Temperature sensitivities of microbial plankton net growth rates are seasonally coherent and linked to nutrient availability

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Summary

Recent work suggests that temperature effects on marine heterotrophic bacteria are strongly seasonal, but few attempts have been made to concurrently assess them across trophic levels. Here, we estimated the temperature sensitivities (using activation energies, $E$) of autotrophic and heterotrophic microbial plankton net growth rates over an annual cycle in NE Atlantic coastal waters. Phytoplankton grew in winter and late autumn ($0.41 \pm 0.16$ SE d$^{-1}$) and decayed in the remaining months ($-0.42 \pm 0.10$ d$^{-1}$). Heterotrophic microbes shared a similar seasonality, with positive net growth for bacteria (0.14–1.48 d$^{-1}$), while nanoflagellates had higher values (> 0.4 d$^{-1}$) in winter and spring relative to the rest of the year (−0.46 to 0.29 d$^{-1}$). Net growth rates activation energies showed similar dynamics in the three groups (~1.07 to 1.51 eV), characterized by maxima in winter, minima in summer and resumed increases in autumn. Microbial plankton $E$ values were significantly correlated with nitrate concentrations as a proxy for nutrient availability. Nutrient-sufficiency (i.e., > 1 μmol l$^{-1}$ nitrate) resulted in significantly higher activation energies of phytoplankton and heterotrophic nanoflagellates relative to nutrient-limited conditions. We suggest that only within spatio-temporal windows of both moderate bottom-up and top-down controls will temperature have a major enhancing effect on microbial growth.

Introduction

Disentangling the factors controlling the dynamics of planktonic microorganisms biomass and productivity is a central topic in marine microbial ecology (Ducklow, 2000; Kirchman, 2016). Regulation by resource availability (bottom-up), mortality including predation and viral lysis (top-down) and temperature explain most of the variability of the standing stocks and activity of phytoplankton, heterotrophic prokaryotes and protistan grazers (Yang et al., 2016). These three types of control operate simultaneously with varying contributions to the observed variability, but some general patterns have been recently suggested (Morán et al., 2017). Strong bottom-up control is only possible when top-down control is minimized and vice-versa (Billen et al., 1990; Ducklow, 1992). Interactions between temperature and nutrients are complex (Cross et al., 2015; López-Urrutia and Morán, 2007; Sin-sabaua and Shah, 2010). Although for heterotrophic prokaryotes these interactions were hypothesized to be more prominent in cold waters (Pomeroy and Wiebe, 2001), whether autotrophic or heterotrophic eukaryotes are favoured by low temperatures is still debated (Caron and Hutchins, 2013).

Observations and modelling efforts suggest that the effect of future warming on phytoplankton and bacterio-plankton will be more pronounced in higher latitude, colder environments (Kirchman et al., 2009; Morán et al., 2017; Ward, 2015), generally characterized by higher inorganic nutrient concentrations (Arrigo, 2005; Sarmiento et al., 2004). At mid-latitudes, the strength of bacterial bottom-up control was inversely related to temperature control (Shiah and Ducklow, 1994; Shiah et al., 1999; Calvo-Díaz et al., 2014), suggesting the alternation of two phases in the seasonal cycle, one dominated by temperature and another one by substrate regulation (Shiah et al., 2003). Temperature appears to play a major role at low temperatures, when substrates are in excess...
due to phytoplankton blooming in spring and autumn after water-column mixing brings new nutrients into the surface layers (Ducklow et al., 2002), whereas nutrient-limited in summer stratified conditions would render temperature unimportant in promoting microbial growth (Calvo-Díaz et al., 2014; Morán et al., 2010). This hypothesis has been recently confirmed experimentally in temperate coastal waters (Huete-Stauffer et al., 2015; Arandia-Gorostidi et al., 2017). On a large geographical scale, temperature regulation of heterotrophic bacteria and archaea decreased toward the Equator (Morán et al., 2017).

The temperature control or sensitivity of planktonic metabolic rates has been traditionally represented by the temperature coefficient $Q_{10}$ (i.e., the ratio of rate increase for a $10{\degree}C$ temperature rise), with values around 2 for maximum growth rates under natural conditions (Eppley, 1972; Chen and Laws, 2017). More recently, since the introduction of the metabolic theory of ecology (MTE, Brown et al., 2004), in which individual metabolic rates scale allometrically with body mass and increase exponentially with temperature according to the Boltzmann factor ($e^{-E/kT}$), activation energies ($E$) have been favoured as a way of representing the response of organisms to temperature. A simple rule of thumb equals a $Q_{10}$ of 2.4 at $20{\degree}C$ to the average $E$ value of 0.65 eV predicted for heterotrophs (Gillooly et al., 2001).

Monthly temperature manipulation experiments in coastal NE Atlantic waters showed a marked seasonality in the activation energies of heterotrophic bacterioplankton specific growth rates (Huete-Stauffer et al., 2015; Arandia-Gorostidi et al., 2017). Values close to the predicted 0.65 eV were only reached in late winter and spring, coincident with the presence of seasonally recurring phytoplankton blooms in the region (Bode et al., 2011), suggesting that resource availability was a pre-requisite for temperature to drive bacterial growth. Results reported in Huete-Stauffer and colleagues (2015) and in Arandia-Gorostidi and colleagues (2017) were based on experiments isolating heterotrophic prokaryotes from larger components of the microbial food web, specifically phytoplankton and protistan grazers, and therefore aimed at determining the response to temperature of specific growth rates. Here, we report on the joint dynamics of phytoplankton, heterotrophic bacterioplankton and heterotrophic nanoflagellates in the parallel experiments conducted with the entire microbial community, thus focused on the effects of temperature on net rather than gross growth rates. We tested two related hypotheses: (i) the temperature sensitivities of the net growth rates of phytoplankton, heterotrophic bacteria and heterotrophic nanoflagellates are temporarily coupled, (ii) their activation energies are intimately linked to the balance between resource and predator controls.

**Results**

**Ambient microbial plankton stocks and net growth rates**

Ambient phytoplankton biomass (chl $a$) showed a bimodal distribution with values greater than 1 µg l$^{-1}$ between March and early May and again from October through December. Major contributions of large-sized cells (i.e., microphytoplankton) were only observed in March and May, when they made most of the total biomass (Fig. 1A). Picophytoplankton contributions ranging from 30% to 60% were observed from June to November, similarly to previous years (Calvo-Díaz et al., 2008). Total chlorophyll $a$ displayed two periods of positive net growth (winter and late autumn, much more marked in the former), generally matching mixing conditions and high inorganic nutrient concentrations in the water column (Table 1), separated by a widespread net decay in between (Fig. 1B). Net chl $a$ values ranged from $-1.23$ to 0.98 d$^{-1}$ and they depended on the initial inorganic nutrient concentrations (Table 1, $r = 0.64$, $p = 0.002$ for nitrate and $r = 0.46$, $p = 0.016$ for phosphate, $n = 12$), so that consistent positive growth only occurred at nitrate concentrations higher than 2.3 and 0.10 µmol l$^{-1}$ of phosphate (0.59 and 0.06 µmol l$^{-1}$, respectively, in November). Inorganic nutrients evolution during the incubations generally matched that of phytoplankton, so that the higher their consumption the higher the chl $a$ net growth rate (e.g., $r = 0.64$, $p = 0.024$, $n = 12$ for NO$_3$ in the in situ temperature incubation). In the in situ temperature treatment nitrate was produced, although at very low amounts, rather than consumed in 4 months, while phosphate was always consumed except in August, when it virtually did not change (Table 1).

Expectedly, the uptake rates (u.r.) of both nutrients covaried strongly: NO$_3$ u.r. $= 0.19 + 17.6$ PO$_4$ u.r. ($r^2 = 0.84$, $p < 0.0001$, $n = 12$). As for phytoplankton, a bimodal distribution was also observed for heterotrophic bacterioplankton abundance (Fig. 1C), although the secondary peak was found earlier in the year and only slightly higher than the spring one (9.93 $\times$ 10$^5$ cells ml$^{-1}$). Bacterial abundance dropped to its annual minimum (1.96 $\times$ 10$^5$ cells ml$^{-1}$) only 3 weeks after the first maximum. HNA bacteria generally dominated the community during the first half of the year (45%–97% of the total), dropping to an mean 38% ± 8% SD thereafter. The contribution of HNA cells (%HNA) was significantly correlated ($r = 0.76$, $p = 0.004$, $n = 12$) with the net growth rates of the total community, which ranged from 0.14 to 1.48 d$^{-1}$ (Fig. 1D). The abundance of heterotrophic nanoflagellates (Fig. 1E) showed a clear peak in late spring-early summer
Fig. 1. Seasonal variability of the abundance and net growth rates of phytoplankton (A, B), heterotrophic bacteria (C, D) and heterotrophic flagellates (E, F) at the surface of the study site at ambient temperature. Error bars represent standard deviation of triplicate measurements.

Table 1. Sampling date, ambient temperature, stratification index (SI) and inorganic nutrient concentrations and their apparent uptake rates at the in situ temperature incubations

| Date   | Temp (°C) | SI (×10⁻³ m⁻¹) | NO₃ concentration (μmol l⁻¹) | PO₄ concentration (μmol l⁻¹) | NO₃ apparent uptake rate (μmol l⁻¹ d⁻¹) | PO₄ apparent uptake rate (μmol l⁻¹ d⁻¹) |
|--------|-----------|-----------------|-----------------------------|----------------------------|----------------------------------------|----------------------------------------|
| 2012   |           |                 |                             |                            |                                        |                                        |
| Jan 18 | 13.8      | 0               | 2.34                        | 0.23                       | 0.983                                  | 0.010                                  |
| Feb 10 | 12.6      | 0               | 4.04                        | 0.10                       | 0.245                                  | 0.026                                  |
| Mar 13 | 12.3      | 2               | 4.20                        | 0.22                       | 1.474                                  | 0.083                                  |
| May 2  | 13.0      | 7               | 1.32                        | 0.11                       | 0.218                                  | 0.018                                  |
| May 23 | 14.2      | 6               | 0.06                        | 0.06                       | −0.048                                 | 0.012                                  |
| Jun 14 | 16.5      | 8               | 0.10                        | 0.08                       | −0.008                                 | 0.012                                  |
| Jul 3  | 18.3      | 17              | 0.46                        | 0.06                       | −0.002                                 | 0.006                                  |
| Aug 2  | 21.2      | 25              | 0.64                        | 0.00                       | 0.021                                  | −0.000                                 |
| Sep 11 | 20.9      | 25              | 0.27                        | 0.06                       | 0.145                                  | 0.043                                  |
| Oct 19 | 18.3      | 3               | 0.21                        | 0.03                       | 0.013                                  | 0.014                                  |
| Nov 15 | 16.3      | 4               | 0.59                        | 0.06                       | 0.013                                  | 0.010                                  |
| Dec 11 | 13.7      | 2               | 3.51                        | 0.22                       | −0.297                                 | 0.006                                  |

Negative values indicate production rather than consumption.
(> 900 cells ml$^{-1}$), following the first maximum of heterotrophic bacteria and likely causing the large decrease in their abundance observed in May (Fig. 1C). A secondary minor peak was found in November (631 cells ml$^{-1}$), with minimum values (ca., 350 cells ml$^{-1}$) observed in January and October. Net growth rates (Fig. 1F) were positive (0.09–1.16 d$^{-1}$), except in July and November, in which their abundances decayed at −0.45 and −0.13 d$^{-1}$ respectively. Although their net growth rates were not further assessed, the abundance of ciliates and dinoflagellates, also potential predators of bacteria and phytoplankton, was also measured in the initial water samples. Ciliates peaked in May and late autumn (> 20 000 cells l$^{-1}$), while dinoflagellates showed higher variability, with high values (> 80 000 cells l$^{-1}$) also in September (Supporting Information Table S2). Minima (< 1000 ciliates l$^{-1}$ and 10 000–20 000 dinoflagellates l$^{-1}$) were usually found in winter. Dinoflagellates were only significantly correlated with total chlorophyll a ($r = 0.58$, $p = 0.046$, $n = 12$), suggesting they were mostly autotrophic or mixotrophic.

**Effect of temperature on microbial plankton net growth rates and nutrient dynamics**

The temperature sensitivity of the three planktonic groups net growth over the year was described by the activation energy of their respective $\mu_{net}$ values. The activation energy of phytoplankton growth in the experiments (Fig. 2A) followed a similar seasonality to the corresponding net growth rates (cf. Fig. 1B), with the minimum negative value (i.e., higher decay at higher temperature) in September (−1.07 eV) and the maximum positive value (i.e., higher growth at higher temperature) in March (1.51 eV). The activation energies of heterotrophic bacterioplankton varied from −0.14 eV in July to 0.67 eV in early May (Fig. 2B). Contrary to phytoplankton, bacterial growth $E$ values were positive in 92% of the months. However, consistently higher (> 0.25 eV) values were only found in the first 5 months of the year, in accordance with the results of the pre-filtered samples (Huetestofer et al., 2015). The temperature sensitivity of heterotrophic nanoflagellates net growth rates also displayed a seasonal pattern with higher values (> 0.65 eV) at the beginning and end of the year (Fig. 2C). Although negative net growth rates at in situ conditions were found in July and November, only once we found a negative $E$ value (i.e., decrease rather than increase in $\mu_{net}$ with higher temperatures), in August.

Inorganic nutrient dynamics within the experiments also showed strong seasonal patterns in their temperature dependence, with higher consumption rates per 1°C increase in the first 3 months, peaking in March for both nutrients, while minima were found in spring as well as in summer for nitrate (Supporting Information Fig. S2). The temperature dependence of nitrate uptake rates was strongly correlated with the activation energy of phytoplankton ($r = 0.73$, $p = 0.008$, Supporting Information Fig. S3A). The activation energies of heterotrophic nanoflagellates net growth rates were significantly correlated with those of phytoplankton ($r = 0.67$, $p = 0.03$, Supporting Information Fig. S3E) and marginally with the temperature.
dependence of nitrate uptake \((r = 0.62, p = 0.054,\) Supporting Information Fig. S3C). The positive correlations between heterotrophic bacteria \(E_{\text{net}}\) values and those of phytoplankton (Supporting Information Fig. S3D) and heterotrophic nanoflagellates (Supporting material Information Fig. S3F) were not significant \((p > 0.05)\).

The clear seasonal shift in the response of microbial plankton net growth to temperature by simultaneously warming and cooling the ambient community (Fig. 1), described by the activation energy shown in Fig. 2, was related to nutrient availability (Table 1). The \(E_{\text{net}}\) values of the three groups were positively and significantly \((p = 0.12\) correlated with nitrate concentration (Fig. 3A). The overall regression with pooled data explained 43\% of the variance in microbial plankton net growth rates \(E\) values. Similar relationships were obtained for phosphate concentration (data not shown).

Figure 3B shows the mean temperature sensitivities of phytoplankton, heterotrophic bacteria and nanoflagellates net growth rates in nutrient-sufficient and nutrient-deficient conditions at the study site, using 1 \(\mu\text{mol NO}_3^-\) L\(^{-1}\) and 0.1 \(\mu\text{mol PO}_4^{3-}\) L\(^{-1}\) as thresholds. \(E\) values were higher under the nutrient sufficiency found from late autumn through early spring (December to early May) for all groups \((t\)-tests, \(p < 0.001\) for phytoplankton, \(p = 0.07\) for heterotrophic bacteria and \(p = 0.02\) for heterotrophic prokaryotes, \(n = 12\)). Differences were also significant with all data pooled \((t\)-test, \(p = 0.001, n = 34\)). The mean activation energy of microbial plankton net growth during those months \((0.68 \text{ eV})\) was indistinguishable from the theoretical value, but close to 0 \((0.02)\) from May through November.

![Fig. 3. A. Relationships between the activation energies of the three planktonic groups and in situ nitrate concentration. Continuous line represents the ordinary least squares linear regression with all data pooled: \(E = -0.06 + 0.24 \times [\text{NO}_3^-], r^2 = 0.43, p < 0.001, n = 34\). Correlation coefficients \((r)\) and significance level are also given next to each group legend. \(*, p < 0.05; ***, p < 0.001;\) ns, not significant. B. Mean \pm SE activation energies of phytoplankton (black bars), heterotrophic bacteria (white bars) and heterotrophic nanoflagellates (grey bars) at nutrient sufficiency and nutrient limitation (nitrate and phosphate concentrations higher and lower than 1 and 0.1 \(\mu\text{mol L}^{-1}\), respectively, \(n = 5\) and 7). Asterisk denote significant differences \((t\)-tests\) between nutrient sufficiency and nutrient limitation conditions: phytoplankton, \(p = 0.02\); heterotrophic nanoflagellates, \(p = 0.03\). Differences for heterotrophic bacteria were marginally significant \((p = 0.07)\).]

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Nitrate and phosphate dynamics in the experiments (mostly consumption, Table 1, Supporting Information Fig. S1) were as expected significantly correlated, with a N:P uptake ratio similar to the Redfield ratio (17.6). Likewise the requirements in other temperate ecosystems (e.g., Joint et al., 2002), inorganic nutrient concentrations below approximately 1 and 0.1 μmol l⁻¹ for nitrate and phosphate, respectively, resulted in algal loss rather than growth (cf. Table 1 and Fig. 1B). Phosphate was consumed even when nitrate was produced and phytoplankton decreased. Since nitrite and ammonium were not included in the analysis, our results suggest that on an annual basis phytoplankton could be co-limited by N and P (Moore et al., 2013). P limitation was recently found in another North Atlantic coastal site (Trommer et al., 2013). Higher temperatures resulted in enhanced phytoplankton growth or loss depending on the environmental conditions. A similar result was reported in an African lake (Tadonléké, 2010), where warming promoted primary production at high phosphate concentrations but inhibited it at low values. Positive activation energies of phytoplankton μnet were always higher than the predicted 0.32 eV (Allen et al., 2005; López-Urrutia et al., 2006), Fig. 2A), supporting the hypothesis that cyanobacteria and eukaryotic algae show higher E than terrestrial plants (Chen and Laws, 2017) because of the presence of CO₂ concentrating mechanisms (Raven et al., 2011), which would approach the E value of net photosynthesis to that of Rubisco maximal enzymatic rate (Tcherkez et al., 2006). In the months of algal mortality, higher temperatures made the negative μnet values even more negative probably by inducing phytoplankton cell lysis (Agustí and Duarte, 2000) or consumption by protists (Chen and Liu, 2015; Rose and Caron, 2007).

The net growth rates of heterotrophic bacterioplankton peaked with higher contributions of HNA cells to total abundance, a percentage that varied widely from 24% to 97% (Fig. 1C). Contrary to other studies showing lagged relationships (Gomes et al., 2015), including our study site (Franco-Vidal and Morán, 2011), in 2012 ambient % HNA explained 58% of the variance in concurrent bacterial μnet values. In order to determine the true temperature dependence of bacterioplankton growth rates it is better to isolate them from heterotrophic nanoflagellates and other possible predators (e.g., Yokokawa and Nagata, 2005; Huete-Stauffer et al., 2015; Sánchez et al., 2017). However, by prefiltering the water samples we would also remove primary producers and the flux of recent photosynthetic, an input that may be important under nutrient limitation (Keller and Hood, 2013). The similar seasonal patterns of heterotrophic bacterioplankton growth rates and activation energies in our treatment and in the 0.8 μm-prefiltered one described in detail by Huete-Stauffer and colleagues (2015) indicates that the temperature dependence of specific growth rates would still hold to net growth rates, suggesting that the opposing effects of eliminating protistan grazers and phytoplankton cancelled each other. However, while ambient bacterial specific growth rates (< 0.8 μm) and our μnet values were indistinguishable (paired t-test, p = 0.14, n = 12), the corresponding E values did differ significantly in both treatments, with consistently higher values in the pre-filtered treatment (paired t-test, p = 0.008, n = 12). The presence of other microbes including HNFs, with their own temperature dependence (Fig. 1C), necessarily affected the temperature dependence of their prey μnet values.

Warming was reported to have greater effects on bacterioplankton growth in the coolest months in Taiwan coastal waters (Tsai et al., 2016). Strong bottom-up control of heterotrophic bacteria in summer at the study site (Morán et al., 2010) was associated with little or no effect of temperature on bacterial production (Calvo-Díaz et al., 2014), demonstrating for NE Atlantic coastal waters the inverse relationship between both modes of control found by Shiah and Ducklow (1995) in a Chesapeake Bay salt marsh. The seasonal switch between temperature and resource regulation of bacterial growth, described for both freshwater (Felip et al., 1996) and marine ecosystems (Calvo-Díaz et al., 2014; Shiah et al., 2003) is further confirmed here with a new experimental dataset, in which we included the entire microbial community. The finding by Huete-Stauffer and colleagues (2015) that only in the late winter–spring period did bacteria specific growth rates approach the theoretical value of 0.65 eV was explained by elevated organic matter concentrations made available by phytoplankton blooms in that period (Bode et al., 2004), while the low summer E values were attributed to strong substrate limitation (López-Urrutia and Morán, 2007; Sinsabaugh and Shah, 2010), in line with previous studies carried out at the study site using different approaches (Morán et al., 2010; Calvo-Díaz et al., 2014). In the experiments reported here, extant DOM could have been supplemented by dissolved primary production (8%–47% of the total in four experiments carried out in 2013, Huete-Stauffer et al., 2018), microzooplankton grazing (Nagata, 2000) and/or algal mortality (Agustí and Duarte, 2000).

Heterotrophic nanoflagellates numbers clearly peaked (ca., 1000 cells ml⁻¹) after the spring maxima of
bacterioplankton and phytoplankton abundance. Similar lags had been described elsewhere (Tsai et al., 2008) and also in the study region (Granda and Anadón Álvarez, 2008). Their growth apparently affected strongly the standing stock of heterotrophic bacteria in the subsequent month (cf. Fig. 1F and 1C, correlation between HNFs $\mu_{\text{net}}$ in 1 month and bacterial abundance in the following one $r = 0.74$, $p = 0.009$, $n = 11$). A similar relationship explaining a higher percentage of variance was found with the abundance of *Synechococcus* and *Prochlorococcus* cyanobacteria ($r = 0.81$, $p = 0.003$, $n = 11$). The response to temperature of HNFs grazing rates was similar to bacterioplankton gross growth rates in coastal waters of Taiwan (Tsai et al., 2008), only slightly larger (0.1–0.6 $\mu$m) than heterotrophic bacteria.

**Different temperature sensitivity of autotrophs and heterotrophs net growth rates?**

A temperature of 16°C was suggested as a threshold value between heterotrophic bacteria being controlled by phytoplankton and the loss of this trophic relationship at the study site (Morán et al., 2010). Incidentally, 16°C was also the limit separating much higher activation energies of heterotrophic bacteria in cool relative to warm waters in the Adriatic (Šolic et al., 2017). High nutrient concentrations in the cooler period (Table 1) permitted consistently positive responses of heterotrophic prokaryotes growth to temperature while this response was weaker in the warm, nutrient-limited conditions of summer and early autumn (Huete-Stauffer et al., 2015; this study). Figures 2 and 3 show that the temporal variation in the temperature sensitivity of their bottom-up (i.e., phytoplankton supplying resources) and top-down (i.e., heterotrophic nanoflagellates preying upon them) control factors were indeed very similar and strongly dependent on initial conditions (Striebel et al., 2016).

High temperature control of the three planktonic groups, represented by $\mu_{\text{net}}$ activation energies approaching or even exceeding the respective theoretical values of 0.32 and 0.65 eV, clearly required nutrient-sufficient conditions (Table 1 and Fig. 3). The role of resource availability in modulating the temperature responses predicted by MTE had been demonstrated elsewhere (López-Urrutia and Morán, 2007; Sinsabaugh and Shah, 2010; Vaquer-Sunyer et al., 2015). Available light rather than nutrients was necessary for community metabolism to show significant, positive $E$ values at high latitudes (Hury et al., 2014). In the tropical and subtropical ocean, relieving nutrient limitation resulted in stronger temperature regulation of heterotrophic prokaryotes productivity from the epipelagic to the bathypelagic zone (Morán et al., 2017).

The activation energy of net growth rate also reflects predator–prey interactions through their respective activation energies, which according to MTE will be the same for heterotrophs (i.e., bacteria and nanoflagellates would be characterized by the same $E$ value for specific growth and mortality rates) or different in the case of autotrophs (i.e., $E$ for specific mortality rate would be higher than phytoplankton intrinsic growth rate, Allen et al., 2005; Brown et al., 2004; López-Urrutia et al., 2006; Rose and Caron, 2007). Our results show that the integration of possibly different $E$ values still results in a consistent seasonal pattern for the temperature sensitivities of the net growth rates of the three groups (Fig. 2). In this regard, Chen and Laws (2017) have recently questioned the idea that planktonic autotrophs and heterotrophs are characterized by different activation energies. The only evidence that heterotrophic $\mu_{\text{net}}$ activation energies were higher than those of autotrophic microbes, is the higher frequency of positive $E$ values (92% for heterotrophic bacteria and heterotrophic nanoflagellates, 50% for phytoplankton, Fig. 2). The fact that the positive temperature sensitivities of heterotrophic bacteria and nanoflagellates $\mu_{\text{net}}$ values held for longer periods than those of phytoplankton (Fig. 2) suggests heterotrophs might be more responsive in the mid to long term (i.e., decades to hundreds of years) to changing environmental conditions. Indeed, although artefacts in our estimated $E$ values and changes in community composition caused by bottle confinement cannot be discounted (Calvo-Díaz et al., 2011; Massana et al., 2001), a decade of monthly samplings at the site has revealed increments in heterotrophic bacteria biomass but unaltered phytoplankton stocks (Morán et al., 2015), consistent with the difference between the two planktonic groups shown here.
Toward a general model for predicting plankton responses to temperature

The combination of large-scale relationships between bottom-up, top-down and temperature controls on heterotrophic prokaryotes (Morán et al., 2017) with the net growth rates of three groups of microbes reported here allows us to propose a conceptual model relating all these variables (Fig. 4). Under strong bottom-up control (e.g., posed by low supply rate of resources) net growth rates can be low or even negative. The same consequence would have strong top-down control, in which predators consume their prey cells at a rate higher than they divide. Turning around the title of Hoekman’s (2010) study, top-down and bottom-up effects influence the relative importance of temperature. If both controls are only moderate (i.e., there are enough nutrients for growth and predation pressure does not overkill the prey), then temperature will play a role proportional to the net growth rate (Fig. 4A). This is confirmed by the significant relationship between net growth rates and their temperature response, explaining 40% of the variance of all data pooled (Fig. 4B). The intercept of the linear regression between net growth rates and their E values was not significantly different from 0 (p = 0.26), meaning that temperature was an irrelevant driver at very strong bottom-up or top-down controls precluding growth. It is also worth mentioning that the higher the negative net growth rate, the higher the effect of temperature in group loss, probably by enhancing the consumption rates of predators.

As discussed before, the temporal window of moderate bottom-up and top-down controls allowing positive, higher responses of microbial plankton $\mu_{\text{net}}$ to temperature seems highly predictable in our ecosystem. Ocean warming experienced by NE Atlantic waters is not uniform but more marked in late spring–summer than in winter or early spring (González-Gil et al., 2015; Holt et al., 2012), when surface temperatures are well below 16 °C at our site (Table 1) and major responses to temperature of heterotrophic bacterial abundance, cell size, biomass and growth rates were observed (Huete-Stauffer et al., 2015, 2016; Morán et al., 2015; this study). Figure 3B shows that in the nutrient-limited months (which are also the warmest at our site), further warming may result in limited or no net growth of other planktonic microbes, according to the low activation energies observed during this period. Conversely, temperature-enhanced growth may be widespread across microbial trophic levels in the next decades as long as nutrient-limited conditions are not encountered.

Notwithstanding the within and between groups variability in temperature sensitivities (e.g. Arandia-Gorostidi et al., 2017 for heterotrophic bacteria), we have found strongly coherent seasonal patterns in agreement with Burnside and colleagues (2014). The subtle equilibrium between bottom-up and top-down controls making temperature a relevant factor is intimately linked to inorganic nutrient concentrations, and likely also organic forms through cascading effects (Goldberg et al., 2017). We concur with Cross and colleagues (2015) that the future reshaping of microbial plankton communities will likely be determined by nutrient availability. Marañón and colleagues (2018) have recently arrived to the same conclusion as ours with phytoplankton photosynthesis and respiration rates. This synchronous variation in the temperature responses of the three microbial groups has other potential implications. In a sort of time-for-space substitution, the latitudinal gradients in the temperature regulation of heterotrophic prokaryotes recently found (Morán et al., 2017) could perhaps be extended to other planktonic groups, from phytoplankton (Ward, 2015) through heterotrophic nanoflagellates (Segovia et al., 2016) to higher trophic levels. The complex interactions between resources, predators and prey within marine microbial food webs are far from having been completely captured in our experimental design. However, we

Fig. 4. A. Conceptual model relating the interplay between bottom-up and top-down controls affecting the net growth rates of the three microbial plankton groups (green, phytoplankton; blue, heterotrophic bacteria; yellow, heterotrophic nanoflagellates) and their response to temperature. The intensity of the red colour represents the degree of the temperature response (i.e., more intense, higher activation energy) of positive and negative net growth rates. B. Relationship between the net growth rates ($\mu_{\text{net}}$) and the corresponding activation energies of phytoplankton, heterotrophic bacteria and heterotrophic nanoflagellates (HNFs) measured in our experiments. Continuous line represents the ordinary least squares linear regression with all data pooled: $E = 0.13 + 0.76 \times \mu_{\text{net}}, R^2 = 0.49, p < 0.001, n = 54.$
hypothesize that future warming may bring elevated microbial plankton stocks only in nutrient-sufficient periods or regions not subject to high bottom-up or top-down control, a situation that is usually associated with cooler temperatures. In the opposite scenario, widespread in tropical and subtropical waters year-round and during summer stratification at higher latitudes, elevated temperatures may envision unaltered or even decreased biomass of planktonic microbes in the upcoming decades.

Experimental procedures

About 12 incubation experiments were performed in 2012 with surface seawater collected from a mid-shelf station off Gijón/Xixón, Spain (Huete-Stauffer et al., 2015). Samples for determining in situ concentrations of inorganic nutrients, fractionated chlorophyll $a$ and the abundance of heterotrophic bacteria and nanoflagellates were collected from Niskin bottles attached to a SeaBird25 CTD probe. Density (sigma-t) data were used to calculate a stratification index (SI, m$^{-1}$) of the water column by subtracting the surface (5 m) value from that at 75 m and dividing it by the depth interval (70 m). A strong negative correlation ($r = -0.79$, $p < 0.001$, $n = 12$) was found between the SI and the depth of the upper mixed layer using the 0.05 kg m$^{-3}$ change over 5 m criterion used in previous works (e.g., Calvo-Díaz and Morán, 2006).

Water for the experiments was passed through a 200 μm mesh on board to remove mesozooplankton and brought in 20 l polycarbonate carboys to the lab usually within 2 h. There, 4 l acid-washed polycarbonate bottles were filled in triplicate with 2 l each and placed into light and temperature-controlled incubators at three different temperatures encompassing a 6°C difference: in situ, 3°C below and 3°C above. Irradiance (ca., 270 μmol photons m$^{-2}$ s$^{-1}$) was saturating for photosynthesis according to Morán and Scharek (2015). The photoperiod was adjusted to that of the sampling date. Incubations lasted between 4 and 7 days depending on the time needed for heterotrophic bacteria to reach stationary phase or start decaying (see, e.g., Supporting Information Fig. S1E and F). The growth of membrane-intact bacteria (Huete-Stauffer et al., 2015) was monitored on quasi-real time and on most occasions the maximum abundance was reached between day 3 and 5. Samples were taken daily for chlorophyll $a$ and heterotrophic flagellates and twice per day for heterotrophic bacteria.

Inorganic nutrients

Inorganic nutrients were sampled at initial, intermediate and final time of the incubation (Supporting Information Fig. S1A and B). Samples (5–10 ml) were frozen upon collection and kept at −20°C until analysis with a Skalar San Plus System autoanalyzer. Nitrate and phosphate concentrations were measured as described in Morán and Scharek (2015). Daily apparent nutrient consumption or production rates (μmol l$^{-1}$ d$^{-1}$) at the different experimental temperatures were calculated as linear regressions of their concentrations during the incubation period.

Chlorophyll $a$

At the onset of the experiments size-fractionated (micro-, nano- and picoplankton) chlorophyll $a$ (chl $a$) samples were obtained by sequentially filtering 50 ml through 25 mm polycarbonate filters of 20, 2 and 0.2 μm pore sizes respectively. Additionally and daily throughout the incubations, 50 ml were filtered through 25 mm GFF glass fibre filters (Whatman) to obtain total chlorophyll $a$ concentrations. Filters were stored at −20°C until analysis in a LS-55 Perkin Elmer spectrofluorometer calibrated with pure chlorophyll $a$, after being left overnight in 90% acetone for pigment extraction. We used the method without acidification.

Heterotrophic bacteria

Heterotrophic bacterial abundances were estimated with a BD FACSCalibur flow cytometer equipped with a blue argon laser (488 nm) using the protocols described in Gasol and Morán (2015). About 1.8 ml was collected twice a day from each temperature incubation, fixed with final concentrations of 1% paraformaldehyde and 0.5% formaldehyde and frozen at −80°C. For analysis, samples were thawed at room temperature, stained for 10 min with SybrGreen I (Molecular Probes) and run through the flow cytometer at low flow rate (15–20 μl min$^{-1}$, calibrated daily) until 10 000 events were acquired. On a plot of green fluorescence (FL1) versus light scatter at 90° (or side scatter, SSC) we separated the bacteria into two groups based on their relative nucleic acid content, bimodal with a clear separation at about 20 relative units of FL1: high nucleic acid (HNA) and low nucleic acid (LNA) cells.

Heterotrophic nanoflagellates

The abundance of heterotrophic nanoflagellates (HNFs) was determined in 5 ml samples fixed with glutaraldehyde (1% final concentration) by flow cytometry using the same equipment as for heterotrophic bacteria. About 1.8 ml subsamples were stained during 10 min at room temperature in the dark with SybrGreen I and analysed at high flow rate (mean 178 μl min$^{-1}$) until 10 000 events were acquired. HNFs were distinguished from bacteria and phytoplankton in the cytograms based on their
relative green and red fluorescence and SSC signals [see details in Christaki and colleagues (2011)].

**Microbial plankton net growth rates and activation energies**

Supporting Information Figure S1 shows the dynamics of nitrate and the abundance of phytoplankton, heterotrophic bacteria and heterotrophic nanoflagellates in the February and October incubations. The net growth rates ($\mu_{\text{net}}$) of each microbial group were calculated for each experimental temperature by determining the slope of the linear regression of ln-transformed cell numbers (total chlorophyll a concentration for phytoplankton) versus time in days during the exponential phase of growth (positive $\mu_{\text{net}}$) or decay (negative $\mu_{\text{net}}$). The exponential phase was operationally defined by all data points conforming to a linear response (i.e., not showing a clear change in slope). Most $\mu_{\text{net}}$ values were significant regardless of their sign (see Supporting Information Table S1 as an example with detailed values for the February and October experiments). Negative net growth was seldom observed except for chlorophyll a (see Results).

The temperature sensitivity of microbial net growth rates was assessed by the activation energy ($E$) in eV, calculated in Arrhenius plots of ln-transformed $\mu_{\text{net}}$ values of the three replicates (2 in the case of HNFs, Supporting Information Table S1) versus experimental temperature ($1/KT$) as described in detail in Huete-Stauffer and colleagues (2015, see their Supporting Information Fig. S2). Although in that study heterotrophic bacteria were isolated through filtration from larger plankton, the coherent dynamics observed in these experiments with the rest of the microbial community present (see Results) justify the calculation of the activation energies of $\mu_{\text{net}}$ values. Here, a positive $E$ value means that the net growth rate (either positive or negative) increased with temperature while a negative $E$ value means that the net growth rate became lower (less positive in the case of positive $\mu_{\text{net}}$ or more negative for a negative $\mu_{\text{net}}$ value) at higher temperatures. The temperature dependence of inorganic nutrients dynamics was simply calculated as the change in concentration per °C increase, with positive and negative values corresponding to consumption and production respectively.

**Statistical analyses**

Pearson correlation coefficients and ordinary least squares linear regressions were performed using Statistica v11 software (StatSoft Inc.). Differences between datasets were assessed by t-tests, which were paired when comparing the results of heterotrophic bacteria growth rates and activation energies obtained with (Huete-Stauffer et al., 2015) and without pre-filtration through 0.8 μm pore-size filters (this study).

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Temperature sensitivity of microbial plankton

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Supporting Information

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