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Contrasting effects of solar radiation and nitrates on the bioavailability of dissolved organic matter to marine bacteria

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\textbf{A B S T R A C T}

We evaluated the role of nitrate (NO\textsubscript{3}\textsuperscript{−}) as a potential photosensitizer and the bacterial responses to dissolved organic matter (DOM) phototransformation from coastal waters in the northwestern Mediterranean Sea. In spring, without any addition of NO\textsubscript{3}−, the exposure of 0.2 μm filtered seawater (DOM-solution) to natural solar radiation (i.e. Full Sun [FS], including photosynthetically available [PAR: 400–700 nm], ultraviolet-A [UVAR: 315–400 nm] and ultraviolet-B [UVBR: 280–315 nm] radiations) stimulated bacterial production (BP) and abundance (BA) in natural assemblages (0.8 μm filtered seawater) by 80 and 20% as compared to unexposed (Dark) DOM-solutions, respectively. This stimulation resulted primarily from the exposure to PAR. When NO\textsubscript{3}− (30 μM) was added to DOM-solution before irradiation, BP and BA increased by 150 and 65% in FS compared to Dark, respectively, due to both PAR and UVBR. By contrast, in summer, the exposure of DOM-solution caused a decrease in BP by 30% but an increase in BA by 23% in FS compared to Dark, regardless of the NO\textsubscript{3}− addition before irradiation. The inhibition of BP resulted mainly from UVAR, whereas the stimulation of BA resulted from PAR. These results suggest contrasting effects along seasons of solar radiation and NO\textsubscript{3}− on DOM bioavailability, depending on its initial chemical composition.

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1. Introduction

In the surface ocean, solar radiation induces the phototransformation of dissolved organic matter (DOM), which affects its bacterial utilization and fate [1,2]. The origin and chemical composition of DOM has been shown to influence its photoactivity and its subsequent bioavailability [2]. In general, the irradiation of terrigenous or refractory deep-water DOM will have a net positive effect on its subsequent bacterial utilization through the formation of low molecular weight (LMW) bioavailable photoproducts, increasing bacterial production (BP) and respiration (BR) by 30–500% [3]. By contrast, the irradiation of freshly produced plankton-derived DOM found in surface waters will have a net negative effect through the formation of bioinert refractory photoproducts, decreasing BP and BR by 10–100% [3].

This phototransformation of DOM proceeds via both direct and indirect reactions. The first involves absorption of photons by the molecular moiety that results in the observed reaction of interest. In most carbon related surface ocean processes, this direct absorption is by chromophoric DOM (CDOM) [4]. Indirect (or sensitized) processes refer to DOM reactions that proceed via free radicals [1] that are produced from a source other than the molecule undergoing the reaction of interest. Very often, this is CDOM that also serves as source of radicals (i.e. photosensitizer) for indirect photochemistry in the surface waters [5].

Nitrate (NO\textsubscript{3}−) could be equally considered a photosensitizer for DOM transformation. Indeed, NO\textsubscript{3}− efficiently absorbs ultraviolet-B radiation (UVBR: 280–315 nm) to produce some free radicals [6] such as the hydroxyl radical (\textit{OH}) [7,8], which is one of the most reactive species in natural waters [9,10]. This photochemical role of NO\textsubscript{3}− may change with season with regard to modifications of solar irradiance spectrum, i.e. higher levels of UVBR in summer could lead to more direct absorption by NO\textsubscript{3}−. Recently, it has been shown that NO\textsubscript{3}− was involved in the photodegradation of dimethylsulfide (DMS) in oceanic waters [11], pesticides in surface waters [12] and in the photoproduction of dicarboxylic acids from unsaturated fatty acid in aqueous solution [13].

The main objective of this work is to assess the effects of solar radiation and NO\textsubscript{3}− on the bioavailability of DOM to bacterio-plankton from coastal waters in the northwestern Mediterranean Sea.
Sea. Irradiation and biodegradation experiments were conducted at two different seasons, i.e. early spring and early summer on NO$_3^-$-amended and unamended seawater samples. Here we investigate, through the measurement of BP and bacterial abundance (BA), the seasonal changes of DOM bioavailability, the role of NO$_3^-$ as a potential photosensitizer and the specific function of photosynthetically available radiation (PAR: 400–700 nm), ultraviolet-A radiation (UVAR: 315–400 nm) and UVBR in these processes.

2. Materials and methods

2.1. Sample collection

Seawater samples were collected in March and June 2003 at 3 m depth at the Station d’Observation Laboratoire Arago (SOLA), a shallow (∼26 m depth) coastal station of the northwestern Mediterranean Sea located ∼500 m offshore of Banyuls-sur-mer, France (42° 29′ N, 03° 08′ E; Fig. 1), using a 5 l Niskin bottle deployed from the research vessel Néreis II. Seawater was transferred into an acid-cleaned 20 l polycarbonate carboy and immediately transported to the laboratory.

2.2. Irradiation experiments

Seawater was prefiltered through precombusted (450 °C, 6 h) GF/A glass fiber filters (90 mm filter diameter, Whatman) and then through 0.2 µm polycarbonate filters (90 mm filter diameter, Nuclepore), using a peristaltic pump with acid-cleaned silicon tubing. The 0.2 µm filtered seawater (hereafter called DOM-solution) was distributed into precombusted 5 l glass bottles and stored at 4 °C in the dark for 12 h before irradiation. A subsample of DOM-solution was then amended with NO$_3^-$ (30 µM final concentration, Fluka). The NO$_3^-$-amended and unamended DOM-solutions were dispensed into precombusted quartz and borosilicate tubes (1 l volume) sealed with acid-cleaned silicone stoppers wrapped in Teflon foil. The tubes were irradiated on 25 March and 27 June for 7 h (10:00 a.m. to 5:00 p.m.) under natural solar radiation in a recirculating water bath (0.1 m depth) maintained at in situ temperature (13.5 ± 1 and 23 ± 1 °C in March and June, respectively) that was set up on the pier near the Harbor of the institute. Four light conditions were simulated using different optical filters: (1) Dark (borosilicate tubes wrapped in aluminum foil), (2) PAR (borosilicate tubes wrapped in Lexan filter), (3) PAR + UVAR (quartz tubes wrapped in Mylar filter) and (4) Full Sun (FS = PAR + UVAR + UVBR; quartz tubes, no filter). Lexan and Mylar have 50% transmittance at 380 and 320 nm, respectively, and both filters have ∼90% transmittance in PAR. Duplicate samples were irradiated for each light/NO$_3^-$ treatment. Incident irradiance was measured in the PAR, UVAR and UVBR domains using a broad band ELDONET radiometer (Real Time Computer, Inc.). In March and June, the PAR, UVAR and UVBR irradiances integrated over the exposure time (doses) were 7377, 1067 and 18 kJ m$^{-2}$, and 8322, 1366 and 30 kJ m$^{-2}$, respectively.

2.3. Biodegradation experiments

Seawater used for irradiation experiments was also filtered under a low vacuum (<50 mmHg) through a 0.8 µm polycarbonate filter (47 mm filter diameter, Nuclepore) to prepare the bacterial inoculum. During the irradiation of DOM-solutions, the bacterial inoculum was kept in the dark at in situ temperature. After exposure, DOM-solutions were inoculated with the (unirradiated) bacterial inoculum (1/10, inoculum/DOM-solution final ratio) to initiate biodegradation experiments. These mixed solutions were incubated in precombusted 11 glass bottles in the dark at 15 ± 1 and 20 ± 1 °C in March and June, respectively. No nutrients were added in the mixed solutions to measure the response of bacteria to “natural” conditions. BP and BA were measured before (T0) and after 48 h (T48) incubation. Duplicate samples were used for each light/NO$_3^-$ treatment.

2.4. Analysis

BP was measured by [$^3$H]leucine incorporation into bacterial proteins [14] and BA by flow cytometry [15]. Chlorophyll a (Chl a) was determined using a PerkinElmer MPF66 spectrofluorometer [16]. Dissolved organic carbon (DOC) was measured using a Shimadzu TOC-5000 carbon analyzer [17]. Fluorescent DOM was determined using a PerkinElmer LS55 spectrophotometer (excitation wavelength: 350 nm, emission wavelength: 450 nm) standardized with a quinine sulfate solution (1 QS U = 1 ppb quinine sulfate in 0.05 M H$_2$SO$_4$) [18]. NO$_3^-$ plus nitrite (NO$_2^-$) were analyzed with a Skalar autoanalyzer [19]. The method from [20] was used for reactive phosphorus (PO$_4^{3-}$) analysis. All analyses were conducted in duplicate or triplicate.

2.5. Statistics

The effects of solar radiation and NO$_3^-$ on BP, BA and cell-specific activity [(CSA = (BP/BA)] after 48 h incubation were assessed by one-way analyses of variance (ANOVA) performed with StatView 5.0 and the statistics package provided in Microsoft Excel 11.0. Samples were first considered as one group in a single classification ANOVA, ignoring the light/NO$_3^-$ treatments. When significant variance components were detected within the group, ANOVA were used to identify individual subgroups that were significantly different from each other [21]. Then, comparisons of BP, BA and CSA were

Fig. 1. SOLA sampling station located ∼500 m offshore of Banyuls-sur-Mer (France) in the northwestern Mediterranean Sea.
Table 1 presents the initial composition of seawater used for the irradiation/biodegradation experiments. Chl a ranged from 0.29 (June) to 0.53 μg L⁻¹ (March). DOC was slightly lower in March (78 μM) than in June (84 μM), whereas fluorescent DOM presented the inverse pattern (1.5 and 1.3 QSU in March and June, respectively). NO₃⁻ and NO₂⁻ were higher in March (1.2 and 0.11 μM) than in June (limit of detection), whereas PO₄³⁻ remained very low (∼0.03 μM). After addition of NO₃⁻, the latter reached 33 and 29 μM in the DOM-solution in March and June, respectively.

Concerning the responses of bacteria to different DOM-solutions, in March, significant variance components were detected within the group of samples for BP, BA and CSA (F(7,8) = 4–21, p < 0.05) (Fig. 2a, c and e). Three subgroups significantly different from each other were identified: (1) Dark, Dark + NO₃⁻, (2) PAR, calculated between the identified subgroups. The significance threshold was set at p < 0.05 for F(k−1,n−k), where k is the number of groups/subgroups and n is the total number of subjects.

3. Results and discussion

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PAR + NO$_3^-$, PAR + UVAR, PAR + UVAR + NO$_3^-$, FS and (3) FS + NO$_3^-$ (F(1,10) = 7–49, p < 0.05; F(1,12) = 11–45, p < 0.01). For the NO$_3^-$-unamended samples, BP increased on average by 80%, BA by 20% and CSA by 40% in PAR, PAR + UVAR or FS (subgroup 2) compared to Dark (subgroup 1) (Fig. 2a, c and e). Clearly, in March, the irradiation of NO$_3^-$-unamended DOM-solution led to an increase in BP, BA and CSA in the mixed solution after 48 h incubation compared to the unirradiated solution. This increase was due essentially to PAR with no significant effect of UVAR and UVBR, some additional LMW substrates may be also produced from CDOM but without any significant enhancement of BP [23]. When NO$_3^-$ was added to the DOM-solution, BP increased on average by 80 and 150%, BA by 20 and 65%, and CSA by 40 and 50% in PAR or PAR + UVAR (subgroup 2) and FS (subgroup 3) compared to Dark (subgroup 1), respectively (Fig. 2a, c and e). These results confirm the role of PAR in the increase of BP, BA and CSA, but in this case, UVBR also played a significant role, whereas UVAR had no effect. Consequently, in March, the addition of NO$_3^-$ during irradiation of the DOM-solution led to a positive effect of UVBR on bacterial growth (Fig. 2a, c and e). This positive effect likely occurs through the action of OH that are released from the UVB photolysis of NO$_3^-$ [7]. When produced in seawater, OH reacts almost exclusively with bromide ion to produce bromide radical (Br$^•$-)1, and to a lesser extent with the carbonate system and DOM [11,24]. As demonstrated for the degradation of DMS [11], Br$^•$- could be one of the radical species involved in the degradation of DOM, leading to the production of other LMW bioavailable substrates that in turn could substantially enhance bacterial growth. Therefore, in March, two different photochemical pathways were observed for the stimulation of BP, BA and CSA: (1) the direct absorption of PAR by CDOM and (2) the reaction between DOM and OH-derived radicals that were produced from the direct absorption of UVBR by NO$_3^-$.

In June, significant variance components were detected within the group of samples for BP, BA and CSA (F(7,8) = 4–11, p < 0.05) (Fig. 2b, d and f). Two different subgroups were identified: (1) Dark, Dark + NO$_3^-$, PAR, PAR + NO$_3^-$ and (2) PAR + UVAR, PAR + UVAR + NO$_3^-$, FS, FS + NO$_3^-$ (F(1,14) = 19–52, p < 0.001) for BP and CSA, and (1) Dark, Dark + NO$_3^-$ and (2) all other light conditions (F(1,14) = 13, p < 0.005) for BA. For the NO$_3^-$-amended and unamended DOM-solutions, BP decreased on average by 30% in PAR + UVAR or FS (subgroup 2) compared to Dark or PAR (subgroup 1) (Fig. 2b and f). On the other hand, BA increased on average by 23% in PAR, PAR + UVAR or FS (subgroup 2) compared to Dark (subgroup 1) (Fig. 2d). Consequently, in June, the irradiation of NO$_3^-$-amended and unamended DOM-solution lead to a decrease in BP and CSA, and to an increase in BA in the mixed solution after 48 h incubation compared to the unirradiated solution. Clearly, UVAR was responsible for the inhibition of BP and CSA, whereas NO$_3^-$, PAR and UVBR had no significant effect. By contrast, the stimulation of BA was due essentially to PAR, whereas NO$_3^-$, UVAR and UVBR had no significant effect (Fig. 2b, d and f). The negative effect of UVAR on BP may be explained by a photomineralization (loss) of biomolecules [25] or their phototransformation into biorefractory compounds (“humification” processes) [26] from the direct absorption of UVBR by CDOM. Although the addition of UVBR may stimulate these photochemical processes (mineralization and humification), it did not lead to supplementary BP inhibition, probably because the UVBR flux was tiny compared with PAR and UVAR. Therefore, in June, the subsequent decrease of BP was only due to the direct absorption of UVAR by CDOM. Note that for both the experiments, the addition of NO$_3^-$ in the dark treatments had no effect on BP (Dark and Dark + NO$_3^-$ in the same subgroup) meaning that NO$_3^-$ alone did not cause a shift in the amount of carbon used for BP.

The difference of bacterial responses between March (stimulation of BP and BA) and June (inhibition of BP and stimulation of BA) probably reflects differences in the DOM quality between these two periods [2]. In June, the fraction of Fluorescent DOM is lower than in March (0.015 and 0.019 QSU mm $^{-1}$), respectively. Indeed, due to seasonal effects, DOM samples in June were likely more photoprocessed than those of March. The degradation/bleaching of the sunlight-absorbing compounds could have an impact on DOM composition. Whereas in March DOM undergoes photoprocessing that increases its bioavailability and leads to the consumption of the relevant molecules, in June the same molecules could be no longer present (or be present in much lower amount) due to the previous, combined photochemical and microbiological processing. Moreover, the role of NO$_3^-$ as a potential photosensitizer, observed in March but not in June (despite the higher UVBR doses at this season), seems to depend on this DOM quality. It is also possible that the variations of temperature between March (13.5 °C) and June (23.0 °C) had an influence on these bacterial responses. This study highlights contrasting effects of solar radiation and NO$_3^-$ on the bioavailability of DOM to bacterioplankton for coastal waters in the northwestern Mediterranean Sea. Our major findings are: (1) in spring, a significant role of PAR in the subsequent stimulation of BP, (2) a potential role of NO$_3^-$ as a photosensitizer (through the action of UVBR) in this BP stimulation and (3) in summer, a significant role of UVAR in the subsequent inhibition of BP without any significant role of NO$_3^-$.

The NO$_3^-$ and humic-rich DOC inputs from the Rhône River, the largest river of the northwestern Mediterranean Sea, may represent about 96 kT NO$_3^-$ year$^{-1}$ and 130 kT C year$^{-1}$ [27,28]. Consequently, the combination of high levels of UVBR with large NO$_3^-$ inputs from estuaries and river plumes or from nutrient-rich deep waters (upwelling) could strongly stimulate photochemical processes such as the production of OH and then participate to the photosensitized transformation of DOM and its subsequent bacterial utilization, as we observed in spring with the addition of 30 μM NO$_3^-$F. For instance, we measured, by using the method described in [7] (i.e. benzoic acid probe introduced in large excess prior to the irradiation of samples), a photoproduction of 100–150 nM·OH$^{-1}$ for water from the Rhône River compared to 2–4 nM·OH$^{-1}$ for marine water from the northwestern Mediterranean Sea exposed to similar solar radiation [Tedetti and Sempéré, unpublished data]. However, the OH production may also result from the photolysis of CDOM. Further studies will be necessary to determine more accurately the respective role of NO$_3^-$ and CDOM as photosensitizers, as well as to better discriminate the role of PAR, UVAR and UVBR in DOM phototransformation.

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References

[1] K. Mopper, D.J. Kieber, Marine photochemistry and its impact on carbon cycling, in: S. de Mora, S. Demers, M. Vernet (Eds.), The Effects of UV Radiation in
the Marine Environment, Cambridge University Press, Cambridge, 2000, pp. 101–129.

[2] M.A. Moran, J.S. Covert, Photochemically mediated linkages between dissolved organic matter and bacterioplankton, in: S.E.G. Findlay, R.L. Simsbaugh (Eds.), Aquatic Ecosystems: Interactivity of Dissolved Organic Matter, Academic Press, San Diego, 2003, pp. 243–262.

[3] K. Mopper, D.J. Kieber, Photochemistry and the cycling of carbon, sulphur, nitrogen and phosphorus, in: D.A. Hansell, C.A. Carlson (Eds.), Biogeochemistry of Marine Dissolved Organic Matter, Academic Press, San Diego, 2002, pp. 455–507.

[4] R.G. Zepp, Environmental photoprocesses involving natural organic matter, in: F.H. Frimmel, R.F. Christman (Eds.), Humic Substances and Their Role in the Environment, John Wiley & Sons, New York, 1988, pp. 193–214.

[5] R.G. Zepp, P.F. Schlotzhauer, R.M. Sink, Environ. Sci. Technol. 19 (2003) 74–81.

[6] J. Mack, J.K. Bolton, J. Photochem. Photobiol. A: Chem. 128 (1999) 1–13.

[7] J. Qian, K. Mopper, D.J. Kieber, Deep-Sea Res. 14 (2001) 741–750.

[8] K. Takeda, H. Takeda, S. Yamaji, K. Ohita, H. Sakugawa, Anal. Sci. 20 (2004) 153–158.

[9] T. Mill, Chemical and photooxidation, in: O. Hutzinger (Ed.), The Handbook of Environmental Chemistry, vol. 2, Part A, Springer, Berlin, 1980, pp. 77–105.

[10] O.C. Zafiriou, J. Joussot-Dubien, R.G. Zepp, R.G. Zika, Environ. Sci. Technol. 18 (1984) 356–371.

[11] R.-C. Bouillon, W.L. Miller, Environ. Sci. Technol. 39 (2005) 9471–9477.

[12] C. Richard, Halle Ater, O. Brahmia, M. Malouki, S. Halladja, Catal. Today 124 (2007) 82–87.

[13] M. Tedetti, K. Kawamura, M. Narukawa, F. Joux, B. Charrière, R. Sempéré, J. Photochem. Photobiol. A: Chem. 188 (2007) 135–139.

[14] D.L. Kirchman, Leucine incorporation as a measure of biomass production by heterotrophic bacteria, in: P.F. Kemp (Ed.), Handbook of Methods in Aquatic Microbial Ecology, Lewis Publishers, Boca Raton, 1993, pp. 509–512.

[15] F. Joux, H. Agogué, I. Obernosterer, C. Dupuy, T. Reinthaler, G.J. Herndl, P. Lebaron, Aquat. Microb. Ecol. 42 (2006) 91–104.

[16] J. Neveux, F. Lantoine, Deep-Sea Res. 40 (1993) 1747–1764.

[17] R. Sohrin, R. Sempéré, J. Geophys. Res. vol. 110, C10S90 (2005), doi:10.1029/ 2004JC002731.

[18] I. Obernosterer, B. Reitner, G.J. Herndl, Limnol. Oceanogr. 44 (1999) 1645–1654.

[19] P. Tréguer, P. Le Corre, Manuel d’analyses des sels nutritifs dans l’eau de mer, Laboratoire d’Océanographie Chimique, Université de Bretagne Occidentale, Brest (1975), p. 110.

[20] L. Murphy, J.F. Riley, Anal. Chim. Acta 27 (1962) 31–36.

[21] R.R. Sokal, J.F. Rohlf, Nested analysis of variance, in: R.R. Sokal, J.F. Rohlf (Eds.), Biometry: The Principles and Practice of Statistics in Biological Research, 2nd ed., WH Freeman and Company, San Francisco, 1981, pp. 271–320.

[22] I. Reche, M.L. Pace, J.J. Cole, Ecosystmes 3 (2000) 419–432.

[23] R.M.W. Amon, R. Benner, Geochim. Cosmochim. Acta 60 (1996) 1783–1792.

[24] M.J. Pullin, S. Bertilsson, J.V. Goldstone, B.M. Voelker, Limnol. Oceanogr. 49 (2004) 2011–2022.

[25] L.J. Tranvik, S. Bertilsson, Ecol. Lett. 4 (2001) 458–463.

[26] R.J. Kieber, L.H. Hydro, P.J. Seaton, Limnol. Oceanogr. 42 (1997) 1454–1462.

[27] T. Mostin, P. Rainhault, H.L. Golterman, B. Coste, Hydrobiologia 373 (1998) 237–248.

[28] R. Sempéré, B. Charriere, G. Cauwet, F. Van-wambeke, Global Biogeochem. Cycles 14 (2000) 669–681.