Deep-Sea Research I 95 (2015) 131–138

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Temperature control of microbial respiration and growth efficiency in the mesopelagic zone of the South Atlantic and Indian Oceans

Ignacio P. Mazuecos [a,c,*], Javier Arístegui [b], Evaristo Vázquez-Domínguez [c,d], Eva Ortega-Retuerta [c], Josep M. Gasol [c], Isabel Reche [a]

[a] Departamento de Ecología, Instituto del Agua, Universidad de Granada, Granada, Spain
[b] Instituto de Oceanografía y Cambio Global (IOCAG), Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
[c] Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, Barcelona, Catalunya, Spain
[d] Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Gijón, Gijón, Spain

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
28 October 2014
Accepted 30 October 2014
Available online 13 November 2014

Keywords:
Mesopelagic zone
Microbial respiration
Prokaryotic growth efficiency
Temperature
Indian Ocean
South Atlantic Ocean

ABSTRACT

We have measured both prokaryotic heterotrophic production (PHP) and respiration (*R*), then providing direct estimates of prokaryotic growth efficiencies (PGE), in the upper mesopelagic zone (300–600 m) of the South Atlantic and Indian Oceans. Our results show that in situ *R* ranged 3-fold, from 87 to 238 μmol C m^{-3} d^{-1}. In situ PHP rates were much lower but also more variable than *R* (ranging from 0.3 to 9.1 μmol C m^{-3} d^{-1}). The derived in situ PGE values were on average ~1.4% (from 0.3% to 3.7%), indicating that most of the organic substrates incorporated by prokaryotes were respired instead of being used for growth. Together with the few previous studies on PGE published before for the Atlantic Ocean and Mediterranean Sea, our findings support the hypothesis that the global mesopelagic zone represents a key remineralization site for export production in the open ocean. We also found a strong correlation between *R* and PGE with temperature across a gradient ranging from 8.7 to 14.9 °C. The derived Q_{10} value of 3.7 suggests that temperature variability in the mesopelagic zone plays a significant role in the remineralization of organic matter.

1. Introduction

Prokaryotes thriving in the mesopelagic zone (200–1000 m depth) are known to play a key role in the mineralization of organic matter transported to the deep ocean (Arístegui et al., 2009; Anderson and Tang, 2010; Giering et al., 2014). Nevertheless, there is still a great lack of information on the regional variability in the percentage of organic matter that is either mineralized as CO_2 (respiration, *R*) or used by prokaryotes for growth (prokaryotic heterotrophic production; PHP) in the global ocean mesopelagic zone. This metabolic balance is frequently expressed as "prokaryotic growth efficiency" (PGE=PHP/(PHP+*R*)), a term that provides a proxy of the efficiency in the recycling of organic matter by prokaryotes. Direct estimations of *R* in the dark ocean, by monitoring oxygen changes inside bottles incubated at in situ temperatures, are cumbersome, due to the low microbial rates and the much lower sensitivity of the *R* methodology, compared with the tracer technique used for PHP. Thus, few studies have provided simultaneous direct estimates of both dark ocean *R* and PHP, being most of them carried out in waters from the North Atlantic Ocean (Biddanda and Benner, 1997; Arístegui et al., 2005b; Reinthaler et al., 2006; Baltar et al., 2010).

Despite the lack of data of actual *R* across the world oceans, it has been estimated that mesopelagic microorganisms mineralize up to 90% of the organic matter exported from the photic zone (Robinson et al., 2010), yielding high *R* at these depths (del Giorgio and Duarte, 2002). This hypothesis is supported by indirect estimates (mostly derived from enzymatic respiratory activities) of global mesopelagic *R*, ranging between 0.6 and 1.4 pmol C y^{-1} (Arístegui et al., 2003; Arístegui et al., 2005a), that would contribute 9–12% of the global ocean respiration (del Giorgio and Williams, 2005). Arístegui et al. (2009) compiled a large data set on metabolic activities from the dark ocean and found that the decrease in PHP with depth was higher than the decrease in *R* inferred from ETS activity, leading to a decreasing trend in PGE as the −0.3 power of depth. Assuming a mean PGE in the epipelagic zone of 15% (del Giorgio and Cole, 2000), the predicted PGE in the deep ocean would thus be ~4%. Similarly low PGE (on average ~2%) derived from

Abbreviations: MTE, metabolic theory of ecology; PA, prokaryotic abundance; PB, prokaryotic biomass; PGE, prokaryotic growth efficiency; PHP, prokaryotic heterotrophic production; *R*, respiration rate

* Corresponding author at: Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain. Tel.: +34 958 241000x20018.
E-mail address: igperez@ugr.es (I.P. Mazuecos).

http://dx.doi.org/10.1016/j.dsr.2014.10.014
0967-0637/

direct *R* estimates were obtained in the meso- and bathypelagic waters of the central North Atlantic (Reinthaler et al., 2006), although higher PGE values have been reported also from the North Atlantic dark ocean (e.g. Arístegui et al., 2005b; Baltar et al., 2010). Nevertheless, in spite of the importance of measuring simultaneously *R* and PHP for addressing the balance between source and sinks of carbon in the water column, there are still very few PGE estimates using direct *R* measurements to corroborate the above prediction across the world oceans.

The metabolism of all organisms is affected by many environmental factors, being temperature a key parameter (Brown et al., 2004). In this sense, marine heterotrophic prokaryotes are not an exception (i. e. Pomeroy and Wiebe, 2001; Vázquez-Domínguez et al., 2007; Sarmento et al., 2010) and temperature changes have been reported to have direct implications for the microbial degradation of organic matter in the surface ocean by modifying PGE (Rivkin and Legendre, 2001; López-Urrutia and Morán, 2007; Vázquez-Domínguez et al., 2007; Wohlers et al., 2009; Kritzberg et al., 2010a, 2010b). As it occurs in the surface ocean, temperature variability in the upper mesopelagic zone would affect the activity of marine microorganisms. Depending on the way in which PGE is modified by temperature in the mesopelagic zone the microbial food web could shift the flow of organic carbon by circulating more C through higher trophic levels or by remineralizing the C as CO_2. Understanding the effects of temperature on PGE and *R* in the mesopelagic zone would thus help to predict future changes on carbon biogeochemistry in a warmer dark ocean, a region poorly investigated until now (Nagata et al., 2001; Arístegui et al., 2009).

In this study, we provide direct estimates of both *R* and PHP, and derived PGE values, from the upper mesopelagic zone of two largely unexplored ocean basins: the South Atlantic and the Indian Oceans. We hypothesized that, as reported from the North-Atlantic Ocean (Arístegui et al., 2005b; Reinthaler et al., 2006; Baltar et al., 2010; Giering et al., 2014), the prokaryotic assemblages in the upper mesopelagic of these two oceans represent active nodes of remineralization of organic matter. We also tested the temperature dependence of *R* and PGE in our in situ data derived from temperature-controlled experiments. Finally, we compiled the existing literature from direct estimates of *R* and PGE to explore the temperature sensitivity of the dark ocean carbon metabolism.

2. Material and methods

2.1. Study site and sampling

The study was conducted on board the "R/V Hesperides" across the South Atlantic and Indian Oceans (Fig. 1 and Table 1), during part of the Malaspina 2010 Expedition (www.expedicionmalaspina.es) from January to March 2011. A total of 12 stations were sampled crossing the two ocean basins (Fig. 1). Water samples for metabolism experiments, as well as for in situ measurements, were collected at

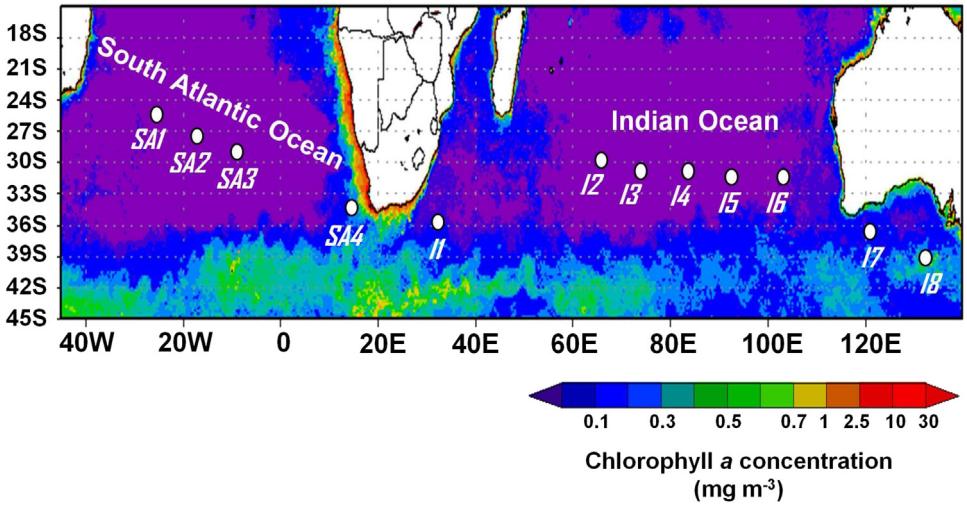

Fig. 1. Location of the stations selected for the respiration experiments (white dots), superimposed to a Moderate Resolution Imaging Spectroradiometer (MODIS) image of chlorophyll averaged for the sampling period (from January to March 2011).
Source: http://modis.gsfc.nasa.gov/.

Table 1
Stations' location in the South Atlantic (SA) and Indian (I) Oceans and water mass properties at the sampling depths where time-series experiments were performed. AOU = apparent oxygen utilization.

Stations	Date (d/m/y)	Latitude	Longitude	Depth (m)	Temperature (ºC)	Salinity (psu)	O_2 concentration (μmol kg^{-1})	AOU (μmol kg^{-1})
SA1	22/01/2011	25.9ºS	27.6ºW	550	9.8	34.78	185.62	94.15
SA2	24/01/2011	27.0ºS	21.4ºW	450	11.8	35.02	195.81	70.29
SA3	27/01/2011	28.7ºS	11.8ºW	585	8.7	34.66	178.83	108.43
SA4	04/02/2011	32.8ºS	12.8ºE	330	11.4	34.95	201.98	64.97
I1	15/02/2011	34.4ºS	31.1ºE	430	14.9	35.42	189.63	58.16
I2	25/02/2011	28.0ºS	63.3ºE	450	12.5	35.15	213.54	46.18
I3	28/02/2011	29.6ºS	72.4ºE	450	11.7	35.03	214.26	50.65
I4	03/03/2011	29.8ºS	82.6ºE	450	10.9	34.91	222.47	47.23
I5	06/03/2011	29.6ºS	93.0ºE	450	10.5	34.83	225.67	46.61
I6	09/03/2011	30.3ºS	103.3ºE	450	9.5	34.71	220.63	57.63
I7	19/03/2011	36.6ºS	120.9ºE	470	9.5	34.68	227.93	50.85
I8	24/03/2011	39.2ºS	135.1ºE	400	9.6	34.71	226.81	52.23

the same depths in the upper mesopelagic zone (from 330 to 585 m, Table 1) using a rosette sampler (provided with 24 12 L-Niskin bottles), implemented with a Conductivity–Temperature–Depth (CTD) instrument (Seabird SBE 9). Before setting up the experiments, water from the Niskin bottle was drawn into a carboy (without any pre-filtration), gently mixed, and left for 2 h inside a temperature-controlled chamber to reach in situ temperature. Following, the water was homogeneously distributed into 28 BOD bottles; 7 for each time sampling interval (0, 24, 48 and 72 h) of the experiment. From these, six bottles were used as replicates for oxygen consumption measurement and one bottle for prokaryotic heterotrophic production.

2.2. Prokaryotic abundance

In situ prokaryotic abundances (PA) were measured by flow cytometry (Gasol and del Giorgio, 2000), using a FACScalibur Becton Dickinson cytometer equipped with a laser emitting at 488 nm. Samples were obtained from the same Niskin bottles where water was collected for experiments. A 1.5 ml aliquot was fixed with 1% of paraformaldehyde + 0.05% glutaraldehyde (final concentrations), deep-frozen in liquid nitrogen and stored at −80 °C until analysis (less than 1 week after collection). Prokaryotic biomass (PB; mg C m^{-3}) was calculated by transforming relative light side scatter (SSC) to cell diameter, using the linear regression model of Calvo-Díaz and Morán (2006) after staining the samples with SybrGreen I. A spherical shape was assumed to derive cellular volumes, which were later converted to biomass with the allometric equation of Gundersen et al. (2002).

2.3. Prokaryotic heterotrophic production

Samples for prokaryotic heterotrophic production (PHP) were obtained both directly from the Niskin bottles (in situ PHP) and from the BOD bottles used in the time-series experiments at different incubation times (0, 24, 48 and 72 h). PHP was measured by ^{3}H-leucine (specific activity = 144.2 Ci $mmol^{-1}$) incorporation into proteins (Kirchman et al., 1985), according to the microcentrifugation protocol proposed by Smith and Azam (1992). Four replicates (1.2 ml) and two trichloroacetic acid (TCA)-killed blanks in microcentrifuge tubes were added L-[4, 5-^{3}H] leucine at 20 nM. Samples and blanks for experiments and Niskin bottles were incubated (for 2–8 h) at in situ temperature. Incubations were stopped by adding 50% TCA, centrifuged (10 min and 14,000 r.p.m.) and again rinsed with 5% TCA and centrifuged. Scintillation cocktail (1 ml Optisafe HiSafe, PerkinElmer) was added and, after 24 h, the emitted radioactivity was counted on board in a liquid scintillation counter (Wallac-PerkinElmer). Leucine incorporation rates (pmol Leu l^{-1} h^{-1}) were converted into carbon by using a theoretical factor of 1.55 kg C mol Leu^{-1} (Simon and Azam, 1989), assuming that isotope dilution was negligible under this saturating concentration of 20 nM of ^{3}H-leucine. PHP rates (average coefficient of variation ~4%) were integrated during the experiments as the area obtained from time 0 to the final time (Fig. 2), and expressed as daily rates (μmol C m^{-3} d^{-1}). Prokaryotic biomass turnover times (days) were estimated as PB/PHP for in situ samples.

2.4. Respiration

Water samples for the respiration experiments were homogeneously siphoned into 24 biological oxygen demand (BOD) bottles (nominal volume 125 ml), that were kept in the dark inside temperature-controlled water baths (±0.1 °C). Six BOD bottles were fixed immediately with Winkler reagents, and left immersed together with the rest of bottles inside the water baths during the dark incubation steps. Other six replicate bottles were fixed at 24, 48 and 72 h. Community respiration was measured at each time series interval (0–24, 24–48 and 48–72 h) from changes in O_2 concentration in the bottles. Finally, like with PHP, integrated respiration rates (R) were estimated as the area obtained from t_0 to final time and expressed as daily rates (μmol C m^{-3} d^{-1}). Dissolved oxygen measurements were carried out by automated microWinkler titrations using a Dissolved Oxygen Analyser (DOA; SiS®) with photometric end-point detection (Williams and Jenkinson, 1982). The coefficient of variation of oxygen concentration among replicated bottles was always < 1%. A respiratory quotient of 1 was used to convert oxygen consumption into carbon respiration (del Giorgio et al., 2006). To minimize the potential effect of growing prokaryotes inside the BOD bottles along the incubations, experimental R was back-scaled to in situ conditions using the power fit obtained between experimentally derived PHP and R, knowing in situ PHP (see details Section 3). For this, we assumed that prokaryotic assemblages were responsible for the whole community respiration. In situ specific R rates were obtained as the ratio between in situ R and the prokaryotic biomass (R/PB).

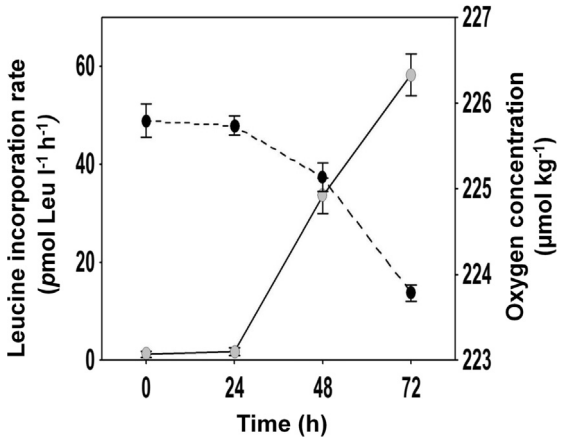

Fig. 2. Representative example of the changes in the leucine incorporation rate (pmol Leu l^{-1} h^{-1}, gray dots) and the O_2 concentration (μmol O_2 kg^{-1}, black dots) along the experiment performed at station I1 located in the Indian Ocean at 430 m depth. Bars represent the standard error of 6 replicates.

2.5. Influence of temperature on respiration

The dependence of R on temperature was tested using the Metabolic Theory of Ecology (MTE) (Brown et al., 2004), assuming that metabolic processes at community-level or ecosystem level depend on ambient temperature (Sarmento et al., 2010). This temperature dependence was examined in our in situ back-scaled R values and also compared to all R data available, derived from direct O_2 consumption, found in the literature

$$\text{Ln metabolic rate } (R) = a - b(1/kT),$$

where k is Boltzmann's constant 8.62 10^{-5} eV K^{-1} and T is the absolute water temperature in K. The negative slope (b) in the Arrhenius plot corresponds to the activation energy (E_a, eV). E_a was further transformed to Q_{10} (the change in metabolic rate for a 10 °C temperature increase) using the algorithm proposed by Raven and Geider (1988), $Q_{10} = \text{Exp}\ (10E_a/RT^2)$, where R is the gas constant (8.314472 mol^{-1} K^{-1}) and T is the mean absolute temperature (K) across the range over which Q_{10} was measured. E_a was converted to J mol^{-1} using the conversion factor of 96,486.9 J eV^{-1} mol^{-1}.

2.6. Statistical analyses

Statistical analyses were performed using Statistica 6.0 (StatSoft Inc, 1997). The dataset were log-transformed to fit the assumptions

of normality and homoscedasticity before performing the regression analysis.

3. Results

3.1. Respiration, prokaryotic production and growth efficiency in the experiments

In all the experiments ^{3}H-leucine incorporation rates increased and O_2 concentrations decreased over time (e.g. Fig. 2). Integrated PHP in the experiments ranged from 1.7 to 96.1 μmol C m^{-3} d^{-1}, while *R* varied from 131 to 467 μmol C m^{-3} d^{-1} (Table 2), being *R* and PHP strongly correlated ($N=10$, $r^2=0.79$ and p-value$=0.001$). The calculated PGE values from *R* and PHP in the experiments were low on average (~5.9%), although variable, ranging from 1.3% to 17.1% (Table 2).

3.2. In situ prokaryotic assemblages and metabolism in the upper mesopelagic environment

In situ prokaryotic abundance (PA) and heterotrophic production (PHP) varied 20-fold (from 0.5 to 11.0 × 10^5 cell ml^{-1}) and 30-fold (from 0.3 to 9.1 μmol C m^{-3} d^{-1}), respectively (Table 3). The highest in situ PA and PHP were found in the western Indian Ocean (stations I1 and I2). However, the highest biomass turnover times were located in the eastern Indian Ocean (stations I6, I7 and I8).

Table 2
Integrated community respiration rate (*R*) and prokaryotic heterotrophic production (PHP) estimated over the incubation times (Time, h) in the experiments (see Section 2). The prokaryotic growth efficiencies (PGE) derived from both integrated PHP and *R* are also included. Temp.=Incubation temperature.

Experiment	Time (h)	Temp. (ºC)	PHP (μmol C m^{-3} d^{-1})	*R* (μmol C m^{-3} d^{-1})	PGE (%)
SA1	72	9.3	24.2	255	8.7
SA2[a]	72	9.2	16.9	–	–
SA3	72	9.0	17.7	254	6.5
SA4	24	10.2	7.2	203	3.4
I1	72	15.1	96.1	467	17.1
I2	72	12.6	28.0	340	7.6
I3	72	11.7	12.4	349	3.4
I4	72	11.2	15.4	274	5.3
I5	72	10.6	8.2	297	2.7
I6	72	9.6	4.1	145	2.7
I7[a]	72	8.6	1.7	–	–
I8	72	8.6	1.7	131	1.3

[a] *R* and consequently PGE estimates were not determined in these experiments.

In situ *R* was estimated by back-scaling experimental *R* to in situ PHP, using the power relationship observed between the integrated experimental PHP and *R* (Fig. 3); where $R=127.24\ (\pm 22.52) \times PHP^{0.283\ (\pm 0.053)}$ ($N=10$, $r^2=0.78$ and p-value$=0.0007$). Back-scaled in situ *R* ranged between 87 and 238 μmol C m^{-3} d^{-1} (Table 3), and represented, on average, about two-fifth, of in vitro experimentally-derived values. However, both in situ and experimental PHP were significantly correlated ($N=12$, slope$=7.65\pm1.59$, $r^2=0.70$ and p-value$=0.0007$) and then in situ PHP can be used to scale in situ *R*. The in situ *R* in the studied stations showed a similar geographical pattern as the experimental *R* estimates, with the highest values in the western Indian Ocean (stations I1 and I2).

The estimated in situ PGE values (derived from back-scaled *R* and in situ PHP) were low in all the study stations, but ranged one order of magnitude, from 0.3% to 3.7% (Table 3), and averaged about 1.4%. The geographic pattern of in situ PGE was similar to PHP and *R*, being the highest observed PGE values in stations I1 and I2.

3.3. Temperature control on R and PGE

Experimental *R* was strongly correlated with temperature during the incubations (Fig. 4; $N=10$, $r^2=0.79$ and p-value$=0.0004$). Fig. 5 plots the Arrhenius trend for all deep ocean data (derived from O_2

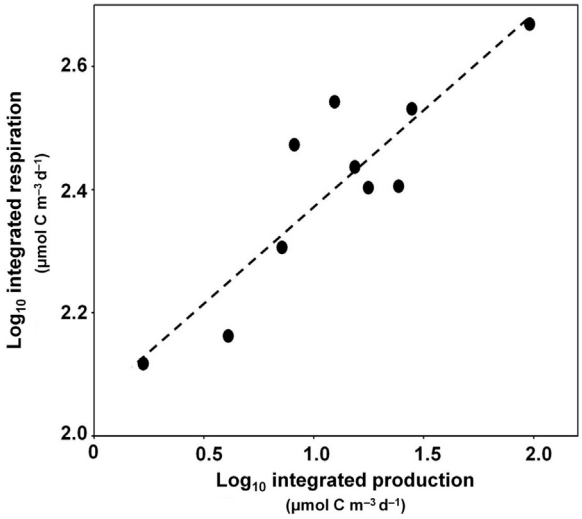

Fig. 3. Relationship between integrated prokaryotic heterotrophic production (PHP) and integrated community respiration (*R*) in the in vitro experiments. The dashed regression line represents the log–log linear transformation of the fitted power function $R=127.24\ (\pm 22.52) \times PHP^{0.283\ (\pm 0.053)}$ ($r^2=0.78$ and p-value$=0.0007$).

Table 3
Prokaryotic abundance (PA), biomass (PB), heterotrophic production (PHP), turnover time, back-scaled respiration rates (*R*) and resulting growth efficiencies (PGE) corresponding to the in situ conditions.

Stations	PA (× 10^5 cell ml^{-1})	PB (mg C m^{-3})	PHP (μmol C m^{-3} d^{-1})	Turnover time (days)	*R* (μmol C m^{-3} d^{-1})	PGE (%)
SA1	0.5	0.30	1.0	24	129	0.8
SA2	0.8	0.47	4.4	9	194	2.2
SA3	0.8	0.44	2.8	13	170	1.6
SA4	3.0	1.62	2.8	48	170	1.6
I1	11.0	6.67	9.1	61	238	3.7
I2	6.0	0.92	6.6	11	218	3.0
I3	1.4	0.86	0.9	80	123	0.7
I4	1.7	0.85	0.8	93	118	0.6
I5	1.7	0.88	1.0	70	129	0.8
I6	1.7	0.88	0.3	278	87	0.3
I7	2.3	1.20	0.3	288	94	0.4
I8	3.0	1.50	1.0	125	127	0.8

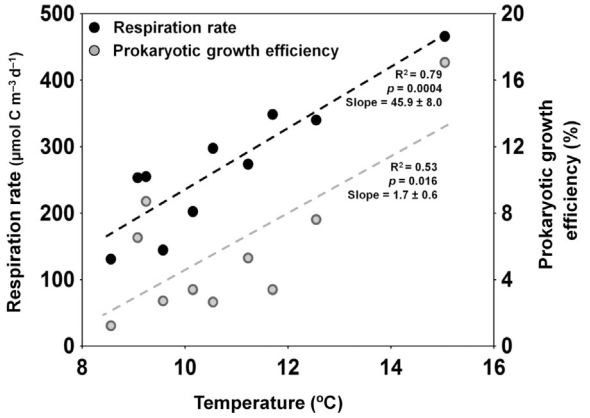

Fig. 4. Relationships between temperature and respiration rates (black dots) and prokaryotic growth efficiencies (gray dots) in the in vitro experiments. The dashed regression lines plot significant relationships (p-values < 0.05).

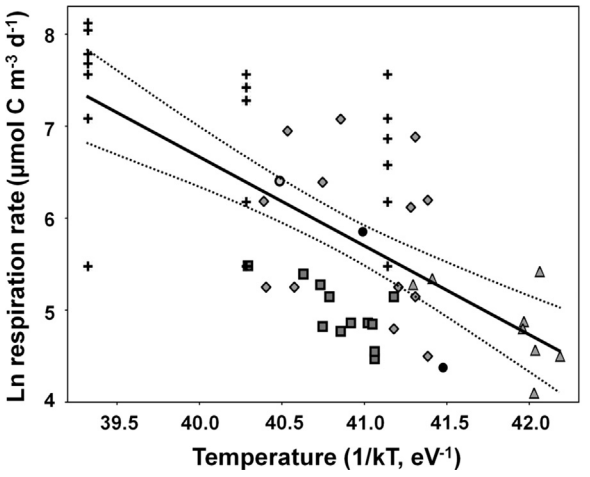

Fig. 5. Arrhenius plot showing the effect of temperature ($1/KT$) on dark ocean R data (derived from O_2 consumption) found in the literature, including also this study data. The black line represents the significant linear relationship (p-value$=0.000001$); dashed lines are the 95% confidence interval of the slope. Biddanda and Benner (1997) (crosses), Reinthaler et al. (2006) (triangles), Baltar et al. (2010) (diamonds), Arístegui et al. (2005b) (black circles), Weinbauer et al. (2013) (gray circles) and this study (squares).

consumption in BOD bottles) found in the literature (see also Table 4) showing a significant temperature dependence ($N=57$ and p-value$=0.000001$) on R across different marine regions. Table 5 also shows the results of the Arrhenius plots and Q_{10} values corresponding to this study and all data in the literature. The estimated activation energy (E_a) and fitted Q_{10} value for this study ($E_a=0.90\pm0.29$ eV and $Q_{10}=3.65$) were consistent with the calculated values from the literature ($E_a=0.98\pm0.16$ eV and $Q_{10}=4.07$). However, the specific rates of the in situ R values (R/PB) were not significantly related to temperature (p-value$=0.418$).

PGE derived from experimental PHP and R was positive and significantly correlated to temperature ($N=10$, $r^2=0.53$ and p-value$=0.016$) (Fig. 4), as well as considering the in situ conditions ($N=12$, $r^2=0.58$ and p-value$=0.004$). However, including all the data found in the literature, we did not obtain a significant relationship between PGE and temperature (p-value > 0.05; Fig. 6).

4. Discussion

Direct determination of R from O_2 consumption during in vitro incubations in the dark ocean requires, in most cases, long-term incubations (>24 h) to detect significant changes in O_2

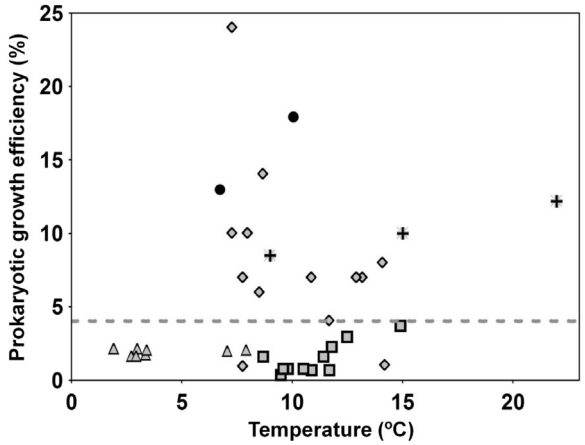

Fig. 6. Scatterplot of prokaryotic growth efficiencies (PGE), derived from direct estimates of R and PHP, as function of temperature for deep ocean prokaryotes found in the literature including this study. The gray dashed line represents PGE$=4$%. The symbols correspond to Biddanda and Benner (1997) (crosses), Reinthaler et al. (2006) (triangles), Baltar et al. (2010) (diamonds), Arístegui et al. (2005b) (circles) and this study (squares).

concentration. This may lead to the generation of artifacts as consequence of the confinement of microbial populations inside bottles, favoring cell growth after 1–2 days and increasing metabolism. In addition, changes in community structure may also take place during the incubations, shifting the community towards opportunistic populations (Massana et al., 2001). Nevertheless, some studies have shown that, in spite of these changes in community structure during daily incubations, metabolic rates can be maintained constant or directly correlated to the increases in biomass (e.g. Baltar et al., 2012). Despite these drawbacks, some previous studies derived in situ R directly from oxygen consumption in vitro incubations (Reinthaler et al., 2006; Baltar et al., 2010). Arístegui et al. (2005b) demonstrated, however, that when long incubations (>24 h) are necessary to obtain significant changes in oxygen consumption, the back-scaling of the experimental results to the in situ conditions is likely the most accurate approach to infer R in the dark ocean. Thereby, experimental R can be back-scaled to in situ conditions when a strong relationship between microbial respiration and production (or biomass) is obtained along the experiment, as it happened in this study (Fig. 3).

Few studies have addressed the effect of hydrostatic pressure on prokaryotic metabolism. In spite of the limited information currently available, Tamburini et al. (2013) reviewed these studies, concluding that decompression of deep-water samples underestimates in situ activity. This conclusion, however, is based on prokaryotic piezophilic populations from the deep ocean (>1000 m depth), adapted to live under high pressures, low temperatures and very low organic matter concentrations, a situation far different to the mesopelagic realm with lower hydrostatic pressure and higher organic matter content.

To date, the few available estimates of dark R obtained from oxygen consumption have been mostly determined for the North Atlantic Ocean and the Mediterranean Sea (Biddanda and Benner, 1997; Arístegui et al., 2005b; Reinthaler et al., 2006; Baltar et al., 2010; Weinbauer et al., 2013). Our back-scaled in situ values of R (ranging from 87 to 238 µmol C m^{-3} d^{-1}) are comparable to the back-scaled R estimates determined by Arístegui et al. (2005b) in the Canary Current region (220 ± 50 µmol C m^{-3} d^{-1}). On the other hand, the experimental R values in this study (from 131 to 467 µmol C m^{-3} d^{-1}) are more similar to other studies (Biddanda and Benner, 1997; Reinthaler et al., 2006; Baltar et al., 2010; Weinbauer et al., 2013) where the back-scaling approach was not applied.

The largest data set of respiration in the global dark ocean is, however, based on potential respiratory activity derived from

enzymatic electron transport system (ETS) measurements. A global average R of 10 μmol C m^{-3} d^{-1} was estimated by Arístegui et al. (2003) for the mesopelagic zone assuming a conservative R/ETS ratio of 0.1, characteristic of bacterial populations in senescent state (Christensen et al., 1980). This global rate represents, however, a lower threshold since prokaryotic communities in the mesopelagic zone can be far from being senescent, presenting R/ETS ratios closer to 1 (Arístegui et al., 2005b), typical of bacterial assemblages in exponential growth phase (Christensen et al., 1980). If so the ETS-derived R (using R/ETS of 1) would be in the range of the back-scaled R obtained in this study.

There are also very few studies in the literature reporting PGE in the dark ocean derived from direct measurements of R and PHP (Table 4). In situ PGE in our study were on average ~1.4%. These low PGE are in accordance with estimates reported by Reinthaler et al. (2006) in the meso- and bathypelagic region of the North Atlantic Ocean (~2%), but without any back-scaling procedure in the direct R estimates. Dumont et al. (2011) found similar low PGE in the South of Tasmania using the equation proposed by del Giorgio and Cole (1998) to predict PGE from PHP. Other studies have estimated the prokaryotic consumption from empirical equations, which had been generated from surface ocean samples (Carlson et al., 2004; Tanaka and Rassoulzadegan, 2004), resulting in higher PGE estimates (5–13% and 19–39%, respectively). Zaccone et al. (2003) and Biddanda and Benner (1997) also found high PGE between 6–11% and 8–12%, respectively. The methodology used in the these later reports, however, could have affected the estimation of in situ R. Zaccone et al. used indirect R measurements from ETS activity and Biddanda and Benner only considered 50% of the measured respiration in the experiments to estimate PGE. Baltar et al. (2010) obtained a wide range of PGE (<1–24%) from a variety of approaches to estimate PGE from in vitro experiments, similar to our experimental range of PGE (1.7–17.1%). Only Arístegui et al. (2005b) used a similar back-scaling procedure to our study to derive R, although PHP was estimated by averaging leucine and thymidine incorporation rates. These authors obtained higher PGE (13–18%), which they attributed, based also on other molecular and metabolic prokaryotic proxies, to the larger organic carbon supply from the continental margins, which likely translated into a more efficient use of carbon for growth in the mesopelagic zone. The low in situ PGE in our study confirm the hypothesis that most of the organic matter transported to the mesopelagic zone in open ocean waters is mineralized, ending up as CO_2; yet more studies reporting direct R estimates are essential for a better knowledge of the regional variability in prokaryotic carbon use throughout the dark ocean.

We observed a strong correlation between water temperature and in situ R, suggesting a significant temperature dependence on prokaryotic metabolism also in the mesopelagic ocean. There are several studies describing the influence of temperature on R and PGE. Nagata et al. (2001), in the mesopelagic waters of the subarctic Pacific, observed temperature dependence of prokaryotic growth, reporting positive significant linear regressions in the upper part of the mesopelagic zone. Bendtsen et al. (2002), based on a model of the microbial food web, postulated that the gradient of dissolved organic carbon in the deep North Atlantic could be explained by the temperature dependence of bacterial metabolism. Iversen and Ploug (2013) used results obtained in laboratory experiments to hypothesize that the carbon flux into deep waters would be reduced in warmer environments due to increased remineralization rates. Our results and all these studies suggest that prokaryotic metabolism in the dark ocean would be affected by rising temperatures. Nevertheless, the influence would be different depending on whether the temperature affects more growth (new biomass generation), being carbon channeled through higher trophic levels, or respiration, being carbon recycled as CO_2 (e.g. Vázquez-Domínguez et al., 2007). The temperature dependence of mineralization rates does not preclude however that the concentration and quality of organic matter might also affect oxygen consumption rates in the dark ocean. Indeed, Nagata et al. (2001) concluded that organic matter was more important than temperature in determining the growth of heterotrophic prokaryotes in the upper mesopelagic zone of the Subarctic Pacific.

The metabolic theory of ecology (MTE) establishes an increment of the organisms' metabolism with increasing temperatures (Brown et al., 2004), as it has been observed in planktonic

Table 4
Reported prokaryotic growth efficiencies (PGE) in the dark ocean, derived from prokaryotic heterotrophic production (PHP) and direct respiration (R) measurements, indicating the different approaches to estimate PHP and R.

Location	Depth (m)	PHP approach	R approach	PGE (%)	Reference
Subtropical North Atlantic Ocean	350–1000	In situ leucine incorporation and prokaryotic biomass yield in experiments	Difference in O_2 concentration in experiments	<1–24	Baltar et al. (2010)
Subtropical North Atlantic Ocean	600–1000	Average of in situ thymidine and leucine incorporation	Difference in O_2 concentration in experiments. Back-scaling to in situ conditions	13–18	Arístegui et al. (2005b)
North Atlantic Ocean	100–4000	In situ leucine incorporation	Difference in O_2 concentration in experiments	~2	Reinthaler et al. (2006)
Gulf of Mexico	100–500	In situ leucine incorporation	Linear regression between time and O_2 concentration in experiments	8–12	Biddanda and Benner (1997)
South Atlantic Ocean and Indian Ocean	300–600	In situ leucine incorporation	Difference in O_2 concentration in experiments. Back-scaling to in situ conditions	<1–17	This study

Table 5
The slopes of the regression (- activation energy, eV) and the parameters of the Arrhenius plot between Ln of respiration rate (R, μmol C m^{-3} d^{-1}) and inverse of temperature ($1/kT$), Ln (R)=$a-b$ ($1/kT$), for in situ data in this study and all data in the literature (Fig. 5). The Q_{10} temperature coefficients are also shown.

	Temperature range (ºC)	Rate	Intercept ± SE	Slope ± SE	R^2	p-Value	N	Q_{10}
This study	8.7–14.9	R	41.86 ± 11.98	−0.90 ± 0.29	0.49	0.012	12	3.65
Literature	1.9–22.0	R	45.89 ± 6.33	−0.98 ± 0.16	0.42	0.000001	57	4.07

communities of the surface ocean (López-Urrutia and Morán, 2007; Regaudie-de-Gioux and Duarte, 2012). Our results also show a significant in situ R dependence on temperature in the range of 8.7–14.9 °C. This temperature dependence is also obtained considering all R data previously reported from the dark ocean (Table 5). In contrast to the expected increase of cell-based metabolism in a warmer environment, we observe in our study a lack of correlation between temperature and specific R (R/PB). Likely a variable fraction of in situ prokaryotes was metabolically inactive (Gasol et al., 1995) and, consequently, specific R in our study was not a good proxy to detect temperature changes. The R activation energy value estimated in this study (E_a of 0.90 ± 0.29 eV) was higher than those reported by López-Urrutia and Morán (2007) for specific R in the surface ocean ($E_a = 0.589$ eV), by Yvon-Durocher et al. (2012) for microbial populations in the global ocean ($E_a = 0.57$ eV) or by Arístegui and Montero (1995) for microplankton R derived from ETS activity ($E_a = 0.70$ eV). However, Regaudie-de-Gioux and Duarte (2012) reported comparable E_a values for R in the Atlantic Ocean ($E_a = 0.92 \pm 0.11$ eV). The Q_{10} values determined in this study (Table 5) were also higher than the Q_{10} range of 1–3 for R reported by Church (2008).

Recent forecasts consider a global warming rate per decade between 0.015 and 0.11 °C in the upper 700 m (Intergovernmental Panel on Climate Change (Rhein et al., 2013)), which could lead to an increase of R of about 4% according to our calculations. Since the global-warming slowdown detected in surface ocean during the 21st century is partially a consequence of the heat transfer to deeper depths (Chen and Tung, 2014), prokaryotic mineralization rates at the mesopelagic zone could be influenced by increasing temperatures in the dark ocean. Therefore, R vs. temperature reported in this study could help to predict future scenarios in the mesopelagic zone.

The positive relationship between PGE and temperature in the experiments (Fig. 4) is not consistent with previous studies that report a decrease of PGE as temperature increases (Rivkin and Legendre, 2001; Kritzberg et al., 2010a, 2010b). Rivkin and Legendre (2001), in a data compilation study, found a negative relationship between these two parameters for a wide range of temperatures (−1.4 to 29 °C). Likewise, Kritzberg et al. (2010a and 2010b) showed a stronger response of respiration to temperature than production, both in an oligotrophic coastal marine system (with a temperature range of 14.4–28 °C) and in Arctic waters (with a temperature range of −0.6 to 5.8 °C). On the contrary, Wohlers et al. (2009) observed in in-door mesocosms' experiments carried out with water from the Baltic Sea, under a gradient of low temperatures (2.5–8.5 °C), that bacterial production was stimulated by temperature increases but community respiration (<3 μm) remained apparently unaffected; then, BGE increasing with rising temperatures. Reinthaler and Herndl (2005) also found higher PGE during the warmer spring and summer periods than during the cooler winter time in the southern North Sea. Other studies, however, had suggested that R and PHP would respond similarly to temperature changes (López-Urrutia and Morán, 2007; Vázquez-Domínguez et al., 2007), suggesting that differences in the response of PHP and R to environmental factors (and consequently to BGE) would be related to the bioavailability of organic resources (López-Urrutia and Morán, 2007) or, as discussed above, to the co-variation effect between temperature and organic matter.

5. Conclusions

Our study supports previous hypotheses (del Giorgio and Duarte, 2002; Arístegui et al., 2009) formulating that organic matter exported to the deep ocean is mostly respired by prokaryotic communities in the mesopelagic zone, instead of being used for growth. Moreover, in addition to the effects of organic matter composition and lability on prokaryotic R, our results suggest that the variability of dark ocean respiration is also dependent on temperature gradients. More effort should be dedicated to explore the effects of temperature variations on metabolic processes (growth and respiration) in the dark ocean, in order to understand how the flow of carbon would be affected by the rising temperatures in the deep ocean.

Acknowledgments

We thank our scientific colleagues and C. M. Duarte for the coordination of Malaspina 2010 expedition, as well as the technical staff and crew members on board the BIO Hespérides, for their support in the field sampling during the different legs of the cruise, and particularly A. Gomes and X.A.G. Morán for providing the in situ PHP data. This paper is a contribution to the INGENIO 2010 CONSOLIDER program (CDS2008-00077). IPM was supported by a JAE Pre-doc fellowship, from the Spanish National Research Council (CSIC) and the BBVA Foundation, Spain. IPM, JA and JMG were partly supported by Project HotMix (CTM2011-30010-C02/MAR).

References

Anderson, T.R., Tang, K.W., 2010. Carbon cycling and POC turnover in the mesopelagic zone of the ocean: insights from a simple model. Deep Sea Res. II 57, 1581–1592.
Arístegui, J., Agustí, S., Duarte, C.M., 2003. Respiration in the dark ocean. Geophys. Res. Lett. 30 (2), 1041. http://dx.doi.org/10.1029/2002GL016227.
Arístegui, J., Agustí, S., Middelburg, J.J., Duarte, C.M., 2005a. Respiration in the mesopelagic and bathypelagic zones of the ocean. In: del Giorgio, P.A., et al. (Eds.), Respiration in Aquatic Ecosystems, pp. 181–205.
Arístegui, J., Duarte, C.M., Gasol, J.M., Alonso-Sáez, L., 2005b. Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. 32, L03608. http://dx.doi.org/10.1029/2004GL021863.
Arístegui, J., Gasol, J.M., Duarte, C.M., Herndl, G.J., 2009. Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54 (5), 1501–1529.
Arístegui, J., Montero, M.F., 1995. The relationship between community respiration and ETS activity in the ocean. J. Plankton Res. 17, 1563–1571.
Baltar, F., Arístegui, J., Gasol, J.M., Herndl, G.J., 2010. Prokaryotic carbon utilization in the dark ocean: growth efficiency, leucine-to-carbon conversion factors, and their relation. Aquat. Microb. Ecol. 60, 227–232. http://dx.doi.org/10.3354/ame01422.
Baltar, F., Lindh, M.V., Parparov, A., Berman, T., Pinhassi, J., 2012. Prokaryotic community structure and respiration during long-term incubations. Microbiologyopen 1 (2), 214–224.
Bendtsen, J., Lundsgaard, C., Middelboe, M., Acher, D., 2002. Influence of bacterial uptake on deep-ocean dissolved organic carbon. Glob. Biochem. Cycles 16 (4), 1127.
Biddanda, B., Benner, R., 1997. Major contribution from mesopelagic plankton to heterotrophic metabolism in the upper ocean. Deep Sea Res. I 44 (12), 2069–2085.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.
Calvo-Díaz, A., Morán, X.A.G., 2006. Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. Aquat. Microb. Ecol. 42, 159.
Carlson, C.A., Giovannoni, S.J., Hansell, D.A., Goldberg, S.J., Parsons, R., Vergin, K., 2004. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnol. Oceanogr. 49, 1073–1083.
Chen, X., Tung, K.-K., 2014. Varying planetary heat sink led to global-warming slowdown and acceleration. Science 345 (6199), 897–903.
Christensen, J.P., Owens, T.G., Devol, A.H., Packard, T.T., 1980. Respiration and physiological state in marine bacteria. Mar. Biol. 55, 267–276.
Church, M.J., 2008. Resource control of bacterial dynamics in the sea. In: Kirchman, D.L. (Ed.), Micobial Ecology of the Oceans, pp. 335–382.
Dumont, I., Schoemann, V., Jacquet, S.H.M., Masson, F., Becquevort, S., 2011. Bacterial abundance and production in epipelagic and mesopelagic waters in the Subantarctic and Polar Front zones south of Tasmania. Deep Sea Res. II 58, 2212–2221.
del Giorgio, P.A., Cole, J.J., 2000. Bacterial energetics and growth efficiency In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans. Wyley, Liss, pp. 289–325.
del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29, 503–541.
del Giorgio, P.A., Duarte, C.M., 2002. Respiration in the open ocean. Nature 420, 379–384.

del Giorgio, P.A., Pace, M.L., Fischer, D., 2006. Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River. Aquat. Microb. Ecol. 45, 55–67.

del Giorgio P.A., Williams P.J.B. 2005. Chapter 14: The global significance of respiration in aquatic ecosystems: from single cells to the biosphere. In: Respiration in Aquatic Ecosystems. Oxford University Press, New York, NY, pp. 267–303.

Gasol, J.M., del Giorgio, P.A., 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci. Mar. 64, 197–224.

Gasol, J.M., del Giorgio, P.A., Massana, R., Duarte, C.D., 1995. Active versus inactive bacteria: size-dependence in a coastal marine plankton community. Mar. Ecol. Prog. Ser. 128, 91–97.

Giering, S.L., Sanders, R., Lampitt, R.S., Anderson, T.R., Tamburini, C., Boutrif, M., Zubkov, M.V., Marsay, C.M., Henson, S.A., Saw, K., Cook, K., Mayor, D.J., 2014. Reconciliation of the carbon budget in the ocean's twilight zone. Nature 507, 480–483.

Gundersen, K., Heldal, M., Norland, S., Purdie, D.A., Knap, A.H., 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic time-series study (BATS) site. Limnol. Oceanogr. 47 (5), 1525–1530.

Iversen, M.H., Ploug, H., 2013. Temperature effects on carbon-specific respiration rate and sinking velocity of diatom aggregates—potential implications for deep ocean export processes. Biogeosciences 10, 4073–4085.

Kirchman, D.L., K'nees, E., Hodson, R.E., 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49, 599–607.

Kritzberg, E.S., Arrieta, J.M., Duarte, C.M., 2010a. Temperature and phosphorous regulating carbon flux through bacteria in a coastal marine system. Aquat. Microb. Ecol. 58, 141–151.

Kritzberg, E.S., Duarte, C.M., Wassmann, P., 2010b. Changes in Arctic marine bacterial carbon metabolism in response to increasing temperature. Polar Biol. 33, 1673–1682.

López-Urrutia, A., Morán, X.A.G., 2007. Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycle. Ecology 88 (4), 817–822.

Massana, R., Pedrós-Alió, C., Casamayor, E.O., Gasol, J.M., 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnol. Oceanogr. 46, 1181–1188.

Nagata, T., Fukuda, R., Fukuda, H., Koike, I., 2001. Basin-scale geographic patterns of bacterioplankton biomass and production in the Subarctic Pacific, July–September 1997. J. Oceanogr. 57, 301–313.

Pomeroy, L.R., Wiebe, W.J., 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat. Microb. Ecol. 23, 187–204.

Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. New Phytol. 110, 441–461.

Regaudie-de-Gioux, A., Duarte, C.M., 2012. Temperature dependence of planktonic metabolism in the ocean. Glob. Biogeochem. Cycles 26, GB1015.

Rhein, M., Rintoul, S.R., Aoki, S., Campos, E., Chambers, D., Feely, R.A., Gulev, S., Johnson, G.C., Josey, S.A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L.D., Wang, F., 2013. Observations: ocean (Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change). In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Reinthaler, T., Herndl, G.J., 2005. Seasonal dynamics of bacterial growth efficiencies in relation to phytoplankton in the southern North Sea. Aquat. Microb. Ecol. 39, 7–16.

Reinthaler, T., van Aken, H., Veth, C., Arístegui, J., Robinson, C., Williams, P.J.B., Lebaron, P., Herndl, G.J., 2006. Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51 (3), 1262–1273.

Rivkin, R.B., Legendre, L., 2001. Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science 291, 2398–2400.

Robinson, C., Steinberg, D.K., Anderson, T.R., Arístegui, J., Carlson, C.A., Frost, J.R., Ghiglione, J.-F., Hernández-León, S., Jackson, J.A., Koppelmann, R., Quéguiner, B., Ragueneau, O., Rassoulzadegan, F., Robison, B.H., Tamburini, C., Tanaka, T., Wishner, K.F., Zhang, J., 2010. Mesopelagic zone ecology and biogeochemistry—a synthesis. Deep Sea Res. II 57, 1504–1518.

Sarmento, H., Montoya, J.M., Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2010. Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? Philos. Trans. R. Soc. B 365, 2137–2149.

Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51, 201–213.

Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs 6, 107–114.

StatSoft Inc, 1997. Statistica for Windows (Computer Program Manual). Tulsa, Oklahoma, USA.

Tamburini, C., Boutrif, M., Garel, M., Colwell, R.R., Deming, J.W., 2013. Prokaryotic responses to hydrostatic pressure in the ocean—a review. Environ. Microbiol. 15 (5), 1262–1274.

Tanaka, T., Rassoulzadegan, F., 2004. Vertical and seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: bottom-up and top-down controls. Deep Sea Res. I 51, 531–544.

Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2007. Ocean warming enhances respiration and carbon demand of coastal microbial plankton. Glob. Change Biol. 13, 1327–1334.

Weinbauer, M.G., Liu, J., Motegi, C., Maier, C., Pedrotti, M.L., Dai, M., Gattuso, J.P., 2013. Seasonal variability of microbial respiration and bacterial and archaeal community composition in the upper twilight zone. Aquat. Microb. Ecol. 71, 99–115.

Williams, P.J.leB., Jenkinson, N.W., 1982. A transportable microprocessor-controlled precise Winkler tritation suitable for field station and siphboard use. Limnol. Oceanogr. 27 (3), 2843–2855.

Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jurgens, K., Kans-Georg, H., Sommer, U., Riebesell, 2009. Changes in biogenic carbon flow in response to sea surface warming. Proc. Natl. Acad. Sci. USA 106, 7067–7072.

Yvon-Durocher, G., Caffrey, J.M., Cescatti, A., Dossena, M., del Giorgio, P., Gasol, J.M., Montoya, J.M., Pumpanen, J., Staehr, P.A., Trimmmer, M., Woodward, G., Allen, A.P., 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487, 472–476.

Zaccone, R., Monticelli, L.S., Seritti, A., Santinelli, C., Azzaro, M., Boldrin, A., La Ferla, R., Ribera, M.D., 2003. Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses. J. Geophys. Res. 108, 8117–8128.