), and the nonhumic fraction (mean EF ~1.5; Wilcoxon, p = 0.035, n = 8). In the North Sea, enhanced bacterial growth was detected only in the bioassay experiments established with bulk DOC (mean EF ~1.6; Wilcoxon, p = 0.05, n = 3) and humic DOC (mean EF ~1.5; Wilcoxon, p = 0.034, n = 4). No difference in bacterial growth was observed on nonhumic DOC (Wilcoxon, p = 0.5, n = 4) between irradiated and dark treatments (Fig. 3). If the different DOC fractions were amended with BSA, the solar irradiation exposed bulk DOC and the nonhumic DOC supported a ~1.2-fold higher bacterial growth as compared to the dark control (n = 1 for bulk DOC; Wilcoxon, p = 0.05, n = 3, for nonhumic DOC). For the humic DOC, however, no difference between BSA-amended irradiated and dark treatments was detectable (Wilcoxon, p = 0.5, n = 3; Fig. 3). If artificial seawater was amended with BSA and subsequently exposed to surface solar irradiation, no differences in the growth patterns in the irradiated and the dark treatment were observed (Fig. 3). In the northern Adriatic Sea, the increase in the bioavailability of the bulk DOC was linearly related to the changes in the optical characteristics of the DOC, thus also to the UVB dose received (Figs. 2 and 4).

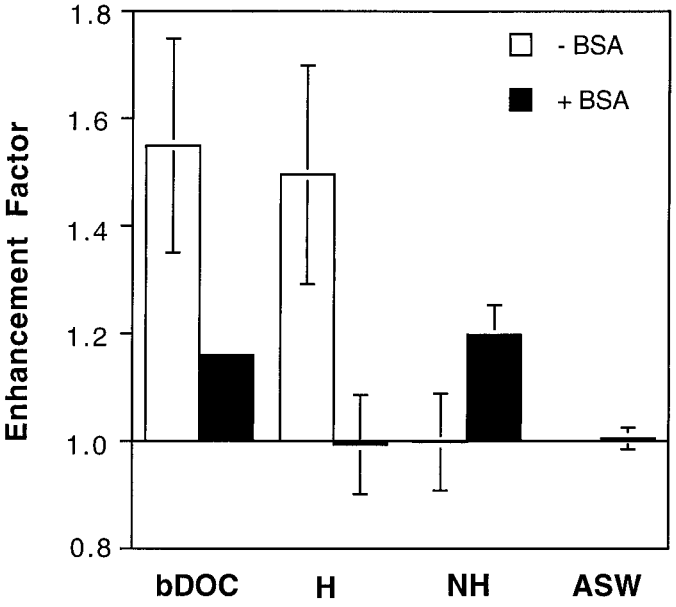

Fig. 3. Enhancement factor (bacterial growth in irradiated : dark treatment) of the different DOC fractions and the BSA-amended treatments from the experiments performed with coastal North Sea water. Mean values ±SD are given. bDOC—bulk DOC. H—humic DOC. NH—nonhumic DOC. ASW—artificial seawater. BSA—bovine serum albumin (916 μM C final concentration).

Photochemical and bacterial oxygen demand—In the northern Adriatic Sea, photochemical O_2 demand of surface water DOC exposed to full solar radiation ranged between 0.21 and 0.62 μmol O_2 L^{-1} h^{-1} (n = 6), whereas for the DOC from the coastal North Sea a photochemical O_2 demand of 0.08–0.16 μmol O_2 L^{-1} h^{-1} (n = 9) was obtained. DOC-normalized photochemical O_2 demand was, on average, ~10 times higher (ANOVA, p = 0.001, n = 15) in the northern Adriatic Sea [mean 2.9 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}, range 2.5–4.1 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}, n = 6; Fig. 5a] as compared to the coastal North Sea [mean 0.2 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}, range 0.1–0.4 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}, n = 9; Fig. 5b]. Shielding off UVB radiation generally decreased the photochemical O_2 demand. In the northern Adriatic Sea, UVB radiation attributed ~80% (range 50–100%, n = 4) to the photochemical O_2 demand, whereas in the North Sea, UVB radiation was, on average, responsible for only ~30% (range 0–60%, n = 5) of the photochemical O_2 demand. When pooling the data from the two study sites, a significant effect of UVB radiation on the photochemical O_2 demand was detectable (Wilcoxon, p = 0.011, n = 9). Bacterial respiration rates (in the 0.8-μm filtrate) varied in the northern Adriatic Sea between 0.13 and 0.64 μmol O_2 L^{-1} h^{-1} (n = 5) and were thus similar to the photochemical O_2 demand. In the coastal North Sea, bacterial respiration

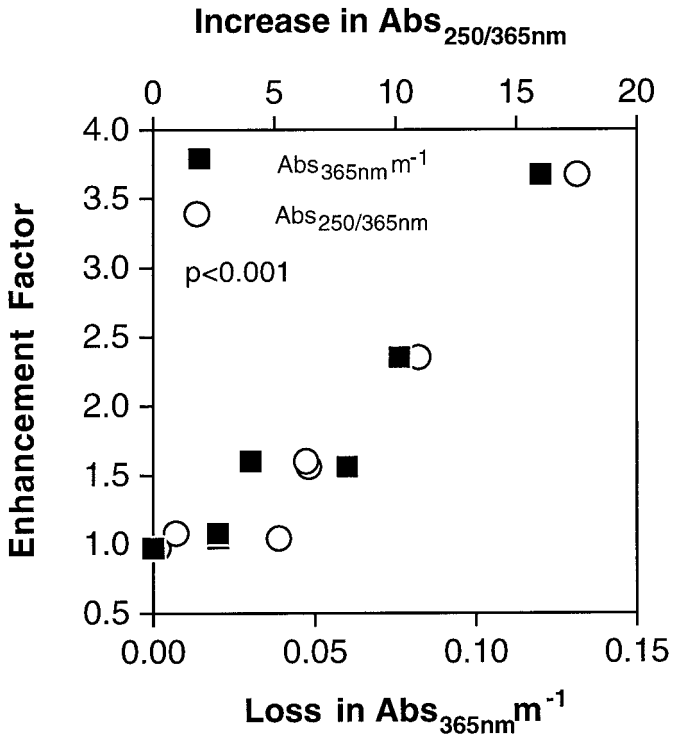

Fig. 4. Relationship between the enhancement factor (bacterial growth in irradiated : dark treatment) and the change in the optical properties of the bulk DOC during the exposure to surface solar irradiation in the northern Adriatic Sea. $Abs_{365\,nm}$ m^{-1}—absorbance at 365 nm. $Abs_{250:365\,nm}$—absorbance ratio 250 : 365 nm.

rates ranged between 0.11 and 0.55 μmol O_2 L^{-1} h^{-1} (n = 7) and were, on average, about threefold higher as compared to rates of photochemical O_2 demand. Normalizing the bacterial O_2 demand to the concentration of DOC resulted in a significantly higher bacterial respiration for DOC originating from the northern Adriatic Sea [range: 2–5 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}] than from the coastal North Sea [0.2–1 nmol O_2 (μmol DOC)$^{-1}$ h^{-1}, ANOVA, p = 0.001, n = 12].

Discussion

Optical properties of the bulk DOC and the humic and nonhumic component—Our results clearly indicate a substantially higher concentration of chromophoric substances in the coastal North Sea as compared to the northern Adriatic Sea. The higher DOC-normalized absorbance and fluorescence and the absorbance ratio of the humic DOC obtained for the northern Adriatic Sea as compared to the North Sea might indicate a different origin of this DOC component at the two study sites. The absorbance ratio is a rough estimate of the average molecular weight distribution of the DOC (Strome and Miller 1978). The comparatively higher absorbance ratio obtained for the northern Adriatic Sea indicates a higher contribution of low molecular weight compounds to the chromophoric DOC pool at this study site. This might be partially attributed to a higher amount of fulvic acids in marine humic DOC (Thurman 1985).

Bacterial growth on bulk, humic, and nonhumic DOC—Humic DOC is operationally defined as the DOC fraction adsorbing to a hydrophobic macroporous resin (i.e., XAD-8) at low pH (Thurman and Malcolm 1981). Isolation of the humic DOC fraction involves significant experimental ma-

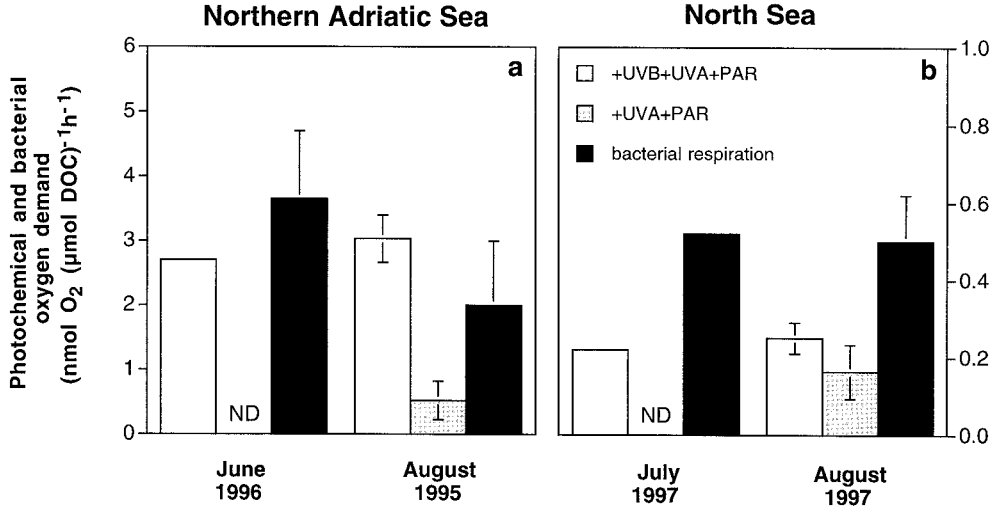

Fig. 5. DOC-normalized photochemical and bacterial oxygen demand of surface water collected in (a) the northern Adriatic Sea (n = 2 for June 1996; n = 4 for August 1995) and (b) in the coastal North Sea (n = 2 for July 1997; n = 7 for August 1997). Samples for the photochemical O_2 demand were exposed to the full range of surface solar radiation (+UVB + UVA + PAR) and to UVA and PAR only (+UVA + PAR) for 5–8 h starting at ~1,000 h. Samples for the bacterial O_2 demand were incubated in the dark. Mean values ±SD are given. UVB—ultraviolet B radiation. UVA—ultraviolet A radiation. PAR—photosynthetic active radiation. UVB radiation was shielded off with Mylar-D foil. ND—not determined.

nipulation, which could affect the properties of the DOC as microbial substrates (Tranvik 1998). Furthermore, contamination due to bleeding of the resin has been reported as a possible source of error (i.e., Moran and Hodson 1990). However, XAD resins have been widely used to investigate the distribution, the chemical characteristics, and the biological availability of humic substances. In the present study, the mean recovery efficiency was ~97%. The sum of the bacterial growth on the humic and the nonhumic DOC exceeded bacterial growth on bulk DOC in the bioassay experiments at both study sites (Table 3). This higher bacterial growth on the fractionated DOC as compared to that on unfractionated, bulk DOC could be an indication of contamination with low amounts of labile DOC during fractionation of the bulk DOC. It might also indicate structural alterations of the humic and the nonhumic DOC during the fractionation step that involved considerable changes in pH. We tentatively assume that the changes in pH and the subsequent DOC fractionation are the main reasons for the observed enhanced bacterial growth on the fractionated DOC as compared to the bulk DOC.

The substantially higher DOC-normalized bacterial growth in the northern Adriatic Sea as compared to the North Sea, where higher concentrations of inorganic nutrients are present, indicates the more refractory nature of the DOC in the coastal North Sea. This is consistent with results of Moran and Hodson (1994), who found a higher biological reactivity of humic substances formed in oceanic systems as compared to vascular plant-derived humic substances.

The effect of irradiation on the optical properties and the bioavailability of bulk DOC, the humic and nonhumic component—The loss in absorbance and the concurrent increase in the absorbance ratio following exposure to surface solar irradiation are directly related to enhanced bacterial growth on irradiated DOC (Figs. 3 and 4; Table 2). Comparing the enhancement factors of the bioassay experiments established with bulk DOC performed at the two study sites at similar incident irradiation reveals an approximately twofold higher stimulation of bacterial growth for solar irradiation exposed DOC of the northern Adriatic Sea as compared to the coastal North Sea. The enhancement of bacterial growth observed on the bulk DOC was, in the northern Adriatic Sea, equally attributable to both the humic and the nonhumic DOC component. In the North Sea, however, only humic DOC stimulated bacterial growth. Evidence for the photochemical production of low molecular weight organic and inorganic compounds and their subsequent bacterial use is accumulating (*see* review by Moran and Zepp 1997), although it has been estimated that only ~20% of the photolysis products have been identified (Miller and Zepp 1995). The type of photoreaction probably depends on the chromophores present. Thus, information on chromophore-specific photoproducts is needed to further elucidate the importance of the origin and age of DOC on its photochemical and biological degradation.

BSA amendment of the bulk and the humic fraction of the DOC prior to exposure to surface solar radiation decreased the stimulating effect as compared to that observed in the corresponding unamended treatments (Fig. 3). In a recent study, Obernosterer et al. (1999*b*) found a reduction in bacterial activity by 50% (as measured by thymidine and leucine incorporation) when growing on BSA-amended, irradiated DOC from mesopelagic waters as compared to the dark control. Unamended, irradiated mesopelagic water significantly stimulated bacterial activity. We suggest that photoinduced reactions between specific DOC components and BSA have rendered BSA biologically unavailable (Obernosterer et al. 1999*b*). The transformation of labile DOC compounds into more refractory constituents has been studied in more detail by Carlson et al. (1985) and Keil and Kirchman (1994), and the importance of solar radiation in these processes has been investigated only recently (Kieber et al. 1997; Benner and Biddanda 1998; Tranvik and Kokalj 1998). BSA-amended artificial seawater supports a different bacterial growth pattern than natural, solar irradiation exposed DOC. This suggests that natural DOC might act as a photosensitizer, involving BSA and other compounds (not absorbing in the wavelength range >300 nm) in photochemical reactions and thereby rendering them less bioavailable. However, in the present study, BSA-amended nonhumic DOC exhibits stimulated bacterial activity when exposed to solar irradiation as compared to the dark control. A similar response was detected in several samples from the chlorophyll maximum layer in a previous study (Obernosterer et al. 1999*b*), which indicates that possible photoinduced changes in the biovailability of this model substrate might be dependent on the composition of the DOC pool.

Photochemical vs. bacterial O_2 demand—Photochemical O_2 consumption has mostly been measured in humic-rich freshwater systems, reporting DOC-normalized O_2 consumption rates similar to those found in the northern Adriatic Sea (Lindell and Rai 1994; Amon and Benner 1996; Reitner et al. 1997). Interestingly, the effect of UVB radiation on the photochemical O_2 demand revealed a pronounced difference between the two study sites. Although UVB radiation contributed ~80% to the photochemical O_2 demand in the northern Adriatic Sea, its contribution was only ~30% in the North Sea. In a recent study, no significant effect of UVB radiation on the photochemical O_2 demand of DOC from the open Atlantic Ocean was detectable (Obernosterer et al. in prep.). Differences in the origin of the DOC at the two study sites probably account for the varying contribution of UVB radiation to the photochemical and bacterial O_2 demand and the 1 order of magnitude higher photochemical O_2 demand for the DOC originating from the northern Adriatic Sea.

Degradation of DOC via photochemical reactions occurs either directly, via the production of carbon gases, or indirectly, via the photochemical production of low molecular weight organic substances and their subsequent uptake and remineralization by bacterioplankton. Most of the direct carbon loss (~94%) is attributable to the production of dissolved inorganic carbon (DIC; Miller and Moran 1997), and production rates of DIC are ~15-fold higher than those measured for CO for both seawater and freshwater (Miller and Zepp 1995). In a recent study (Miller and Moran 1997), a linear relationship between the loss in absorption at 350 nm and the photochemical production of both DIC and CO was observed. Applying this relationship (Miller and Moran

1997) to our study, we estimate a CO_2 production of ~2.2 and 2.5 μmol L^{-1} d^{-1} for surface water of the northern Adriatic Sea and the North Sea, respectively (for the northern Adriatic Sea, this estimation is based on data obtained during August 1995). Photochemical production of CO would account for ~0.15 μmol L^{-1} d^{-1} at both study sites. Among the different DIC constituents, CO_2 is suggested to be the major end product of photooxidation (Miller and Zepp 1995). In order to compare CO_2 formation derived from DIC production with that based on O_2 consumption, our photochemical O_2 consumption rates can be converted to CO_2 production rates using a $CO_2:O_2$ ratio of 2 (Langford et al. 1973). Calculated photochemical CO_2 production would be ~6 and 3 μmol L^{-1} d^{-1} for surface water of the northern Adriatic Sea and the coastal North Sea, respectively.

Amon and Benner (1996) have shown that, on a molar basis, photochemical O_2 consumption approximately equals the loss of DOC ($O_2:DOC = 1.13$). If this is valid also for our study sites, a DOC loss of 4.5 and 1.1 μmol L^{-1} d^{-1} for surface water of the northern Adriatic and the coastal North Sea, respectively, can be calculated. The indirect contribution of photochemical transformation to the biological degradation of DOC can be estimated from the difference in the bacterial carbon consumption between the irradiated and the dark treatment using a bacterial carbon content of 20 fg $cell^{-1}$ (Lee and Fuhrmann 1987) to convert the increase in cell numbers into biomass production, a growth efficiency of 50%, and a mean enhancement factor of 1.6 (Figs. 3 and 4). Our estimations then indicate that ~3 and 2.5 μmol C L^{-1} d^{-1} in the uppermost surface layer of the northern Adriatic Sea and the North Sea, respectively, are consumed by bacterioplankton due to the photochemical activity on the DOC pool. If the photochemical production of low molecular weight organic acids is about equal to the CO photoproduction (Mopper et al. 1991; CO production ~0.15 μmol L^{-1} d^{-1} vs. bacterial carbon consumption ~3 μmol L^{-1} d^{-1}), then only ~5% of the bacterial carbon consumption can be attributed to identified photolytically produced organic carbon.

This rough estimate indicates that biological activity at the expense of photochemically produced compounds and direct loss of DOC via the photochemical production of carbon gases are approximately equal, which is consistent with the conclusion of Miller and Moran (1997). The estimated daily total DOC loss through photochemical activity, including direct and indirect pathways, accounts for ~7 and 1% of the total DOC in surface water of the northern Adriatic Sea and the coastal North Sea, respectively.

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