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Growth of marine bacterioplankton on river and seawater dissolved organic carbon in a Mediterranean coastal system

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Abstract: The growth of marine bacteria and the degradation of bulk river and seawater dissolved organic carbon (DOC) were investigated in a coastal system of the Aegean Sea, Eastern Mediterranean. Batch cultures were set up for each season under study (summer to winter) and incubated at in situ temperature. Changes in inorganic nutrients and DOC concentrations, bacterial abundance and biomass were monitored for a maximum of ca. 17 days. Bacterial specific growth rate (μ) ranged between 0.011 – 0.023 h^{-1} in the river water cultures and between 0.012 - 0.022 h^{-1} in the seawater cultures. In most cases, bacterial abundance, bacterial productivity (BP) and bacterial growth efficiency (BGE) were higher in the river water cultures than in the seawater ones. However, on average, marine utilizable DOC (UDOC) was higher (42.2%) than the riverine UDOC (38.7%). Inorganic nutrients seemed not to be limiting for bacterial growth and in most of the incubations final nitrogen and phosphorous concentrations were similar or higher than the initial ones. Temperature did not correlate with any of the bacterial parameters but it correlated positively with DOC degradation rates in both treatments, suggesting that temperature had no direct effect on bacterial growth on either riverine or marine DOC.

Résumé : *Croissance du bactérioplancton marin à partir du carbone organique dissous de l'eau de rivère et de l'eau de mer dans un système côtier méditerranéen*. La croissance des bactéries marines et la dégradation du carbone organique dissous (COD) originaire de la rivière et de l'eau de mer ont été étudiées dans un système côtier en mer Egée, Méditerranée orientale. Pour l'étude, une série de cultures bactériennes a été mise en place et incubée à des températures in situ à chaque saison, de l'été à l'hiver. Les variations des concentrations en nutriments inorganiques, COD, abondance et biomasse bactériennes ont été suivies pendant une durée maximum de 17 jours. Le taux de croissance bactérien (μ) varie de 0,011 à 0,023 h^{-1} pour les cultures d'eau de rivière et de 0,012 à 0,022 h^{-1} pour l'eau marine. L'abondance bactérienne et la production bactérienne (BP) ainsi que l'efficacité de croissance (BGE) étaient, dans la plupart des cas, supérieures dans les cultures d'eau de rivière à celles d'eau de mer. Cependant, la proportion de COD marin utilisable (UDOC) était, en moyenne, supérieure (42,2 %) à l'UDOC de rivière (38,7 %). Les nutriments inorganiques semblent ne pas limiter la croissance bactérienne et, dans la plupart des incubations, les concentrations finales de phosphore et d'azote étaient semblables ou supérieures aux concentrations initiales. La température n'est pas corrélée avec les paramètres bactériens mais elle est corrélée positivement avec le taux de dégradation du COD dans les deux types d'eau, suggérant que la température a un effet indirect sur la croissance bactérienne par le biais du COD de rivière et de mer.

Keywords: Bacteria, dissolved organic carbon, nutrients, degradation, Mediterranean.

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Introduction

Dissolved organic carbon (DOC) represents the largest pool of reduced carbon in the ocean. Regarding its origin, it is characterized as auto- or allochthonous. Autochthonous DOC is produced from biological processes within the marine environment such as phytoplankton exudation, sloppy feeding, dissolution of sinking particles, lysis of phytoplankton and bacterial cells, mostly after viral attack, while allochthonous DOC is introduced to the sea, mainly from land via riverine discharge (Williams, 2000). River derived DOC is considered to be a quantitatively important component of the oceanic carbon budget (Ludwig et al., 1996) especially in coastal and estuarine systems (Hopkinson et al., 1998).

It is generally considered that the main labile DOC source for bacteria is derived from phytoplankton photosynthesis (Cole et al., 1988 and references therein). This paradigm is being changed. Although it is now known that only a small fraction (0.7 to 2.4%) of oceanic dissolved organic matter (DOM) is of terrestrial origin in the open ocean (Opsahl & Benner, 1997), it has been shown that in coastal and estuarine areas terrestrial DOM is used as an energy source for bacterial production (Kuparinen et al., 1996; Hopkinson et al., 1998; Moran et al., 1999) and it can trigger off the whole microbial food web, especially in waters with low primary production (Carlsson et al., 1995; Kuparinen et al., 1996). In addition, the progress that has been made in identifying individual chemical species of DOC and subsequent bioavailability experiments, have shown that substances such as humic acids (Moran & Hodson, 1994) or other high molecular weight DOC (Amon & Benner, 1996), that usually were considered as refractory material, can sustain significant bacterial growth in the marine environment. Finally, the important synergism that has been identified between biological and photochemical degradation, i.e. the alteration of DOC by exposure to natural sunlight and the production of a variety of biologically labile photoproducts (Miller & Moran, 1997), enhances the biological significance of river-transported DOC.

The importance of river discharge to the oligotrophic Greek seas is shown by the fact that the total annual surface run-off is 51.8 km^3 which corresponds to 20% of the European run-off in the Mediterranean (Skoulikidis et al., 1998). However, data on DOC are rather scarce for the Eastern Mediterranean and especially for the Aegean Sea (Kormas et al., 1998; Obernosterer et al., 1999) and usually are reported only standing stocks that do not provide detailed information on the dynamics of the DOC pool. In order to understand DOC dynamics, the processes comprising DOC production and mineralisation must be quantified and correlated to hydrographic and/or biological variables. Finally, there is limited information in the literature regarding the study of direct degradation of freshwater DOC by marine or estuarine bacteria (Wikner et al., 1999).

In this direction, the aim of this study was to investigate the bioavailability of allochthonous DOC to marine bacteria in a semi-enclosed coastal system, Maliakos Gulf, Eastern Mediterranean. In a previous study Kormas et al. (1998), found a linear relationship between bacterial abundance and phytoplankton biomass in Maliakos Gulf, which suggests utilisation of phytoplanktonic DOC for bacterial growth (Cole et al., 1988). However, this relationship does not exist in winter, implying that other sources of DOC might sustain bacterial growth. The present study aims at the investigation of the consumption of riverine DOC by marine bacteria of a coastal system in the eastern Mediterranean Sea. Batch cultures of marine bacteria growing on river and seawater were incubated in the dark at in situ temperatures for a maximum period of ca.17 days. The experiments took place before and at the beginning of the maximum river inflow; the latter coincides with the winter phytoplankton bloom in the gulf. The changes in DOC, inorganic nutrient concentrations and bacterial abundance and biomass were monitored.

Materials and methods

Study area

Spercheios River has a length of 82.5 km and it ranks tenth in Greece in terms of mean annual flow and seventh in annual sediment discharge; no data exist for its DOC concentration and DOC discharge rate. Its estuary covers 100 km^2 and most of it is intensively cultivated - mainly rice fields. Mainly dry cropland and grasslands occupy the land around the estuary while olive trees cover only a small portion. Close to the mouth of the river, the estuary forms a small (5 km^2) shallow lagoon-like embayment, with a maximum depth of 5 m.

Maliakos Gulf (Fig. 1) is a semi-enclosed embayment on the east coast of Greece. It covers an area of ca. 110 km^2 and it is divided by headlands in two parts. At the eastern end, the gulf is connected to the Aegean Sea through the Oreoi Channel and to the Evoikos Gulf through the Knimida Channel. This part has an average depth of 36 m. The western part, where Spercheios River meets the sea, forms a basin with a maximum depth of 27 m, while closer to the river mouth the depth does not exceed 10 m. Spercheios is the major source of terrigenous material in the gulf, however, temporal streams in other points might be important occasionally.

Field measurements

Temperature was measured with a Hydrobios thermometer

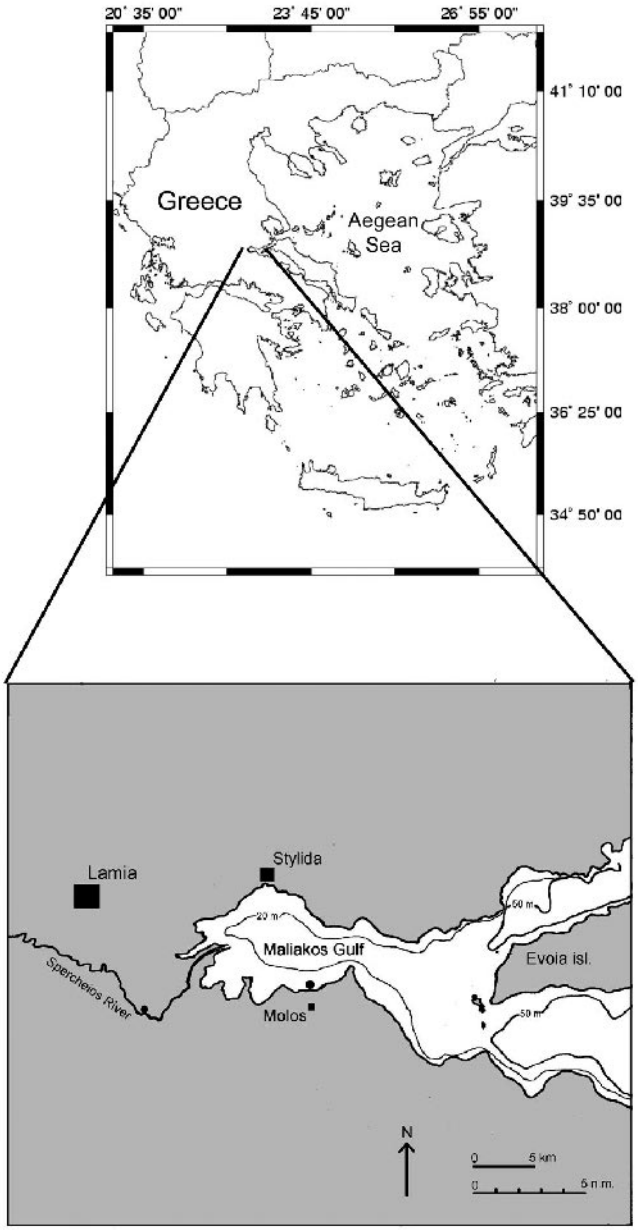

Figure 1. Study area and sampling points.
Figure 1. Zone d'étude et sites de prélèvements.

(± 0.1^{0}C). Chlorophyll *a* (chl *a*) and phaeopigments (phaeo) were determined spectrophotometrically using the acetone extraction method (Parsons et al., 1984) on Whatman GF/F filters (approximately 0.7 µm retain capacity).

Batch cultures

River (salinity = 0) and seawater (salinity = 36.5-37) batch cultures were used according to Ammerman et al. (1984) with slight modifications. In brief, river water was collected 7.5 km upstream from the river mouth and seawater was collected from a pier on the south coast of the gulf (Fig. 1). During collection, the water was successively filtered through 63 and 20 µm mesh nets and recovered in acid-washed (overnight in HCl 10%, rinsed with copious amounts of Milli-Q water) polyethylene 25 l carboys. The carboys were kept cool and in darkness until return to the laboratory (ca. 3 h). In the laboratory, approximately 14 l from each carboy was filtered through precombusted (450°C, 4 h) Whatman GF/C (1.2 µm nominal pore size) and acid-washed Millipore 0.2 µm PTFE filters under low vacuum (≃ 150 mm Hg). These filtrates served as the culture media. The filtration procedure caused insignificant changes between the DOC field values and initial concentrations in the cultures, while no flagellates were found at the initiation of the incubations (data not shown). For the river water cultures (RWC), salinity was fixed to that of seawater (35-37) with addition of NaCl. The inoculum was prepared after filtration of ca. 10 l of seawater through Whatman GF/F filters and then through Millipore 0.2 µm PTFE filters. The residue on the 0.2 µm filters (mostly bacteria from 0.2 to 0.7 mm size) were resuspended in 50 ml of culture media and then added to the cultures. The resulting initial bacterial numbers, in the batch cultures, were ca. 30-40% of the in situ seawater values. Tests on GF/F filters showed that less than 10% of the bacterial cells were retained on these filters (data not shown). Inoculation took place no later than 10 hours after collection. Each treatment was run in triplicate 4 l conical flasks, except for the September ones that were run in duplicate. The cultures were incubated in the dark, at in situ temperatures (Table 1).

Table 1. Environmental parameters at the sampling points of Spercheios River and Maliakos Gulf. Temp = temperature, DOC = dissolved organic carbon, Chl *a* = chlorophyll *a*. The number in parenthesis represents the percent of chl *a* to total [chl al + phaeopigments].

Tableau 1. Paramètres environnementaux aux stations de prélèvements dans la rivière Spercheios et dans le golfe de Maliakos. Temp = température, DOC = carbone organique dissous, Chl *a* = chlorophylle *a*. Les nombres entre parenthèses représentent la contribution de la chl *a* aux pigments totaux [%, chl *a* + phaeopigments].

		Temp (°C)	DOC (mM)	Chl *a* (µg l^{-1})
Sep. 1999	Spercheios	21.0	1.94	0.41 (69%)
	Maliakos	23.0	1.34	0.56 (42%)
Nov. 1999	Spercheios	13.2	0.87	0.34 (78%)
	Maliakos	15.4	0.63	1.61 (51%)
Jan. 2000	Spercheios	9.2	0.50	0.25 (81%)
	Maliakos	10.0	0.42	3.10 (59%)

Two ethanol-washed and well-rinsed with Milli-Q water microscopic slides were suspended in the cultures in

September 1999 to test bacterial wall growth. One slide was examined for attached bacteria at the middle and one at the end of the incubation period. Upon retrieval, the slides were air-dried (< 1 hour) and covered with a cover slip of known surface, after the addition of one drop of DAPI solution. The total number of bacteria was determined on each slide as described below. No bacterial cells were detected on in 50-60 optical fields of the slides, indicating that the batch cultures were dominated by free-living bacteria.

Laboratory measurements

Samples for the determination of inorganic nutrients (i.e. phosphate, nitrate, nitrite and ammonium) were stored at -20°C until analyses (usually less than five days after sampling). The nutrient concentrations were determined spectrophotometrically as described in Parsons et al. (1984).

Samples for DOC measurements were collected directly in pre-combusted glass vials, two drops of 37% HCl were added and the samples were stored in the dark at 4°C until analysis (< 1 week). DOC was measured by high-temperature combustion (Sugimura & Suzuki, 1988) in a Dohrmann DC-190 analyser. The stored samples were filtered through 25 mm acid-washed and well rinsed (Milli-Q) PTFE filters of 0.2 µm pore size (Millipore). From the filtrate, three to five replicates (100 µl injections) for each sample were analysed. Standard error for replicate injections varied from 1.0 to 9.4%. Blank samples (Milli-Q water) ranged from 60 to 90 µM DOC. Calculation of carbon concentrations was made using sucrose as a standard.

In our batch culture experiments, utilizable DOC (UDOC) is defined as the amount of DOC removed by bacteria during their exponential growth phase. It is expressed as % of total DOC. DOC degradation rate was calculated the DOC concentration difference during exponential growth phase divided by the respective time difference. Bacterial counts were measured using epifluorescence microscopy as described in Turley (1993).

Biovolume (BV) of bacterial cells were measured at the beginning and the end of the exponential growth phase and were determined following a modified protocol by Massana et al. (1997). Cells were classified either as cocci (spheres) or rods. The biovolume of the rods was calculated according to the formula (Bratbak, 1985):

$$\text{volume} = \pi / 4 \times w^2 (1 - w/3), \quad (1)$$

where l is the length and w is the width of the cells. A total of 200-700 cells was measured per triplicate sample. The carbon content (C) of the cells was calculated from BV using the formula (Thiel-Nielsen & Søndergaard, 1998):

$$C = 243 \cdot BV^{0.56} \quad (2)$$

Bacterial specific growth rate (µ) was calculated as the slope of the Ln transformed bacterial numbers versus time during the exponential growth phase. The lag portion of the curves was omitted and the end of the logarithmic growth was defined by the highest value before the curve reached the plateau phase. The bacterial growth efficiency (BGE) was calculated by the formula (Wikner et al., 1999):

$$BGE = (Y_b / \Delta DOC) \times 100 \quad (3)$$

where Y_b is the bacterial biomass maximum yield in the beginning of the plateau phase of the growth curve and ΔDOC is the net change in the concentration of DOC in the experimental flasks during the same time period. Degradation rate was calculated by dividing ΔDOC by the time of the exponential growth phase. Bacterial production (BP) was calculated from the difference in biomass during the exponential growth phase.

All statistical analyses were performed with the STATISTICA (StatSoft Inc., USA) software package.

Results

In Table 1, the environmental conditions of the river and marine sampling points are summarized. Temperature showed the expected seasonal pattern with decreasing temperatures from September to January. Both the river and seawater had similar values. DOC lowest values occurred in January while the highest were in September. Chl *a* in the river slightly decreased from September to January. The opposite pattern was observed in the sea where, in addition, phaeopigments had a smaller contribution to total chl *a* equivalents.

Mean bacterial abundance and DOC concentrations during incubations are shown in Figure 2 (SE bars are not shown for clarity reasons). At the end of the exponential growth phase, bacterial abundance was always higher in the river water cultures (from $25.2 \times 10^5 \pm 5.0 \times 10^5$ cells ml^{-1} to 107.2×10^5 cells ml^{-1}) than in the seawater (from $14.4 \times 10^5 \pm 1.3 \times 10^5$ cells ml^{-1} to 48.6×10^5 cells ml^{-1}). DOC concentrations decreased during the exponential growth phase, after which, both bacterial abundance and DOC concentrations did not follow any clear pattern. Changes in BV at the beginning and the end of the exponential growth phase are shown in Table 2; there was a 10-fold increase of the biovolumes in the latter phase. No statistical differences were found between treatments in all of the experiments.

Mean values of DOC degradation rates during the exponential growth phase (Fig. 3a) were higher in the SWC (0.32 mM d^{-1}) than in the RWC (0.14 mM d^{-1}) in September but the opposite was observed in November and January when the lowest values were observed (0.07 and 0.11 mM d^{-1} in the SWC and RWC respectively). UDOC (Fig. 3b) showed the highest values in November for both the seawater (64%) and the river water cultures (58%), while the lowest values were observed in January (16% and 26% respectively).

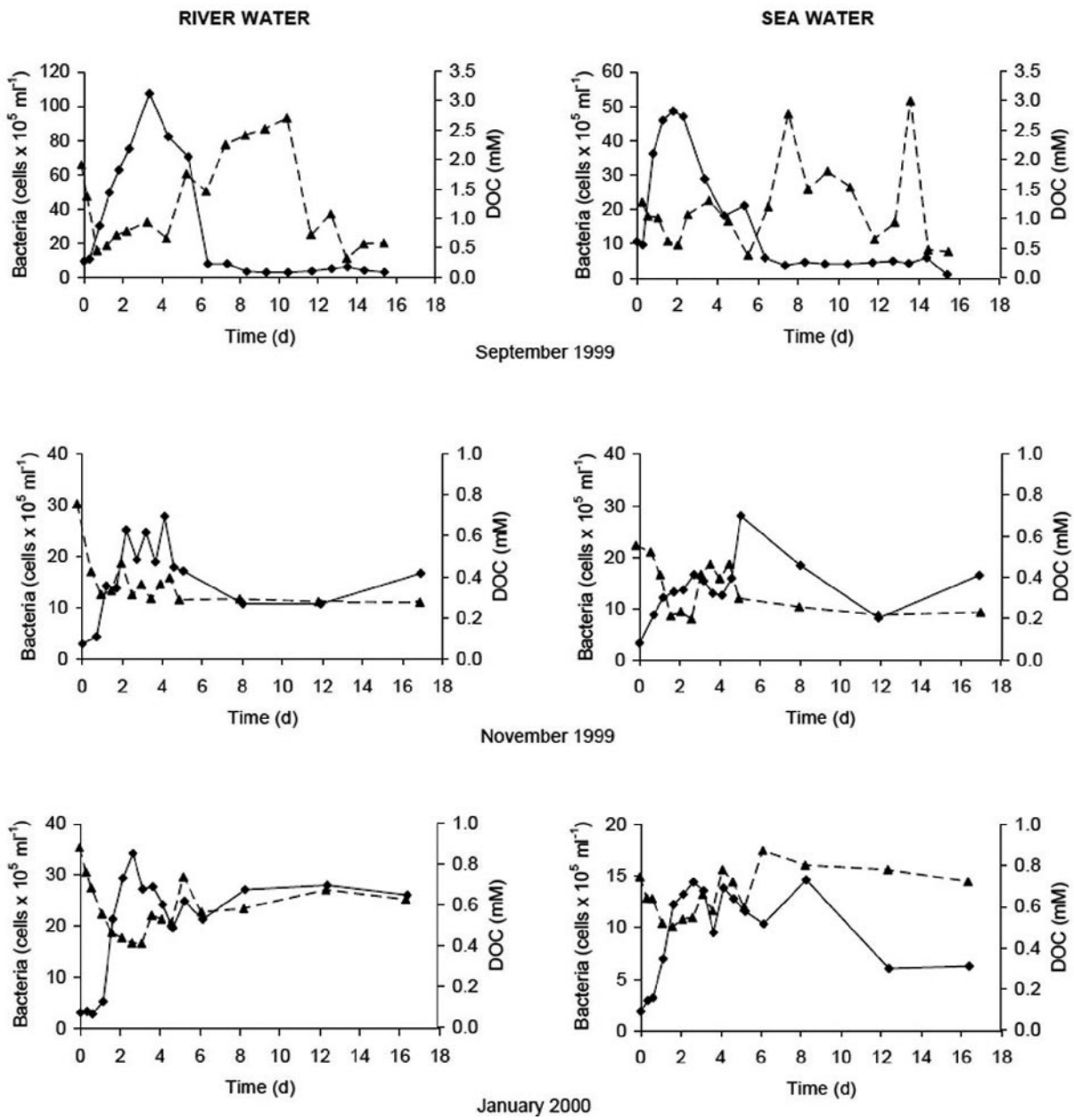

Figure 2. Mean values of dissolved organic carbon (DOC) concentrations (dashed line) and bacterial abundance (solid line) in Spercheios River (left panel) and Maliakos Gulf water (right panel) batch cultures.

Figure 2. Valeurs moyennes des concentrations en carbone organique dissous (DOC, pointillés) et des abondances bactériennes (trait plein) dans les cultures réalisées sur les eaux de la rivière de Spercheios (à gauche) et du golfe de Maliakos (à droite).

Bacterial specific growth rate (μ) (Fig. 4) showed an almost similar pattern with DOC degradation rate with higher values for the SWC (0.022 h^{-1}) than the RWC (0.012 h^{-1}) in September, while the opposite was observed in January (0.016 and 0.023 h^{-1} respectively). Lowest values for both types of culture were measured in November. Bacterial production (Fig. 5) was always higher in the RWC (149-623 μgC l^{-1} d^{-1}) than in the seawater ones (107-520 μgC l^{-1} d^{-1}). Highest values in both cases occurred in September and lowest in November. BGE was higher (18.3 and 10.4%) (Fig. 6) in the RWC than in the SWC in September and November but the opposite occurred in January (10.4 and 21.9% for the RWC and SWC respectively).

Concentrations of inorganic nutrient changes are shown in Fig. 7. Phosphate showed little variation in the RWC and SWC in September and January. However, both types of culture showed a considerable increase from the beginning

Table 2. The bacterial biovolumes at the beginning and the end of the exponential growth phase in Spercheios River and Maliakos Gulf water batch cultures.

Tableau 2. Biovolumes bactériens au début et à la fin de la phase exponentielle de croissance bactérienne dans les cultures réalisées sur l'eau de la rivière Spercheios et du golfe de Maliakos.

		Bacterial biovolume (μm^3)	
		Spercheios	Maliakos
Sep. 1999	start	0.062	0.061
	end	0.697	0.544
Nov. 1999	start	0.055 ± 0.003	0.051 ± 0.001
	end	0.687 ± 0.012	0.613 ± 0.008
Jan. 2000	start	0.103 ± 0.009	0.032 ± 0.001
	end	0.538 ± 0.010	0.691 ± 0.009

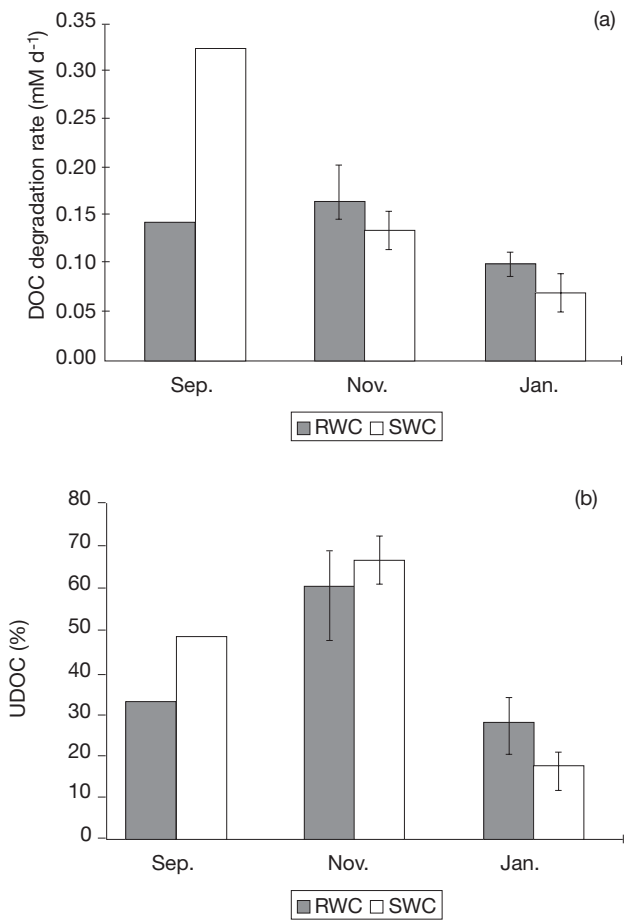

Figure 3. Dissolved organic carbon (DOC) degradation rate (a) and percentage of utilizable DOC (UDOC) in Spercheios River (RWC) and Maliakos Gulf (SWC) water batch cultures (b). Bars indicate standard error.

Figure 3. Taux de dégradation du carbone organique dissous (DOC) (a) et pourcentage du DOC labile (UDOC) (b) pour les cultures réalisées sur les eaux de la rivière de Spercheios et du golfe de Maliakos. Les barres représentent l'erreur standard.

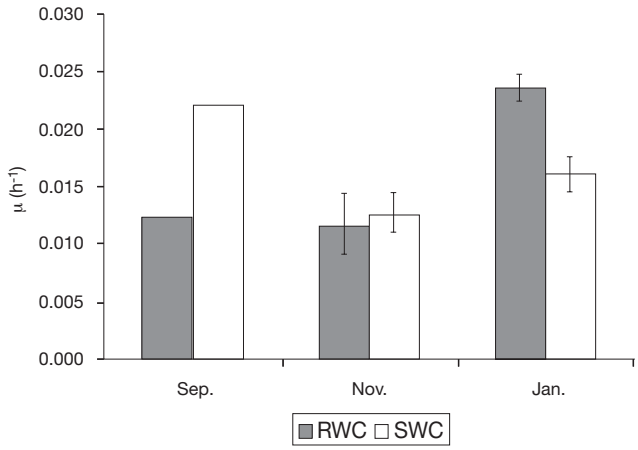

Figure 4. Bacterial specific growth rate (μ) in Spercheios River (RWC) and Maliakos Gulf (SWC) water batch cultures. Bars indicate standard error.

Figure 4. Taux de croissance bactérien (μ) dans les cultures réalisées sur les eaux de la rivière de Spercheios et du golfe de Maliakos. Les barres représentent l'erreur standard.

to the end of the experiment in November. Nitrate was always much lower in SWC than in RWC and stayed practically unchanged during the three incubation periods. In RWC, they stayed unchanged in September, decreased considerably during the first days in November and increased considerably during the first days in January. Ammonium showed similar patterns in both SWC and RWC. In September it slightly decreased during the first days of the incubation and then reached initial levels again. In November it stayed practically unchanged during the first five days of the incubation and then increased regularly until the end of the experiment; and in January, it showed a rather constant increase throughout the incubation period.

Temperature was positively correlated with DOC degradation rate ($r = 0.82$, $p < 0.01$, $N = 12$). No other correlations were found.

Discussion

Our experiments on the growth of marine bacterioplankton from Maliakos Gulf on unamended riverine and marine DOC, covered a period of increasing trophic state, i.e. from the summer with low chl *a* values to winter when a 5.5-fold increase in chl *a* is observed, which corresponds to the winter phytoplankton bloom of the gulf (Kormas et al., 1998). The river flow rate also increases from summer to winter/spring (Kormas, 1998). Hence, it was hypothesised, that DOC of different origin, (riverine vs. seawater) and therefore of different quality, in addition with the changes in the growth conditions (i.e. mostly in temperature and inorganic nutrients) and river supply, would result in

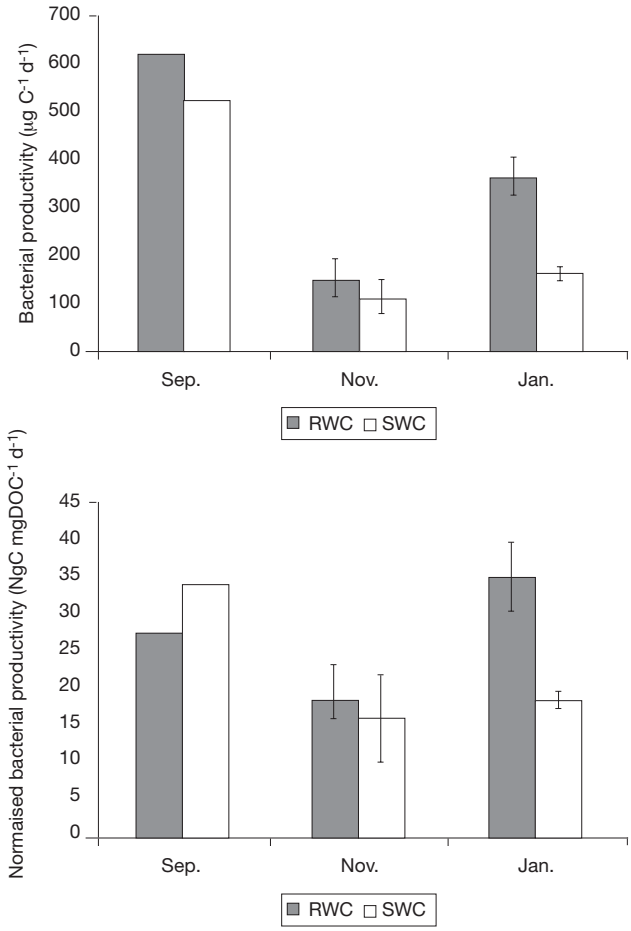

Figure 5. Bacterial production in Spercheios River (RWC) and Maliakos Gulf (RWC) water batch cultures. Bars indicate standard error.

Figure 5. Production bactérienne dans les cultures réalisées sur les eaux de la rivière de Spercheios (RWC) et du golfe de Maliakos (SWC). Les barres représentent l'erreur standard.

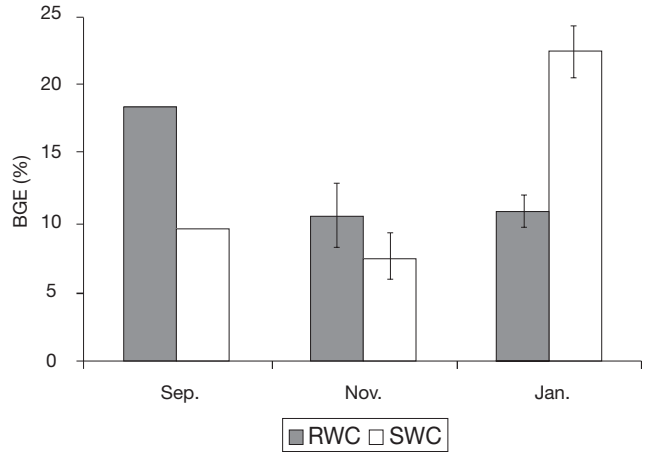

Figure 6. Bacterial growth efficiency (BGE) in Spercheios River (RWC) and Maliakos Gulf (SWC) water batch cultures. Bars indicate standard error.

Figure 6. Rendements de croissance (BGE) dans les cultures réalisées sur les eaux de la rivière de Spercheios (RWC) et du golfe de Maliakos (SWC). Les barres représentent l'erreur standard.

differences in the growth of marine bacteria and the degradation of allochthonous versus autochthonous DOC.

In our experiment, we did not add inorganic nutrients to the culture media. The reason for this was that previous studies (Kormas, 1998), showed that the initial concentrations of inorganic nutrients of the present study would not be limiting for bacterial growth in batch cultures. Also, it has been reported that nutrient enrichment seems to have no or marginal effect on DOC degradation rates in freshwater (Søndergaard et al., 2000a), estuarine (Wikner et al., 1999) and marine planktonic systems (Søndergaard et al., 2000b). Also, unamended natural water is considered the most appropriate growth medium to study natural bacterial populations in bacterial growth experiments, avoiding problems such as chelating processes, adsorption, etc. (Schut et al., 1997). However, in the present study inorganic nutrients concentrations were monitored periodically to check for a possible depletion. During all of the experimental incubations, most nutrient concentrations did not fall below initial concentrations (Fig. 7) and in most cases there was an increase of nutrient concentrations at the end of the incubations as a result of remineralization processes.

In the RWC, the bacterial abundances reached the higher values and in both cultures (RWC and SWC) during their exponential growth DOC concentrations decreased (Fig. 2) as a result of DOC degradation and utilisation for bacterial growth. After the end of the exponential growth phase, bacterial abundances and DOC dynamics fluctuated considerably. Our experimental design could not eliminate the presence of marine viruses. In each incubation after ca. five days, we could observe virus-like particles on the filters and usually the incubations were terminated at the point when no reliable bacterial counting could be done due to the increase of these virus-like particles. Because no aggregates were observed at the end of the incubation periods and the role of virus in regulating bacteria has already been recognized (Fuhrman, 1999), we believe that viral lysis of bacterial cells dominated the bacterial abundance and, consequently, DOC dynamics after the stationary phase of the growth.

Marine bacteria growing on riverine DOC showed comparable (November), higher (January) or lower (September) values of specific growth rate than those growing on marine DOC but BP was always higher in the RWC (Fig. 4 and 5). This shows that river water DOC can support growth of marine bacteria, as already found in other

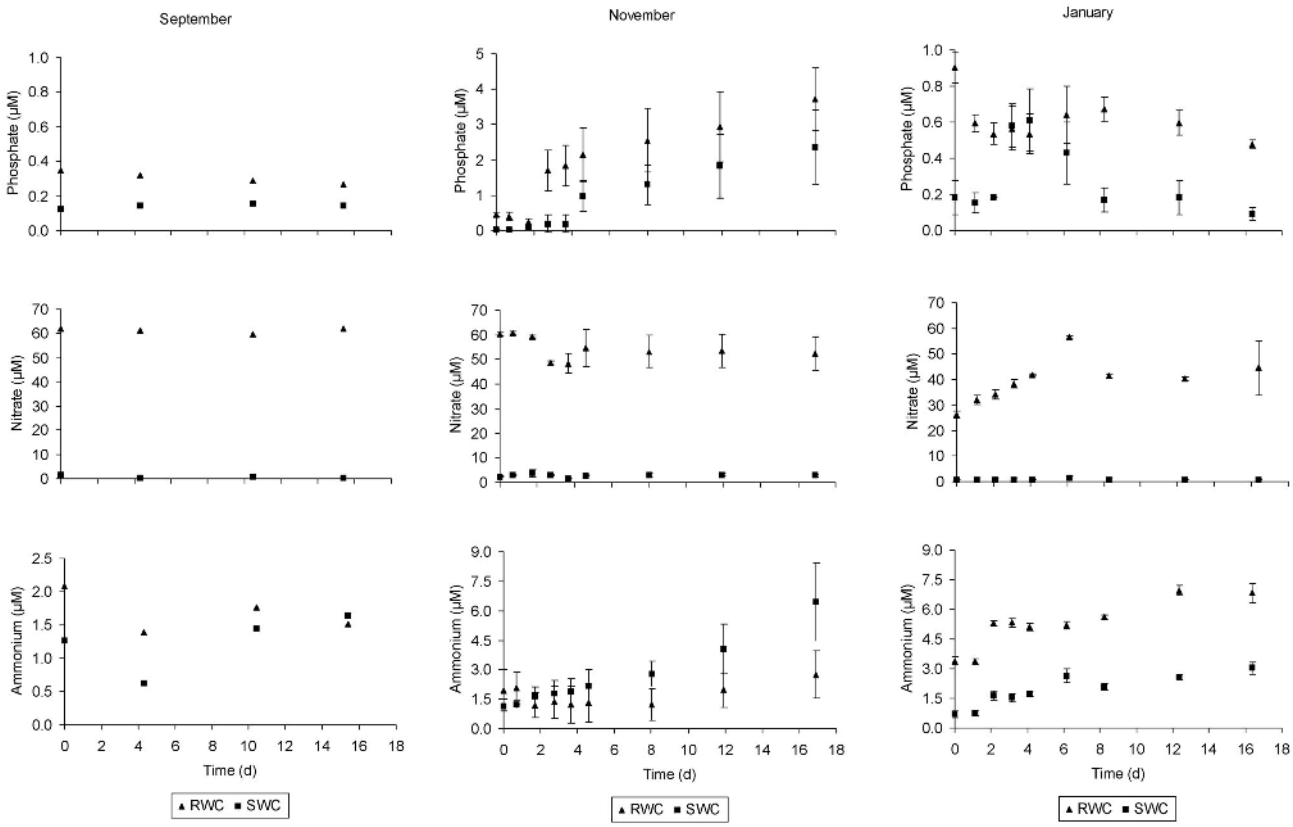

Figure 7. Inorganic nutrient concentrations during the incubation of Spercheios River (RWC) and Maliakos Gulf (SWC) water batch cultures. Bars indicate standard error.

Figure 7. Concentrations des nutriments inorganiques pendant l'incubation des cultures réalisées sur les eaux de la rivière de Spercheios (RWC) et du golfe de Maliakos (SWC). Les barres représentent l'erreur standard.

studies (Moran & Hodson, 1994; Wikner et al., 1999). Growth on riverine DOC also gave higher BGE than growth on marine DOC in September and November. Although when interpreting bacterial BGE values that were calculated with different methods might be biased, the average BGE obtained in the RWC (13%), was lower than the average of estuarine environments reported in the literature (34%, del Giorgio & Cole, 1998), but it matched well with the values of Wikner et al. (1999) (12%). Like Wikner et al. (1999), we also suggest that our lower average riverine BGE was partly due to the different experimental design, offering to the marine bacterial assemblages in the RWC only riverine DOC. Our average seawater BGE (12.8%) fell in the lowest range of marine coastal systems (del Giorgio & Cole, 1998).

In September, the UDOC fraction and DOC degradation rates in the river water were lower than in the seawater but the opposite trend was observed in January (Fig. 3), implying that marine bacteria can degrade riverine DOC at least as fast as marine DOC. Although differences in DOC composition between freshwater and marine environments suggest that the former is more recalcitrant (Williams, 2000), there is a good body of evidence that riverine DOC can be degraded efficiently in the marine environment (Hopkinson et al., 1998; Moran et al., 1999; Wikner et al., 1999). It is believed that marine bacteria are rapidly respiring a very small pool of highly labile, recently produced DOC (Fuhrman, 1987) resulting in higher degradation rates of high molecular weight DOC, that prevail allochthonous DOC, versus low molecular weight (Amon & Benner, 1996). The differences in UDOC, apart from differential utilisation by marine bacteria, are partly due to the differences in temporal composition of DOC in both Spercheios River and Maliakos Gulf. Unfortunately, up to date, there are no available data on the chemical characterisation of DOC in the area studied. In addition, the higher bacterial activity observed in January in the RWC could be the result of an increased availability of riverine DOC, due to the increase of the ionic strength of the medium after the addition of NaCl, through structural changes in molecules (e.g. Stumm & Morgan, 1996) or

through flocculation (Sholkowitz, 1976) -although neither phenomena was observed in our batch cultures- and subsequent enhanced bacterial degradation (Travnik & Sieburth, 1989). Another possible factor that could mask the true lability of riverine and marine DOC, is that different species selection is promoted at the beginning of the cultures, resulting in different bacterial communities with different DOC degrading capabilities (Cottrell & Kirchman, 2000), an issue that needs further investigation.

In our experiments, we would expect UDOC to be higher in the SWC in January, when the phytoplankton bloom has started and fresh algal DOC is present in the seawater, assuming that river DOC does not mask the former during the phytoplankton bloom, especially since the sampling point was quite far from the river mouth (Fig. 1). However, this was not observed and we believe that although higher degradation rates of marine DOC could have occurred because the low winter temperatures (Table 1) can result in limited substrate uptake (Nedwell, 1999). Kirchman & Rich (1997) suggested that bacteria respond more slowly at colder temperatures in DOC supplies. This inability of marine bacteria to utilise algal cell exudates during the winter phytoplankton bloom in Maliakos Gulf, could be the cause for the observed mismatch between bacteria and chl *a* reported by Kormas et al. (1998). The lack of correlation between temperature and any of the bacterial parameters measured, might imply that temperature is indirectly involved in bacterial-DOC relationships, having an impact mostly on the substrate affiliation and uptake rate by the bacterial cells (Nedwell, 1999).

Pomeroy & Wiebe (2001) have suggested that substrate-temperature interactions appear to become a dominant factor in growth and fitness only at the extremes of the ranges of concentrations and/or temperature ranges in any environment. Reduced bacterial production in early stages of phytoplankton blooms may involve not only some inhibition by low temperature as a requirement for a higher substrate concentration, sensu Nedwell (1999), but also a lag phase prior to the onset of bacterial division which may last from a day to a month (Pomeroy & Wiebe, 2001 and references therein).

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