Temperature regulation of marine heterotrophic prokaryotes increases latitudinally as a breach between bottom-up and top-down controls

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Abstract

Planktonic heterotrophic prokaryotes make up the largest living biomass and process most organic matter in the ocean. Determining when and where the biomass and activity of heterotrophic prokaryotes are controlled by resource availability (bottom-up), predation and viral lysis (top-down) or temperature will help in future carbon cycling predictions. We conducted an extensive survey across subtropical and tropical waters of the Atlantic, Indian and Pacific Oceans during the Malaspina 2010 Global Circumnavigation Expedition and assessed indices for these three types of controls at 109 stations (mostly from the surface to 4,000 m depth). Temperature control was approached by the apparent activation energy in eV (ranging from 0.46 to 3.41), bottom-up control by the slope of the log-log relationship between biomass and production rate (ranging from -0.12 to 1.09) and top-down control by an index that considers the relative abundances of heterotrophic nanoflagellates and viruses (ranging from 0.82 to 4.83). We conclude that temperature becomes dominant (i.e. activation energy >1.5 eV) within a narrow window of intermediate values of bottom-up (0.3–0.6) and top-down 0.8–1.2) controls. A pervasive latitudinal pattern of decreasing temperature regulation towards the Equator, regardless of the oceanic basin, suggests that the impact of global warming on marine microbes and their biogeochemical function will be more intense at higher latitudes. Our analysis predicts that 1°C ocean warming will result in increased biomass of heterotrophic prokaryoplankton only in waters with <26°C of mean annual surface temperature.

KEYWORDS
bacterioplankton, bottom-up, heterotrophic prokaryotes, latitudinal gradients, microbial oceanography, ocean warming, temperature control, top-down

1 | INTRODUCTION

Planktonic heterotrophic prokaryotes, although small (usually <0.6 μm in diameter), make up the largest living biomass of the oceans. As mediators in most organic matter transformations, they have a fundamental role in global biogeochemical cycles (Arrigo, 2005). Hence, a comprehensive knowledge of the drivers regulating heterotrophic prokaryotes abundance and metabolism is essential to
predict their future role in a changing ocean characterized by increasingly warmer temperatures and decreased nutrient inputs from deep waters (Hoegh-Guldberg & Bruno, 2010). Heterotrophic prokaryoplankton include bacteria and archaea, but archaea are largely outnumbered by bacteria in most marine environments. The contribution of archaea to total prokaryotic biomass and activity generally increases in the meso- and bathypelagic layers (Herndl et al., 2005).

As for every living organism on Earth, various physical (e.g. light, temperature), chemical (e.g. nutrients, oxygen) and biological (e.g. predation, parasitism) factors act on planktonic heterotrophic prokaryotes. On a global scale, three major types of control are held responsible for the variability of heterotrophic prokaryoplankton biomass and activity: temperature, resource availability (i.e. bottom-up control) and losses to predators and viral pathogens (i.e. top-down control) (Hunter & Price, 1992). Temperature regulates the velocity of metabolic processes and, ultimately, growth rates, thereby modulating resource demands (Angilletta, Steury, & Sears, 2004; Savage, Gillooly, Brown, West, & Charnov, 2004). The question of bottom-up and top-down controls of heterotrophic prokaryotes has been the subject of many studies, both empirical and theoretical (Pernthena, 2005). The hypotheses that loss processes preferentially regulate biomass while resource availability determines productivity (Pace & Cole, 1996) and that the weight of both regulation modes varies consistently along productivity gradients (Gasol, Pedrós-Alió, & Vaqué, 2002; Li, Head, & Harrison, 2004; Vaqué et al., 2014) have met frequent exceptions.

Numerous studies have addressed the importance of temperature, bottom-up and top-down controls in marine ecosystems, either separately or, less frequently, in combinations of two (Dufour & Torróton, 1996; Pace & Cole, 1994; Shiah & Ducklow, 1994). Only a few studies have addressed the three types of control simultaneously (Bouvy et al., 2011; Hoekman, 2010; Shurin, Clasen, Greig, Kratina, & Thompson, 2012), but we are not aware of any systematically doing so in open ocean environments. Previous work suggesting a seasonal switch between temperature and bottom-up controls (Shiah & Ducklow, 1994) was recently hypothesized to be general in temperate coastal ecosystems (Calvo-Diaz, Franco-Vidal, & Morán, 2014). However, both these studies were limited to single locations.

Here, we concurrently estimated the strength of temperature, bottom-up and top-down controls on heterotrophic prokaryotic communities across the global ocean between 35°N and 40°S. We did so with a data set obtained during the Malaspina 2010 Global Circumnavigation Expedition, which sailed the tropical and subtropical Atlantic, Indian and Pacific Oceans between 2010 and 2011 (Duarte, 2015). The cruise sampled prokaryotic heterotrophic biomass (PHB) and production (PHP) down to 4,000 m depth for a total of 109 stations. We quantified the strength of bottom-up control from the relationship between PHB and PHP (Billen, Servais, & Bécquevort, 1990). By temperature control, we will refer to the thermal sensitivity of the community, which was characterized by the apparent activation energy of PHP (Lønborg et al., 2016). Finally, we used the mean abundances of heterotrophic nanoflagellates and free viruses as a proxy for top-down control. After investigating the general relationships between temperature, bottom-up and top-down controls, we searched for latitudinal patterns in the data set and used a simple model to predict the biomass of planktonic heterotrophic prokaryotes in surface waters with a 1°C warming over current conditions.

2 | MATERIALS AND METHODS

The Malaspina 2010 Global Circumnavigation Expedition consisted in seven legs, which covered >50,000 km of the subtropical and tropical oceans between December 2010 and July 2011 (Duarte, 2015). The northern Atlantic was sampled in late autumn and early summer, while the north Pacific was sampled in spring. All the southern hemisphere stations were sampled from summer through early spring. 109 of the 127 stations sampled for heterotrophic prokaryotes abundance and activity were included in this analysis. The remaining 18 stations were either characterized by bottom or maximum sampling depths shallower than 3,000 m or lacked sufficient detail in bacterial data over the entire water column. 30 stations had maximum sampling depths comprised between 3,000 and 4,000 m, while the rest were sampled down to 4,000 m. At each station, water samples were taken from 6–10 depths (average 8.1) from Niskin bottles attached to a CTD probe, usually evenly distributed between the upper 100 m and the rest of the water column. The sampling depths generally included the surface (3 m), 20% and 7% of surface photosynthetically active radiation, the deep chlorophyll maximum (DCM), 20 m below the DCM, the oxygen minimum, the deep scattering layer, the salinity minimum and 4,000 m.

2.1 | Heterotrophic prokaryotes biomass and production

The abundance and cell size of heterotrophic prokaryotes was determined by flow cytometry in SybrGreen I stained samples as detailed elsewhere (Gomes et al., 2015). Cellular carbon content was calculated for each sample and depth (range 2.4–26.7 fg C/cell) and subsequently used to estimate prokaryotic heterotrophic biomass (PHB, μg C/L). Prokaryotic heterotrophic production rates (PHP) were estimated with the centrifugation method (Smith & Azam, 1992) of 3H-leucine incorporation (Kirchman, K’nees, & Hodson, 1985). As no empirical leucine to carbon conversion factors were available for the entire water column (Teira et al., 2015), we converted leucine (Leu) uptake rates to PHP using the theoretical value of 1.5 Kg C mol/Leu.

2.2 | Abundance of heterotrophic nanoflagellates and viruses

The abundance of heterotrophic nanoflagellates was measured by a combination of microscopy and flow cytometry as described in Pernice et al. (2015). The mesopelagic and bathypelagic data set (from 200 m to 4,000 m) of Pernice et al. (2015) was complemented by epifluorescence microscopy counts from surface and DCM samples at most stations. Yet, data on heterotrophic nanoflagellates abundance were not as exhaustive in terms of vertical resolution (seven depths at
most) as for heterotrophic prokaryotes. Total viral abundance, estimated by flow cytometry (E. Lara, D. Vaqué, E. L. Sà, J. A. Boras, A. Gomes, E. Borrull, C. Diez-Vives, E. Teira, M. C. Pernice, F. C. García, I. Forn, Y. M. Castillo, A. Peiró, G. Salazar, X. A. G. Morán, R. Massana, T. S. Catalá, G. M. Luna, S. Agustí, M. Estrada, J. M. Gasol, & C. M. Duarte, unpublished data), was generally available for the same depths of heterotrophic prokaryotes but for slightly fewer (103) stations.

2.3 | Bottom-up, temperature and top-down control indices

At each station, the bottom-up control index was calculated as the slope of the ordinary least squares (OLS) linear regression between log-transformed PHB and PHP across all depths (Fig. S1a–c) following Billen et al. (1990). Ducklow (1992) suggested the following categories in bottom-up control strength according to the slope value: weak (0.2–0.4), moderate (0.4–0.6) and strong (>0.6). The rationale for this interpretation is that at high bottom-up control (and minimal top-down control), PHB can be converted into PHB with a log-log slope close to 1, while at decreasing bottom-up control (due to increasing top-down control) increases in PHP do not translate into PHB but into predator biomass or get respired through the viral loop.

As for the strength of bottom-up control, the degree of heterotrophic prokaryotes sensitivity to temperature was evaluated based on the OLS linear regression slope rather than on its coefficient of determination, although both the slope and $r^2$ values are intimately linked. The temperature control index was calculated as the apparent activation energy $E$ (eV units) in Arrhenius plots of natural log-transformed PHP vs. $1/\text{KT}$ (Fig. S1d–f), in which $T$ represents absolute temperature in Kelvin and $k$ is the Boltzmann’s constant ($8.62 \times 10^{-5}$ eV/K). The linear regressions for calculating bottom-up and temperature control indices were generally significant ($p < .05$) except in the few stations (18 and 14, respectively) for which low slopes were found. With high mean $r^2$ values of $0.70 \pm 0.25$ (bottom-up) and $0.78 \pm 0.12$ (temperature), both PHP and temperature explained substantial percentages of the variance of the respective dependent variables PHB and PHP.

The abundances of heterotrophic nanoflagellates and viruses were used to independently estimate a top-down control index. For viruses, we directly calculated the mean abundance (weighed by depth) at each station from the available data set. We did the same for heterotrophic nanoflagellates (HNFs). However, we had to previously estimate their abundance at 2–4 additional depths per station in order to have the same vertical resolution as that of heterotrophic prokaryotes and viruses. To that end, we used OLS linear regressions between log-transformed abundances and depth calculated for each leg as detailed in Table S2. The vertical distribution of HNFs abundance was very consistent throughout the cruise: it increased slightly between the surface and the DCM depth, and then decreased steadily downwards as shown by Pernice et al. (2015) for the 200–4,000 m depth range. Ratios of the mean abundances at each station to the corresponding global means of 56 HNFs per mL and $1.37 \times 10^6$ viruses per mL were subsequently calculated. During the Malaspina cruises, E. Lara, D. Vaqué, E. L. Sà, J. A. Boras, A. Gomes, E. Borrull, C. Diez-Vives, E. Teira, M. C. Pernice, F. C. García, I. Forn, Y. M. Castillo, A. Peiró, G. Salazar, X. A. G. Morán, R. Massana, T. S. Catalá, G. M. Luna, S. Agustí, M. Estrada, J. M. Gasol, and C. M. Duarte, (unpublished data) performed 11 experiments of the concurrent impact of HNF grazing and viral lysis on heterotrophic prokaryotes at three depths (surface, DCM and 4,000 m). An analysis of these data showed that, on average, HNF and viruses were responsible for 40% and 60%, respectively, of the total mortality of heterotrophic prokaryoplankton.

We applied the relative impact of both mortality factors so that the top-down control index was finally calculated as:

$$\text{Top-down control index} = [(\text{mean station HNFs abundance/mean Malaspina 2010 HNFs abundance}) \times 0.40 + (\text{mean station viruses abundance/mean Malaspina 2010 viruses abundance})] \times 0.60).$$

A top-down control value substantially >1 means that HNF and/or viruses were more abundant than the global mean whereas a value close to 0 means that they were comparatively less abundant. As bottom-up and top-down control indices bear no units, we omitted the eV units when representing temperature control index values. It should be borne in mind that actual values of the three types of microbial control are not directly comparable.

The effect of a 1°C temperature rise on surface heterotrophic prokaryotes biomass was estimated by combining the site-specific temperature and bottom-up control indices. We first estimated PHP at 1°C above the in situ value with the apparent activation energy $E$. Then we applied the log PHB versus log PHP linear regression of the bottom-up control index to this estimated PHP value to predict the heterotrophic prokaryotic biomass under warmer conditions. Finally, the ratio of the predicted PHB at 1°C over the ambient value to the actually measured PHB value was calculated to evaluate the response of heterotrophic prokaryoplankton to future warming in the different ocean basins.

2.4 | Statistics

Ordinary least squares (Model I) linear regressions and Pearson correlation coefficients between variables were performed with Statistica software. Geographical differences in the strength of the three types of control were assessed by one-way ANOVAs and post hoc Tukey HSD tests after grouping the stations by ocean basin and latitude: North Atlantic (legs 1 (partial) and 7), South Atlantic (legs 1 (partial) and 2), Indian Ocean (legs 3 and 4), South Pacific (leg 5 (partial)) and North Pacific (legs 5 (partial) and 6). Details on the stations position along the cruise track are presented in Fig. S2a.

3 | RESULTS

Heterotrophic prokaryotes biomass and productivity decreased steeply with depth at virtually all stations, following an average surface–depth temperature gradient of $22.9 \pm 0.3°C$ (SE) (Table S1). The bottom-up control index varied from $-0.12$ (i.e. decrease rather than
increase in heterotrophic prokaryotes biomass with higher production rate, found at only two stations) to 1.09, with a mean value of 0.47. The apparent activation energy of PHP, characterizing the strength of temperature control, ranged from 0.46 to 3.41 eV and averaged 1.49 eV. The extent of bottom-up and temperature regulations varied across the circumnavigation (Fig. S2). Strong bottom-up control (i.e. >0.6) was observed in 21% of the stations, mostly located in the Equatorial and South Atlantic (legs 1 and 2), while no significant bottom-up control (i.e. <0.2) was patent primarily in the South Pacific (Fig. S2b). The strongest temperature sensitivity was in turn observed in the communities sampled in the Great Australian Bight (leg 4) in the Indian Ocean, the coldest region sampled in our study, and the lowest temperature control was generally observed in communities sampled in the Pacific (legs 5 and 6, Fig. S2c).

The relationship between temperature and bottom-up control indices was unimodal, with temperature control peaking at intermediate bottom-up control values and declining at lower and higher values (Figure 1a). Above a threshold bottom-up control index of ca. 0.30, we found a clear switch between both modes of control. An exponential fit explained 34% of the variance of this negative relationship, which operated similarly in the three ocean basins. Temperature control approached a minimum of ca. 1 eV at bottom-up control values exceeding 0.8. Conversely, when bottom-up control fell below 0.6, temperature began to play a more important role, with $E$ values >2 eV only observed within a narrow range of bottom-up control indices (approx. 0.3–0.6). This inverse relationship failed to be observed at low bottom-up control values, as temperature sensitivity was also low at the few stations, mostly located in the Pacific, showing bottom-up control indices <0.2. According to the theoretical framework we used, top-down control takes over when bottom-up control disappears (Billen et al., 1990; Ducklow, 1992). As an independent verification of that balance between bottom-up versus top-down regulation, we used the vertically averaged mean abundances of heterotrophic nanoflagellates and viruses. The mean abundances of both groups were significantly and negatively correlated with the average bottom-up control indices in the seven Malaspina legs (Figure 1b). Indeed, high average bottom-up (low top-down) as well as high average top-down (low bottom-up) indices resulted both in low average temperature control, whereas moderate average values of bottom-up and top-down controls maximized temperature control.

**FIGURE 1** Relationships between temperature, bottom-up and top-down controls of marine heterotrophic prokaryotes. (a) Relationship between bottom-up (B-U control) and temperature control (temp control) indices of heterotrophic prokaryotes at all the stations sampled per ocean and leg during the Malaspina 2010 Global Circumnavigation Expedition. The fitted line includes all bottom-up control values higher than 0.3 (temp control = 0.89 × B-U control$^{0.76}$, $r^2 = 0.34$, p < .001, n = 91). The three-degree polynomial fitting (dashed line) is also shown for reference (b) Relationships between mean (±SE) values of bottom-up control (B-U control) and depth-averaged concentrations of heterotrophic nanoflagellates (HNF, black dots) and viruses (grey dots) at each of the seven legs sampled during Malaspina 2010. Stations per leg ranged from 10 (leg 4) to 22 (leg 6). The fitted lines represent ordinary least squares linear regressions: HNF = 69.3–26.6 B-U control, $r^2 = .64$, p = .030; viruses = $2.70 \times 10^6 - 2.93 \times 10^6$ B-U control, $r^2 = 0.69$, p = .020, n = 7. (c) Schematic representation of the interplay between the three types of control on marine heterotrophic prokaryotes using mean values for each Malaspina leg. The intensity of the contour plots (z-axis) represents the strength of the temperature control in the space defined by bottom-up (x-axis) and top-down (y-axis) controls.
In other words, temperature acted when both bottom-up and top-down controls were modest. Figure S3 shows the mean values of the bottom-up, temperature and top-down control indices in the three oceans, distinguishing between the northern and southern hemispheres in the Atlantic and Pacific. Although not all basins shared the same spatial coverage (Fig. S2a), the South Pacific consistently differed from the other oceans in the three types of control. The lowest mean bottom-up control characterizing these waters (0.05) was significantly lower than in the rest (ANOVA, $F = 10.07$, $p \ll .001$, $n = 5$) due to the concurrent maximum in mean top-down control (1.97, $F = 7.48$, $p = .0007$, $n = 5$). As a consequence, this basin was also the only one that differed significantly in temperature control. With a mean value of 0.92 eV, the temperature control index of the South Pacific was significantly lower than the N Atlantic and the Indian oceans ($F = 5.26$, $p = .0007$, $n = 5$).

Our analysis also showed a pervasive latitudinal gradient in the strength of temperature control, which was highest in subtropical waters and declined towards the Equator in the three oceans (Figure 2a). A consistent relationship emerged when data were binned by 2° latitude irrespective of the ocean basin, in which the highest temperature control indices (defined here as $E$ values >1.5 eV) were usually found at latitudes higher than 20°N and 30°S (Figure 2b).

With a 1°C increase in sea surface temperature (SST), the predicted biomass of heterotrophic prokaryotes in surface waters was in general higher than current measurements. A mean value of 1.13 was found for the ratio between the 1°C warmer PHB estimates and the current PHB values (PHB+1°C:PHB, range 0.42–4.70). When averaged per ocean basin, this PHB+1°C:PHB ratio displayed a significant negative relationship with mean SST ($r = -.89$, $p = .045$, $n = 5$). The modelled values ranged from a 29% higher biomass of heterotrophic prokaryotes in the cooler Indian waters to a 20% decrease in the warmer South Pacific (Figure 3).

4 | DISCUSSION

Temperature, microbial biomass and activity peaked in the upper layers (<200 m depth) while minimum values were consistently found at greater depths (>2,000 m), as well known (Aristegui, Gasol, Duarte, & Herndl, 2009). This vertical gradient allowed us to estimate indices for temperature, bottom-up and top-down controls of heterotrophic prokaryoplankton at each station of the seven legs into which the Malaspina 2010 cruise was divided (Fig. S2). Although the composition

![Figure 2](image)

**Figure 2** Relationship between latitude and temperature control of heterotrophic prokaryotes in the global ocean. (a) Relationship between temperature control index and latitude for all Malaspina data pooled. (b) Mean ± SE temperature control (Temp C) index versus latitude for all data binned by 2° latitude. Polynomial fitting shown for reference: Temp C = 1.1444 + 0.0081 × lat + 0.0008 × lat², $r^2 = .52$, $p < .01$, $n = 38$. The dashed line represents the 1.5 value used as a threshold for major control by temperature (see the text for details), predicted to be exceeded in the red dashed areas north of ca. 20°N and south of ca. 30°S.

![Figure 3](image)

**Figure 3** Relationship between the mean ratio of predicted prokaryotic heterotrophic biomass (PHB) at +1°C to the measured value vs. mean sea surface temperature (SST) in the five ocean basins sampled in the Malaspina cruises (see Figure 2). Error bars represent ± SE. See the text for details. Fitted line represents ordinary least squares linear regression: PHB+1°C:PHB ratio = 3.25 – 0.09 SST, $r^2 = .79$, $p = .045$, $n = 5$ ([Colour figure can be viewed at wileyonlinelibrary.com])
and biogeochemical role of the prokaryotic assemblages change markedly with depth (Giovannoni, Rappé, Vergin, & Adair, 1996), and surface and deep processes are not connected at short timescales, the use of the same vertical range for all stations permitted us to assess the relative differences between sites and oceanic regions when integrated from the epipelagic through the bathypelagic zones. By pooling all data collected at the same location to estimate bottom-up and temperature regulation we implicitly considered each station as a self-contained spot. Integrating microbial abundance and activity across depth is justified by the fact that sinking organic carbon is the main support for heterotrophic processes in the entire water column (Arístegui et al., 2002). Values in the upper layers and deeper in the water column of most variables considered here were significantly and positively correlated. For instance, integrated PHB and PHP in the upper 200 m and in the 200–4,000 m interval had correlation coefficients of 0.48 and 0.57, respectively, \( p < .001, n = 106 \) and 110). Surface primary production also showed positive correlations with mesopelagic fish biomass (Irigoien et al., 2014), further supporting the validity of an integral water-column approach.

The mean log PHB versus log PHP linear regression slope of 0.47 indicates an overall moderate bottom-up control of heterotrophic prokaryotes in subtropical and tropical waters (Ducklow, 1992; Gasol et al., 2002). This finding supports the contention that their biomass is largely constrained by resource availability in the global ocean (Ducklow, 2000; Gasol & Duarte, 2000), particularly so in the deep ocean (Arrieta et al., 2015). We used the apparent activation energy (\( E \)) of PHP over the same depth interval as the temperature control index. The mean \( E \) obtained in this study was more than twice the mean value assumed for heterotrophic organisms (0.65 eV, Gillooly, Brown, West, Savage, & Charnov, 2001). Recent experimental work on the activation energy of the specific growth rates of heterotrophic prokaryotes has shown that \( E \) can vary widely on a seasonal scale with low values in periods of nutrient limitation (Huete-Stauffer, Arandia-Gorostidi, Díaz-Pérez, & Morán, 2015). Spatially, higher \( E \) values are usually found in cold environments (Mazuecos et al., 2015; Vaquer-Sunyer, Duarte, Santiago, Wassmann, & Reigstad, 2010). In this regard, we systematically included in our analyses data from the base of the bathypelagic zone, characterized by uniformly low temperatures (1-4°C, Table S1). In a recent study compiling data from the global ocean, Lønborg et al. (2016) have shown that the activation energy of prokaryotic heterotrophic production was significantly higher in cold bathypelagic waters (2.36 eV) than in the epipelagic zone (0.39 eV).

The same inverse relationship between temperature and bottom-up control indices for values of the latter higher than 0.3 (Figure 1a) had been consistently observed in estuarine (Shiah & Ducklow, 1994) and coastal (Calvo-Díaz et al., 2014) waters. However, slight differences in the methods used and in the extension of the water column being considered (surface or a few 10s of metres vs. >1,000 m here) make our present numbers not directly comparable to theirs. \( E \) values higher than 2 eV coincident with moderate to low bottom-up controls (≤0.4) were recently reported for the Arctic (Maranger, Vaqué, Nguyen, Hebert, & Lara, 2015). Similar to the annual variability found in a temperate ecosystem (Huete-Stauffer et al. 2015, Calvo-Díaz et al., 2014), we interpret the inverse temperature control versus bottom-up control relationship as a relief of resource availability stress. That is, when dissolved organic matter is no longer limiting, prokaryotes will not respond to further increases in substrate inputs and then temperature takes the rule in the expected way, that is warming results in increased metabolic activity due to acceleration of the cellular enzymatic machinery. The loss of the negative relationship between temperature and bottom-up controls at low values of the latter index points directly at strong top-down control as the most plausible cause. Indeed, significantly negative relationships were found when we compared the abundances of the organisms responsible for most of the losses of heterotrophic prokaryotes with the mean bottom-up control indices at each leg (Figure 1b). It might be argued that viral and HNFs abundances are rough proxies for actual top-down control. However, given the well-known covariance between the abundances of both planktonic groups and that of heterotrophic prokaryotes (Weinbauer & Peduzzi, 1995), a higher abundance of predators or parasites relative to their prokaryotic prey is frequently indicative of stronger control (Gasol et al., 2002). In support of this, the experiments of bacterial mortality performed at 11 stations (mostly 3 depths each) distributed along the different basins (E. Lara, D. Vaqué, E. L. Sà, J. A. Boras, A. Gomes, E. Borrull, C. Diez-Vives, E. Teira, M. C. Pernice, F. C. García, I. Forn, Y. M. Castillo, A. Peiró, G. Salazar, X. A. G. Morán, R. Massana, T. S. Catalá, G. M. Luna, S. Agustí, M. Estrada, J. M. Gasol, & C. M. Duarte, unpublished data) showed positive, significant correlations between the abundance of viruses and viral lysis (\( r = .47, p = .009, n = 30 \)) and the abundance of HNFs and grazer mortality (\( r = .93, p < .001, n = 23 \)). We argue that the strong top-down control on heterotrophic prokaryotes found in the South Pacific was the likely reason offsetting the negative relationship between bottom-up and temperature controls found elsewhere (Figure 1a). Moreover, both bottom-up (by definition in the model we used) and temperature controls played a minor role when top-down control reached its maximum. The lowest values of bottom-up and temperature control indices coincided with the highest top-down control in those South Pacific stations (Fig. S3), all three variables significantly different from either all or most of the rest of the oceanic basins surveyed.

Superimposed upon site-specific differences in the bottom-up versus top-down balance, we identified an effective increase in the strength of temperature control with latitude, such that the most temperature-sensitive communities were consistently found northern of 20°N and southern of 30°S (Figure 3b). This zonal asymmetry is coherent with the northward displacement of the equatorial upwelling, injecting inorganic nutrients into the upper layers and relieving the general oligotrophy (and strong bottom-up control) of tropical and subtropical waters. Previous studies (Gasol, Vázquez-Dominguez, Vaqué, Agustí, & Duarte, 2009; Hoppe, Gocke, Koppe, & Begler, 2002) had shown that heterotrophy (i.e. bacterial carbon demand exceeding concurrent primary production) was more pronounced and tended to extend further at southern than at northern
latitudes, while temperature was more strongly correlated to bacterial activity in the N Atlantic.

Globally, surface temperature covaries negatively with inorganic nutrient inputs in the ocean (Kamykowski & Zentara, 1986), with the conspicuous exception of the equatorial region. Hence it would be tempting to ascribe the observed latitudinal increase in temperature control to increased inorganic nutrient availability. High nutrient concentrations would result in higher bioavailable DOM from enhanced primary production, thereby potentially allowing temperature to have a major impact on heterotrophic prokaryotes productivity. However, no significant relationships were found between the temperature control index and phytoplankton biomass or production rates (Estrada et al., 2016; Marañón et al., 2016; Pinedo-González et al., 2015) during the Malaspina circumnavigation ($p > .30, n = 96$ and 106, respectively). This can be explained by the fact that the high nutrient, high phytoplankton biomass and productivity stations located between 20°N and 10°S were also characterized by very high top-down or bottom-up control indices (Fig. S2), thus diminishing temperature control.

By combining the temperature and bottom-up control indices at each site (Figure 1a, Fig. S2b,c) we were able to predict the effect of a future 1°C temperature rise on the biomass of heterotrophic prokaryotes in surface subtropical and tropical waters. The negative relationship found between the average PHB$_{1-3}$-PHB ratios and the mean surface temperature in the basins surveyed (Figure 3) suggests that increases in the standing stocks of heterotrophic prokaryotes are more likely to be met in cooler, higher latitude waters than in the tropical region. No significant increases in PHB are expected at mean annual temperatures at the surface higher than 26°C, according to our model. In this regard, a temperate North Atlantic site, with a mean annual temperature of 15.8°C, has already undergone a decadal increase in the biomass of low nucleic acid content bacteria (typically made up of oligotrophic taxa), concurrent with a widespread reduction in cell size, in seeming response to warming (Morán et al., 2015). This hypothesis of a greater effect of future ocean warming on microbial standing stocks at middle to high latitudes compared with the tropics agrees with a recent phytoplankton model (Ward, 2015). It may also help focus the predicted increase in the contribution of small cells, especially cyanobacteria, to total phytoplankton (Flöambaum et al., 2013; Pittera et al., 2014; S. Agustí, personal communication). Thus, while picophytoplankton biomass will likely increase with warming in temperate and subpolar waters (Morán, López-Urrutia, Calvo-Díaz, & Li, 2010), in the tropical and subtropical region cyanobacteria and small autotrophic eukaryotes might be subject to the same loose control by temperature as their heterotrophic counterparts shown here. However, the projected higher impact of global warming in polar regions may itself slow down as they reach milder temperatures, although these hypotheses will have to be confirmed by sustained observations.

Temperature regulation of marine heterotrophic microbes could be seen as a latitudinal opening in the otherwise solid edifice built by the mutually opposing bottom-up and top-down controls. Only within a narrow window or “breach” of intermediate (i.e. not too high or too low) bottom-up and top-down values (approximately between 0.3 and 0.6 and between 0.8 and 1.2, respectively, in this analysis, Figure 1c) did temperature have a preponderant role in enhancing heterotrophic prokaryoplankton biomass production. The subtropical and tropical regions sampled by the Malaspina expedition comprise 70% of the total area covered by the oceans. Consequently, the highly structured relationships between temperature, bottom-up and top-down regulation revealed in Figure 1 are likely to be universal, providing a novel, comprehensive framework with which to assess the future role of marine microorganisms in biogeochemical cycling. Neglecting the interactions between the biological and temperature controls found in this study may result in biased predictions of the response of ocean biota to global change. We conclude that the effects of global warming on marine planktonic prokaryotes will be strongly dependent on the prevailing mode of regulation, which in turn appears to be closely associated with latitude.

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