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Ocean warming enhances respiration and carbon demand of coastal microbial plankton

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Abstract

The increase of anthropogenic CO_2 during this century is expected to cause warming of large regions of the ocean. Microbes lead the biological role in the CO_2 balance of marine ecosystems, their activity is known to be influenced by temperature, and it is important to constrain and quantify these effects on bacterial carbon use. Furthermore, if warming were to enhance the carbon demand (production + respiration) of planktonic microbes but would maintain their efficiency low (as it generally is), then most carbon consumed would end up respired. We designed a strategy in which we measured bacterial production and respiration throughout a seasonal cycle in a coastal Mediterranean site, and determined experimentally the effects of ca. 2.5 °C on these processes. We show that warming will increase nearly 20% the total carbon demand of coastal microbial plankton without any effect on their (commonly low) growth efficiency, which could generate a positive feedback between coastal warming and CO_2 production.

Keywords: bacterial production, carbon biogeochemistry, coastal ocean, growth efficiency, respiration, warming

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Introduction

Atmospheric CO_2 has increased by nearly 30 ppm between 1960 and 1990 due to the effect of industrialization and deforestation (Siegenthaler & Sarmiento, 1993), and this rate of increase is assumed to stay or even accelerate (Houghton *et al*., 2001). With the present concentration of CO_2 in the atmosphere and the expected increase, the concentration of CO_2 may double during this century. If this were the case, climate models within the most conservative scenario predict that the Earth surface temperature would increase on average by nearly 2.5 °C (Houghton *et al*., 2001). Under such warming, large regions of the ocean surface would be affected in the next 100 years by an increment in average temperature of approximately 2 °C (Timmermann *et al*., 1999), which would most probably drive changes in the function of marine ecosystems (McGowan *et al*., 1998; Goes *et al*., 2005). The magnitude of such warming depends on the increment in concentration of atmospheric CO_2 that is partially modulated by the capability of the oceans to drawdown CO_2 from the atmosphere, itself constrained by physical, chemical, and biological factors (Siegenthaler & Sarmiento, 1993). The biological carbon retention of the oceans relies on the balance between primary production and microbial respiration (del Giorgio & Duarte, 2002; Karl *et al*., 2003). Therefore, our capability to model changes in the drawdown of CO_2 from the atmosphere by the oceans depends on the understanding of the effect of warming on the carbon process of planktonic microorganisms.

Microbes are essential in the transfer and degradation of carbon in aquatic systems (Azam *et al*., 1983), and temperature is an extremely influencing factor on microbiological processes such as production (Rivkin *et al*., 1996), growth rate (White *et al*., 1991), and growth efficiency (Rivkin & Legendre, 2001). Some analysis predict with increasing temperatures a rise in bacterial respiration rates (R) without a concomitant increase in bacterial production (P) (Rivkin & Legendre, 2001), while others suggest that bacterial growth efficiency ($BGE = P/P + R$) is mainly determined by substrate quality and not much affected by temperature (del Giorgio & Cole, 1998). Small increments of temperature

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in surface oceanic waters have the potential to greatly modify the microbe's role in the global carbon cycle, but the direction and the magnitude of that modification are subject to controversy due to the lack of direct empirical determinations of such effects.

If warming affects positively the rates of bacterial production and respiration, but respiration rates increase more abruptly than production rates, bacterial growth efficiency would decline with increasing temperatures, and a higher proportion of the carbon demand ($CD = P + R$) would be respired (Rivkin & Legendre, 2001), implying that more of the organic carbon consumed would be remineralized to CO_2. Conversely, if warming affects more the production than the respiration rates, bacterial growth efficiency would increase and a higher proportion of the carbon consumed by bacteria would be repackaged into biomass; and thus, more biomass would be available to higher trophic levels through a more efficient microbial loop (Azam *et al.*, 1983). However, if temperature affects similarly bacterial production and respiration rates but BGE stays low, more carbon would be consumed and used by microbes but most of it would end up as CO_2. Although all these scenarios could produce a net increase of CO_2 in the system if primary production does not counteract bacterial respiration, the way in which this increase would be produced is different, as different are their effects. Thus, the correct interpretation of this regulation is relevant for ecologists and ecosystem modelers, who aim to understand and track changes in the biogeochemistry of oceanic systems (Laws *et al.*, 2000).

Coastal ecosystems represent nearly 7% of the global oceanic surface, and are recognized as important sites of planktonic metabolism (Wollast, 1998). They comprise between 14% and 30% of total oceanic primary production (Gattuso *et al.*, 1998), and account for about 30% of oceanic respiration (Smith & Hollibaugh, 1993). The relative shallowness of continental shelves make them particularly vulnerable to warming (Harley *et al.*, 2006). Therefore, the study of the influence of temperature on coastal bacterial carbon demand may be very relevant for our understanding of marine carbon biogeochemistry. Within this context, we conducted a series of experiments aimed at measuring the effects of warming on the rates of bacterial production (P) and dark respiration (R) in the Bay of Blanes. This is a well-characterized coastal oligotrophic system with dominance of heterotrophic processes during most part of the year (Duarte *et al.*, 2004). We studied there the effects of small increases of temperature, such as those predicted for the ocean to suffer in a century (ca. 2 °C) (Timmermann *et al.*, 1999), on the amount of carbon consumed by bacteria ($CD = P + R$) and on the fate of such processed carbon, being either respired or repackaged into bacterial biomass (measured as bacterial growth efficiency, $BGE = P/P + R$). In brief, we intended to quantify the impact of warming on the carbon demand of planktonic coastal systems.

Material and methods

Sampling and experimental set up

Experiments were established with water collected at the Blanes Bay Microbial Observatory (41°39′N, 2°48′E), a coastal Mediterranean oligotrophic site where metabolic balances have been well studied (Duarte *et al.*, 2004). Samples of about 50 L were collected from 1 mile offshore and prescreened with a 200 μm Nylon mesh to avoid the presence of mesozooplankton. *In situ* water temperature was measured with a calibrated mercury thermometer. Upon transportation to the laboratory (about 1 h), one half of the water was left still and the other was immediately filtered trough 0.8 μm (47 mm AAWP filter, Millipore, Billerica, Maine, USA) at low pressure (ca. 100 rpm) using a peristaltic pump (1.6 MM-WT head, Watson-Marlow, Falmouth, Cornwall, UK). Filtration of 25 L of water took between 4 and 5 h. The filter retained nearly 50% of the prokaryotes present in the 200 μm fraction and only <1% the oxygenic phototrophic prokaryotes or eukaryotes crossed the filter. Then, 8 L of each fraction (<200 and <0.8 μm) were disposed in four acid-clean polyethylene containers per fraction, 2 L per container. The remaining sample from these fractions was siphoned inside boro-silicate glass bottles to obtain respiration rates (see sections below). Two containers of each fraction were placed inside each of two isothermal walk-in chambers; one equilibrated at ambient seawater temperature, and the other at temperatures which were on average 2.7 °C above the *in situ* value. Temperatures inside the chambers were controlled several times during the 48 h of incubation with a precision digital thermometer (± 0.1 °C).

Production rates

Bacterial production rates were determined from each sample at time 0, and from the polyethylene containers at times 24 and 48 h, with the use of the ^{3}H-leucine incorporation method (Kirchman *et al.*, 1985; Smith & Azam, 1992). Leucine incorporation was converted to carbon production rate with a standard factor of 3.1 kg C mol leucine^{-1} (Simon & Azam, 1989). In each measurement we dispensed four 1.2 mL replicates plus two TCA-killed controls (5% final concentration). A final concentration of 40 nM of leucine with a 1 : 10

(hot : cold) mix was used; the incubation lasted 2 h, and was done in the temperature-regulated chambers. This concentration of leucine has been shown to be saturating in Mediterranean coastal waters. The incubation was stopped with 50% TCA (5% final concentration). We sometimes measured simultaneously the production of bacteria in the polyethylene containers and the boro-silicate glass bottles, and we did not find differences between the production rates of prokaryotes inside both types of containers (paired t-test, $n = 35$, $P > 0.1$). To compare the effect of warming on bacterial production rates we use all the measurements of production rates performed during the incubations.

Respiration rates

Respiration rates were obtained by linear regression of oxygen concentration vs. time in incubations of ca. 130 mL of sample maintained in boro-silicate glass bottles. The $<200\,\mu m$ dark respiration values correspond to the whole microbial food web, while the values of the $<0.8\,\mu m$ correspond to the fraction of prokaryotes passing through the $<0.8\,\mu m$ filter. Between 38 and 48 bottles were used per sample, and the bottles were siphoned twice with sample before they were closed with their stoppers. After that, four to eight bottles of each fraction were immediately fixed with Winkler reagents to estimate the initial oxygen concentration. The remaining bottles were submerged inside dark coolers that had been previously filled with tap water and prestabilized to the correct temperature during at least 12 h. The coolers provided temperature stability and darkness while they were placed in the two isothermal chambers. At 24 and 48 h we fixed eight to 10 bottles for each fraction and temperature. Dissolved oxygen was determined with an automatic titrator, based on potentiometric endpoint detection (Outdot *et al*., 1988). The average standard error between bottles was $0.53\,\mu mol\,O_2\,L^{-1}$. Oxygen consumption rates were transformed to carbon units assuming a respiratory quotient of 1. All oxygen decrease rates were significant with a $P < 0.05$, except in one occasion, in which $P = 0.07$, indicating that our assumption of linearity in the consumption of oxygen with time held in most of our determinations.

Carbon demand and growth efficiency

Since we were interested in the instantaneous CD and BGE, we used the initial bacterial production rates and extrapolated the oxygen consumption rate between 0, 24, and 48 h to time 0. The rate of bacterial carbon demand was estimated as $CD = P_0 + R$, and BGE as $BGE = 100 \times P_0/CD$, where P_0 was the bacterial production rate observed at time 0. Such estimation overestimates bacterial carbon demand and growth efficiency in the $<200\,\mu m$ fraction, because ca. 15% of the respiration in this fraction was not observed in the $0.8\,\mu m$ fraction and, thus, it should not be exclusively related to bacteria.

Finally, the percentage of change in initial bacterial production, dark respiration, carbon demand, and growth efficiency with increased temperatures was computed as 100 times $(X_b - X_a)/X_a$, where X_a and X_b were the variables measured at ambient temperature (X_a) or ca. 2° above ambient temperature (X_b). While the Q_{10} function was computed as $Q_{10} = (X_b/X_a)^{10/(t_b - t_a)}$, in which X_b and X_a are rates of metabolic activity at temperature t (in °C) and $t_b > t_a$ (Sherr & Sherr, 1996).

Results

Monthly between March 2003 and February 2004, we measured the variables related to carbon demand in waters from the Bay of Blanes under ambient, as well as increased temperatures. *In situ* water temperature in the Bay varied between 12.5 °C in February 2004 and 25 °C in July 2003 and, while this does not cover the whole range of water temperatures in the world's oceans, it does offer a relatively large temperature shift. Our experiments were performed in the laboratory inside isothermal chambers regulated at an average (± SE) temperature that varied between 12.8 ± 0.3 and 25 ± 0.1 °C in the chamber equilibrated at the ambient temperature, and between 15.5 ± 0.6 and 27.3 ± 0.2 °C in the warmed chamber. There were not significant differences between the temperatures measured *in situ* and those inside the chamber at ambient temperature (paired t-test, $n = 12$, $P > 0.1$), while there were with those inside the warmed chamber (paired t-test, $n = 43$, $P < 0.01$). The average difference of temperature between the ambient and warmed chambers was 2.7 ± 0.3 °C, a difference nearly two times higher than the 1.3 °C that has been observed in the Bay during day–night cycles performed in summer 2004.

The initial concentration of dissolved oxygen in the $<200\,\mu m$ fraction varied between a minimum of $197.2\,\mu M$ O_2 in summer and a maximum of $263.3\,\mu M$ O_2 in winter, suggesting that dissolved oxygen in the Bay was mainly related to temperature. If we exclude a single exceptionally high bacterial production during July (2.61 ± 0.20 and $5.88 \pm 0.37\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 0.8 and 200 μm fractions, respectively); the average initial bacterial production at ambient temperature varied between 0.07 ± 0.02 in the $0.8\,\mu m$ fraction and $0.22 \pm 0.06\,\mu g\,C\,L^{-1}\,h^{-1}$ in the $200\,\mu m$ fraction. Thus, the percentage of initial bacterial production in the smaller fraction was near 30% of that found in the

larger fraction. Dark respiration rates at the same temperature averaged $1.37 \pm 0.27\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 0.8 μm fraction and $1.65 \pm 0.27\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 200 μm fraction; then, respiration rates varied seasonally much less than the production rates. Average bacterial respiration (i.e. <0.8 μm) was 89 ± 13% of the total microbial community respiration (i.e. <200 μm). While total carbon demand by bacteria averaged $2.34 \pm 0.54\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 200 μm fraction and $1.54 \pm 0.36\,\mu g\,C\,L^{-1}\,h^{-1}$ the 0.8 μm fraction, and bacterial growth efficiencies averaged 18.2 ± 6.4% in the 200 μm fraction and 11.9 ± 5.6% in the 0.8 μm fraction; both variables attaining maximum values in spring–summer and with exceptional high values coincident with elevated production rates measured on July. After 24 h of incubation at ambient temperature, the bacterial production rates averaged $2.75 \pm 0.76\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 0.8 μm fraction and $2.42 \pm 0.76\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 200 μm fraction, while at 48 h averaged $3.11 \pm 1.05\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 0.8 μm fraction and $2.29 \pm 0.67\,\mu g\,C\,L^{-1}\,h^{-1}$ in the 200 μm fraction. Thus, bacterial production rates at 24 or 48 h were near 10-fold those measured at time 0. Conversely, the respiration rates did not change significantly whether they were estimated between 0 and 24 h or between 0, 24, and 48 h (Student *t*-test, $n = 46$, $P > 0.1$).

If we consider the initial production rates and the respiration rates between 0, 24, and 48 h, a positive effect of the small temperature increments on bacterial production rates was observed in our experiments (Fig. 1a, Table 1). The same positive effect was observed for

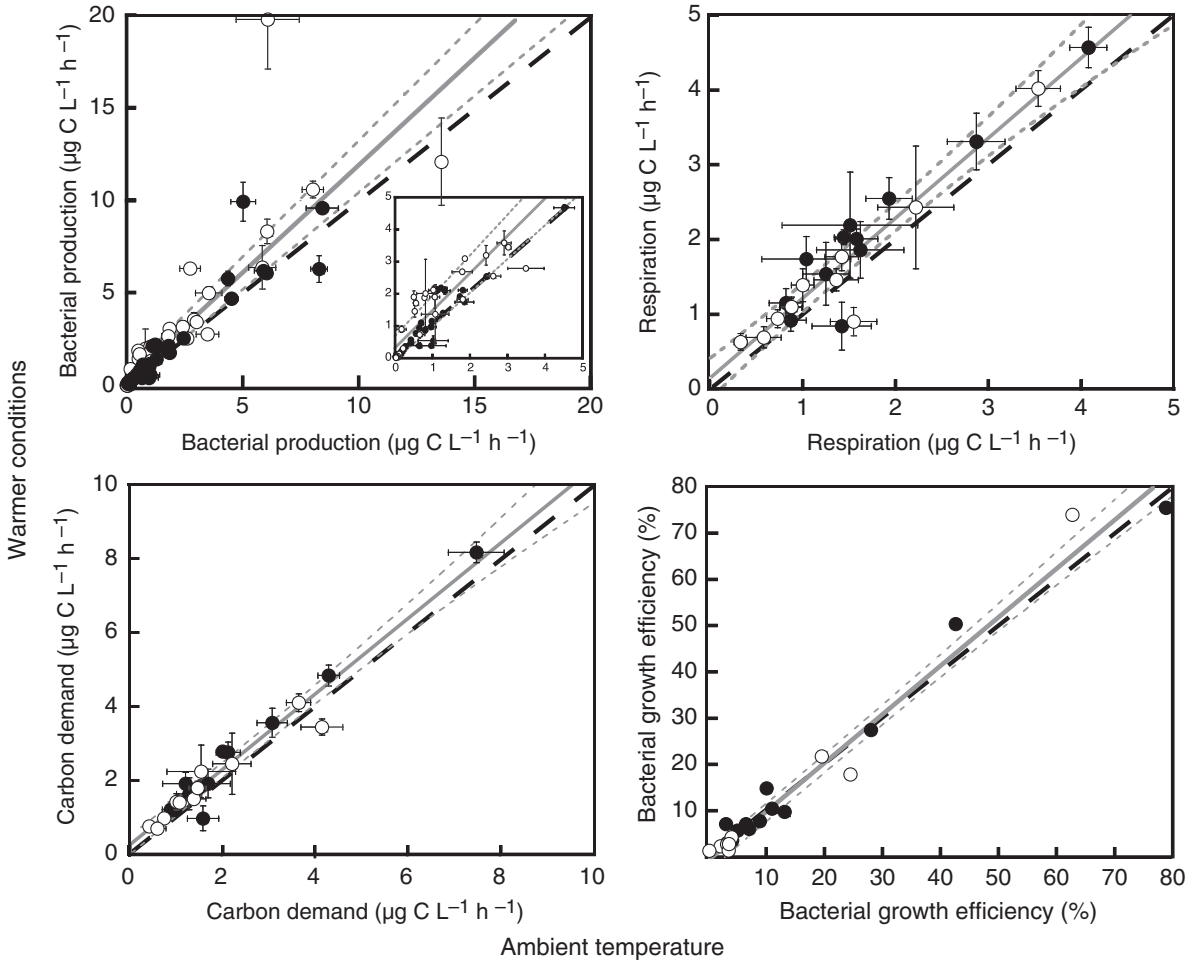

Fig. 1 (a) Bacterial production, (b) microbial dark respiration, (c) bacterial carbon demand (CD), and (d) bacterial growth efficiency (BGE) measured in the two fractions, <0.8 μm (open symbols) or <200 μm (bold symbols), and temperatures: ambient temperature or ca. 2° above ambient temperature ('warmer conditions'). Error bars correspond to one standard error (SE). SE for CD were estimated as $SE_{P_0} + SE_R$, and for BGE as (Toolan, 2001) $(P_0 + R)^{-2} \times (R^2 SE_{P_0}{}^2 + P^2 SE_R^2)^{1/2}$. In the latter case, the error bars are not visible because they are smaller than the symbols. The continuous line corresponds to the regression fit (Table 1), the dotted line indicates the confidence limits of this fit, and the slashed line indicates the 1 : 1 correspondence. The inset in Fig. 1a magnifies bacterial productions rates between 0 and $5\,\mu g\,C\,L^{-1}\,h^{-1}$.

Table 1 Linear regressions ($X_b = c + dX_a$, Model I) describing the relationship between variables measured at ambient temperature (X_a) and at ca. 2° above ambient temperature (X_b)

	C	*D*	*n*	r^2	Paired *t*
P	0.32 (0.26)	1.16 (0.08)	72	0.73	<0.01
R	0.15 (0.14)	1.07 (0.08)	23	0.90	<0.01
CD	0.28 (0.13)	1.02 (0.05)	23	0.95	<0.01
BGE	−0.29 (0.96)	1.05 (0.04)	23	0.97	>0.10

The *c*'s and *d*'s coefficients corresponds to the intercept and the slopes of the regressions, respectively. All data available were included in the analysis, independently of the fraction studied. Variables tested are bacterial production (*P*, $\mu g C L^{-1} h^{-1}$), microbial dark respiration (*R*, $\mu g C L^{-1} h^{-1}$), bacterial carbon demand (CD, $\mu g C L^{-1} h^{-1}$), and bacterial growth efficiency (BGE, %). All regressions are significant ($P < 0.001$) and, with the exception of BGE, the means of sample differences between pairs of readings are significantly different from 0 (paired *t*-tests, $P < 0.01$), thus, indicating a positive effect of temperature. Values between parentheses correspond to one standard error of the estimate. For this analysis, all measurements of bacterial production (including those at t_0, t_{24}, and t_{48}) are considered.

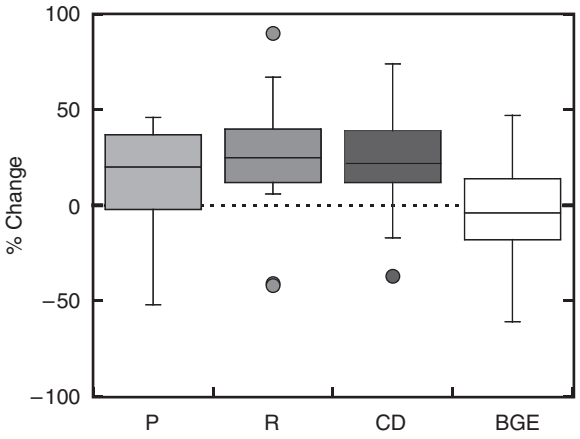

Fig. 2 Percentage of change in initial bacterial production (*P*), microbial dark respiration (*R*), bacterial carbon demand (CD), and bacterial growth efficiency (BGE) between samples incubated at warmer conditions with respect to the value observed at ambient temperature. All data were pooled, and while two outliers are not shown in the graph, they were considered in the computation of the distributions.

rates of dark respiration and bacterial carbon demand (Figs 1b and c, and Table 1). However, we did not observe any enhancement of bacterial growth efficiency which, in most cases, remained below 30% (Fig. 1d, Table 1). Our results show (Fig. 2) that the increase in temperature of ca. 2.5 °C produces an average increase of $29.9 \pm 11.2\%$ in bacterial production rates, $22.8 \pm 5.8\%$ in dark respiration rates, and $23.2 \pm 4.8\%$ in the total amount of carbon demanded by the planktonic communities of Blanes Bay; all these increases were significantly different from 0 (Wilcoxon's signed-rank tests, $P < 0.01$). Whereas, the average increase of $16.1 \pm 17.1\%$ in BGE was not different from 0 (Wilcoxon's signed-rank test, $n = 23$, $P > 0.10$) but was significantly different from that observed in *P*, *R* and CD (Wilcoxon/Kruskall–Wallis test, $P < 0.01$).

Discussion

General considerations

We estimated bacterial carbon demand and growth efficiency using the initial bacterial production, and the respiration rates measured between 0, 24, and 48 h. The main drawback of this approach is that production and respiration rates are determined over different temporal scales; bacterial production on the order of hours, and respiration rates on the order of days (i.e. Briand *et al*., 2004; Alonso-Sáez *et al*., 2007). The rationale behind this approach, which is in fact the one dominant in the literature, is to use initial bacterial activity rates which are as close as possible to the *in situ* conditions, because long incubation periods commonly imply modifications in activity and diversity (e.g. Massana *et al*., 2001). Oxygen disappearance was also assumed to be linear and respiration constant and, in fact, we did not observe significant differences between respiration rates computed between 0 and 24 h and those computed between 0 and 48 h. Finally, the changes in production and respiration rates related to the increase in temperature can be due either to a bacterial community adapting its physiology, or to a bacterial community that shifted its taxonomic composition, although the latter change is very improbable into the initial samples.

Ecological implications

Our results are in agreement with observations for Blanes Bay and NW-Mediterranean waters. The bacterial production rates were on the higher end of those reported for inshore NW Mediterranean waters (Pedrós-Alió *et al*., 1999), and the community respiration values were slightly smaller than those previously measured in the Bay (Duarte *et al*., 2004), but well within the range of interannual variation that had been reported (Satta *et al*., 1996). Bacterial respiration was on average 89% of the total microbial community respiration, which is not significantly different from the $67 \pm 9\%$ previously observed in the open NW-Mediterranean (Lemée *et al*., 2002). Besides, most of the

variability in carbon demand and bacterial growth efficiency was driven by respiration, with production explaining little of the variation, as has often been reported (del Giorgio & Cole, 1998). Finally, if we consider the bacterial growth efficiency of the $<0.8\,\mu m$ fraction as that of the bacterial community, the observed values are in the lower range of the values reported in the literature, but well within those of natural oligotrophic planktonic systems (del Giorgio & Cole, 1997).

The positive effect of the small temperature increments on bacterial production rates observed here is concordant with previous studies (Rivkin *et al.*, 1996; Pomeroy & Wiebe, 2001) and points toward a constant magnitude of change regardless of the ambient temperature, a pattern already shown in the Chesapeake Bay (Shiah & Ducklow, 1994). Conversely, the lack of change in bacterial growth efficiency related to temperature found in this coastal study is in contrast to what was found in a recent compilation of mostly open-sea measurements (Rivkin & Legendre, 2001), but in accordance with the notion that bacterial growth efficiency is modulated by both, temperature and the rate of supply of the limiting nutrients (Shiah & Ducklow, 1994; del Giorgio & Cole, 1998; Apple *et al.*, 2006). Therefore, our study underlines that the relation between temperature and bacterial growth efficiency found at a global scale (Rivkin & Legendre, 2001) should not be used to forecast the effect of warming on the rates of bacterial production, dark respiration, bacterial carbon demand and bacterial growth efficiency of marine systems. From our point of view, the relationship between metabolic rates and small increases of temperature should be better approximated by using a Q_{10} function (Sherr & Sherr, 1996). Different studies in aquatic systems have found a value close to 2 for the Q_{10} for the metabolic rates of microorganisms such as prokaryotes (White *et al.*, 1991), protists (Choi & Peters, 1992), phytoplankton (Eppley, 1972) and zooplankton (Ikeda *et al.*, 2001). The model of Rivkin and Legendre (Rivkin & Legendre, 2001) predicts a Q_{10} of 1.85 for bacterial respiration (derived from their equation; $R = ((BP/(0.374-0.0104\,T))-BP)$, and our results experimentally determine a median Q_{10} of 1.9 for dark respiration rates, 2.5 for bacterial production, 2.0 for bacterial carbon demand and 0.8 for BGE. If we consider the model of the microbial food web depicted by Strayer (1988), with all phytoplankton production flowing through bacteria, protists, and zooplankton, 100 arbitrary units of carbon produced by primary producers would be consumed at ambient temperature by three steps of secondary producers (bacteria + protists + zooplankton) with a 20% efficiency to maintain a secondary production of 24.8 (20 + 4 + 0.8) units of carbon and a dark respiration of 99.2 (80 + 16 + 3.2) units of carbon. Whereas, assuming a warming of 2.5 °C and a Q_{10} of 2, 120 arbitrary units of carbon would be needed to compensate the increased total consumption: 29.7 (24 + 4.8 + 0.9) units of carbon would go to secondary production and 119 (96 + 19.2 + 3.8) units of carbon would go to respiration, thus ca. 20 more units of CO_2 would be produced. We have to point out, however, that this warmer scenario depends on the supply of the limiting nutrient(s). The predicted increase in production and respiration rates should only happen if there is an increase in the availability of dissolved organic carbon and a concomitant lack of limitation by other nutrients (N, P). In this sense, a recent study in the Southern Ocean has demonstrated an increase in the release of dissolved organic matter by phytoplankton when submitted to warmer conditions (Morán *et al.*, 2006), and the availability of inorganic nutrients could increase if changes in river outflow occur (Béthoux *et al.*, 1998). We suggest, therefore, a positive feedback between anthropogenic CO_2 raise and CO_2 production by planktonic organisms through global warming. A positive feedback that should be modulated by the effect of temperature on the primary production of the ecosystem (López-Urrutia *et al.*, 2006; Rochelle-Newall *et al.*, 2007).

If these results were of general applicability, they would have deep implications in the carbon cycling of the coastal ocean. First of all, CO_2 production would increase under global warming due to the rise in dark respiration rates, an increment that should not be necessarily linked to a decrease in the efficiency of carbon transfer. Second, as bacterial carbon demand seems to be more affected by temperature than primary production (Hoppe *et al.*, 2002), the increase in microbial respiration related to warming would shift the balance between primary production and dark respiration, thus increasing the trend of coastal systems toward being net sources of CO_2 (Smith & Hollibaugh, 1993), or decreasing their capability to become sinks of CO_2 (Borges, 2005). And third, the low BGE and the increase in carbon demand would promote the consumption of dissolved organic carbon and, consequently, less export through advection to nearby open ocean waters (Tsunogai *et al.*, 1999; Arístegui *et al.*, 2003).

As it has been suggested in previous studies (Pomeroy & Wiebe, 2001), the effect of temperature on marine systems is far from being simple. A direct physical consequence of the temperature rise of marine waters is the loss of CO_2 related with the lower solubility of gases, being of the order of a 6.25% decrease with a 2.5 °C increase (Rivkin & Legendre, 2001). With the same temperature increment, we have experimentally

determined a physiological response of 20% increase in production and respiration rates, without any collateral effect on bacterial growth efficiency. Biology may, therefore, respond to ocean warming at high rates, and this fact should be considered in carbon biogeochemistry models of future warmer scenarios.

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