Testing the Metabolic Theory of Ecology with marine bacteria: Different temperature sensitivity of major phylogenetic groups during the spring phytoplankton bloom

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Although temperature is a key driver of bacterioplankton metabolism, the effect of ocean warming on different bacterial phylogenetic groups remains unclear. Here, we conducted monthly short-term incubations with natural coastal bacterial communities over an annual cycle to test the effect of experimental temperature on the growth rates and carrying capacities of four phylogenetic groups: SAR11, *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes*. SAR11 was the most abundant group year-round as analysed by CARD-FISH, with maximum abundances in summer, while the other taxa peaked in spring. All groups, including SAR11, showed high temperature-sensitivity of growth rates and/or carrying capacities in spring, under phytoplankton bloom or post-bloom conditions. In that season, *Rhodobacteraceae* showed the strongest temperature response in growth rates, estimated here as activation energy (E , 1.43 eV), suggesting an advantage to outcompete other groups under warmer conditions. In summer E values were in general lower than 0.65 eV, the value predicted by the Metabolic Theory of Ecology (MTE). Contrary to MTE predictions, carrying capacity tended to increase with warming for all bacterial groups. Our analysis confirms that resource availability is key when addressing the temperature response of heterotrophic bacterioplankton. We further show that even under nutrient-sufficient conditions, warming differentially affected distinct bacterioplankton taxa.

Introduction

One of the current challenges in marine ecology is to predict how global change will affect the structure and metabolism of marine biota. Ocean warming may increase surface temperature between 1 and 6°C depending on the CO₂ emissions scenario (Meehl *et al.* 2007; Collins *et al.*, 2013), posing a threat to future marine food webs (Edwards and Richardson, 2004; Wiltshire and Manly, 2004; Sarmiento *et al.*, 2010) and biogeochemical processes (Sarmiento *et al.*, 1998; Laws *et al.*, 2000). Since heterotrophic prokaryotes represent the largest living biomass in the oceans (Whitman *et al.*, 1998) and play a key role in all biogeochemical cycles, the effects of ocean warming on these planktonic organisms may have large consequences for ecosystem functioning (Kirchman *et al.*, 2005; Degerman *et al.*, 2013; Huete-Stauffer *et al.*, 2015). However, there is still no consensus about the metabolic response of heterotrophic bacteria to increasing temperatures (e.g. Li *et al.*, 2004; Sarmiento *et al.*, 2010, Morán *et al.*, 2011), and to what extent it is comparable to that of other organisms.

In the last decade, the Metabolic Theory of Ecology (MTE, Brown *et al.*, 2004) has emerged as a valuable framework to test the metabolic response to changes in temperature of different organisms, including heterotrophic bacteria (Daufresne *et al.*, 2009). The MTE assumes that the metabolic rates of all organisms are a combination of the allometric scaling of their body mass (West, 1997) and biochemical kinetics, which are temperature dependent as described by the Van't Hoff-Arrhenius relation (Arrhenius 1889; Gillooly *et al.*, 2001). Therefore, the MTE merges the effect of temperature and mass into a single equation that can be used from organisms to communities, allowing the effect of temperature to be tested experimentally. Although the MTE has successfully predicted the increase in metabolic rates with temperature in different organisms (López-Urrutia *et al.*, 2006; Bailly *et al.*, 2014), the predicted activation energy for heterotrophic metabolism (0.65 eV, Gillooly *et al.*, 2001; Brown *et al.*, 2004) has not been agreed upon in bacteria, for which variations between 0 and >1 eV have been reported (Sinsabaugh and Shah, 2010; Morán *et al.*, 2011; Huete-Stauffer *et al.* 2015). Yet, studies attempting to measure activation energies of environmental microorganisms are still scarce.

The MTE aims also at predicting the response to warming of other biological properties, such as the carrying capacity (i.e., the maximum density or biomass that the environment can sustain with the available resources). The carrying capacity has been hypothesized to decrease with temperature (Savage *et al.*, 2004, Brown *et al.*, 2004), since at higher metabolic rates, and thus higher resource requirements, the same energy supply will sustain a lower organism abundance. However, the

response of the carrying capacity to temperature is not free of controversy (Jiang and Morin 2004), as external forcing, especially resource availability and predation pressure, may affect the outcome of this relationship (Eiler *et al.*, 2003; Šolić *et al.*, 2009).

In general, substrate availability has been described as a key factor governing the temperature dependence of heterotrophic bacterial metabolism (López-Urrutia and Morán, 2007). A recent study has shown that the overall dependence of heterotrophic bacterial communities on temperature has a seasonal component, with stronger temperature effects in periods with high nutrients and phytoplankton biomass (Huete-Stauffer *et al.*, 2015). Yet, marine bacterial communities are highly diverse, and some of the widespread, abundant groups are markedly different ranging from oligotrophs to copiotrophs (Koch *et al.*, 2001; Lauro *et al.*, 2009; Yooseph *et al.*, 2010). Although disparity in the *in situ* growth rates of different phylogenetic groups of marine bacteria, such as SAR11, *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes*, has been reported previously in a few studies (see review in Kirchman 2016), the temperature-sensitivity of specific taxa is unclear and it has been addressed mostly with bacterial isolates (Ratkowsky *et al.*, 1982; Cho and Giovannoni, 2004). Thus, our knowledge of how temperature affects the growth and carrying capacity of dominant phylogenetic groups of environmental bacterial communities is still very limited.

In this study, we address the seasonal variability in the growth rates and carrying capacities of broad phylogenetic groups of coastal heterotrophic bacteria, hereinafter referred to as bacteria, while assessing their experimental responses to temperature. In order to test the predictions of the MTE with specific taxa, we incubated surface natural samples from the southern Bay of Biscay continental shelf at three different temperatures encompassing 6°C variability around the ambient value. We targeted in the analysis typical oligotrophs such as SAR11, as well as more resource-dependent (i.e. copiotrophic) bacteria such as *Bacteroidetes* or *Rhodobacteraceae*, in order to assess whether a systematic differential response to temperature, according to their trophic strategy, could be identified. Our results indicate that the taxonomic composition of marine bacteria communities may be highly relevant for predicting the microbial response to warming in a future ocean.

Results

Environmental setting

Surface seawater samples for temperature controlled incubations were collected monthly between January and December 2012 at a continental shelf station located in the southern Bay of Biscay, where effects of coastal pollution and river discharges are negligible. After collection, seawater was filtered by 0.8 μm pore-size filters to remove predators. *In situ* temperature ranged from 12.8°C in March to 21.2°C in August (Fig. 1a). *In situ* inorganic nutrients concentration (Fig. S1), peaked during winter (January and March for phosphate and dissolved inorganic nitrogen, DIN) and also in late autumn (December for silicate). Cell-specific bacterial heterotrophic production (sBP) was also measured at the onset of the experiments as an estimation of carbon bioavailability (Fig. 1a). sBP showed maximum values in spring (with a pronounced peak in April) and minimum values in summer and early winter. Total chlorophyll *a* concentration was measured in unfiltered seawater samples, as an estimation of the state of the phytoplankton community in environmental conditions. Maximum chlorophyll *a* concentrations were found in late autumn (December, 1.8 $\mu\text{g L}^{-1}$) and spring (March and April, 1.4 $\mu\text{g L}^{-1}$), while minimum values were found in summer (July, 0.1 $\mu\text{g L}^{-1}$), coinciding with the increase in water column stratification. We also took samples for estimating the abundance of heterotrophic nanoflagellates (HNF) by flow cytometry (Christaki *et al.*, 2011) before and after the pre-filtration treatment in order to assess the efficiency of 0.8 μm pore-size filters in removing potential bacterial predators. We found that filtration removed on average 98.4 ± 0.8 % of HNFs but only 8.4 ± 15.5 % of bacteria.

Seasonal dynamics of the in situ abundance and cell-size of bacterial groups

Abundance of heterotrophic bacteria at the initial time of the incubations showed a high variability, ranging from $2.60 \pm 0.04 \cdot 10^5$ cells mL^{-1} in May to $1.02 \pm 0.01 \cdot 10^6$ cells mL^{-1} in September (Fig. 1b). The contribution of SAR11, *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes* to the bacterial community (measured as the percentage of hybridized cells by CARD-FISH) was on average 54%, with a minimum in January (25%) and a maximum in March (68%). SAR11 was the most abundant group for most of the months, reaching up to 51% of total cells in August, but it dropped to 8% in May. *Bacteroidetes* was the second most abundant group ranging from 4% in August to 29% in May. *Rhodobacteraceae* and *Gammaproteobacteria* showed minimum contributions in November (1.5% and 3.2%, respectively), and maxima in April (16%). Seasonality of SAR11 abundance was clearly different from the other groups, as maximum abundances were found in summer rather than in spring. Accordingly, temperature was positively correlated with

SAR11 abundance (Table S1, Pearson $r=0.63$, $p\text{-value}=0.017$, $n=12$) and negatively with *Bacteroidetes* (Pearson $r=-0.60$, $p\text{-value}=0.023$, $n=12$).

Significant differences in the initial cell-size were found between different phylogenetic groups (Fig. 2 i,j,k,l). SAR11 ($0.038 \pm 0.007 \mu\text{m}^3$ excluding size of May) and *Gammaproteobacteria* ($0.038 \pm 0.010 \mu\text{m}^3$) showed the lowest biovolumes year-round, while cell-size of *Rhodobacteraceae* was significantly higher ($0.049 \pm 0.018 \mu\text{m}^3$, ANOVA, $p\text{-value}<0.001$). Non-significant differences were found between *Bacteroidetes* and the other analysed groups, and its average size was very similar to that found for *Rhodobacteraceae* ($0.048 \pm 0.016 \mu\text{m}^3$). The average cell-size of the bacterial community (for all DAPI-stained cells) was negatively correlated with *in situ* temperature (Pearson $r=-0.60$, $p\text{-value}=0.037$, $n=12$), with maximum values in spring (May, $0.044 \mu\text{m}^3$), and minimum values in summer (August, $0.027 \mu\text{m}^3$, Fig. S2).

Seasonal dynamics of in situ bacterial growth rates and carrying capacities

Every month, seawater was incubated at *in situ* temperature conditions during ca. 1 week in order to measure the growth rates and carrying capacities of each phylogenetic group. To calculate bacterial growth rates, we took 2 or 3 samples every day during the exponential growth phase of the bacterial community for CARD-FISH analysis (see experimental procedures). SAR11 showed the lowest growth rates at *in situ* temperature (Fig. 2 e,f,g,h, ANOVA, $p\text{-value}=0.002$, $n=12$), with a mean value of $0.50 \pm 0.38 \text{ d}^{-1}$. Their growth rates showed low variability year-round, but unusually high values ($>1 \text{ d}^{-1}$) were found in spring (April and May). Average growth rates of *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes* were higher, generally close to 1 d^{-1} , but showed different seasonalities. While maximum growth rates in *Gammaproteobacteria* and *Bacteroidetes* were found in spring (2.10 d^{-1} in May for both groups), *Rhodobacteraceae* had maximum growth rates in winter (1.50 d^{-1} in January). Occasionally, growth rates were very low at the end of the stratified season (September and October) for these three groups (0.25 d^{-1} to non-detectable growth).

The carrying capacity (K) was measured as the maximum bacteria abundance reached by the different phylogenetic groups during the incubations (see Fig S3). K showed contrasting seasonalities between SAR11 and the rest of the groups at *in situ* temperature. While SAR11 K values were minimum in Spring (May) and maximum in summer (July, Fig. 2a), the rest of the groups showed minimum K values in late summer-autumn (September for *Gammaproteobacteria* and October for *Rhodobacteraceae* and *Bacteroidetes*), and maximum K values in Spring (March for *Gammaproteobacteria* and April for *Rhodobacteraceae* and *Bacteroidetes*).

Temperature dependence of growth rates and carrying capacities of target bacterial groups

During the incubation experiments, we also determined the growth rates and carrying capacities of the different groups under three experimental temperatures (*in situ* temperature and 3°C below and above *in situ* temperature). To test the effect of temperature on these parameters, we followed the equations by the MTE (see Experimental Procedures). The effect of temperature on growth rates (Fig. 3 e,f,g,h) was summarized by the activation energy (E) of specific growth rates. All phylogenetic groups consistently showed their highest E values in months with high chlorophyll a concentrations: April for *Gammaproteobacteria* (0.89 ± 0.04 eV), May for *Rhodobacteraceae* (1.46 ± 0.06 eV), and December for SAR11 and *Bacteroidetes* (0.86 ± 0.12 eV and 1.03 ± 0.11 eV), but non-significant differences were observed between the mean annual E values of the different phylogenetical groups (ANOVA, p -value=0.06, n =12). Minimum E values (not statistically different from 0, indicative of no effects of temperature on growth rate) were usually found in summer. In spring, significant differences were found among the E of all groups (ANOVA, p -value<0.01, n =12). The lowest E values were found for SAR11 (0.55 ± 0.12 eV) and the highest for *Rhodobacteraceae* (1.43 ± 0.05 eV), with intermediate values for *Gammaproteobacteria* and *Bacteroidetes* (0.81 ± 0.13 eV and 0.89 ± 0.04 eV, respectively). Activation energies of *Gammaproteobacteria* and *Bacteroidetes* were positively correlated with their initial mean cell-sizes (Table S1, Pearson's r =0.60, p -value=0.023, n =12 and r =0.55, p -value=0.038, n =12 respectively).

The ratio between the growth rates in the +3°C and -3°C treatments was also calculated for each group in order to better visualize the magnitude of change within the temperature range assessed (Fig. 4). In accordance with the activation energies, this ratio showed conspicuous seasonal variations for all groups. Maximum values were found in spring, yet with significant differences between phylogenetic groups (ANOVA, p -value<0.001, n =4). *Rhodobacteraceae* showed the highest ratios (3.4) and SAR11 showed the lowest (1.7). The latter group, together with *Gammaproteobacteria*, yielded mean ratios below 1 in summer (i.e. decrease rather than increase in growth rate with a 6°C temperature rise) (Fig. 4).

Regarding the carrying capacities, either non-significant effect or an increase in K with temperature (i.e. positive K -RT values) was found for all bacterial groups, but some negative values (close to 0) were sporadically found (Fig 3 a,b,c,d). Similar to other variables, K -RT values tended to show seasonal variability. Maximum K -RT was found in winter for *Bacteroidetes* (January), spring (April and May) for SAR11 and *Rhodobacteraceae*, and in April and October for *Gammaproteobacteria*. K -RT was significantly correlated with E for *Rhodobacteraceae* (Pearson r =0.87, p -value=0.026) and cell-size for SAR11 (Pearson r =0.74, p -value=0.006, n =12).

Discussion

The response of heterotrophic bacterioplankton to increasing temperatures may largely influence biogeochemical processes in the future ocean (Cho and Azam, 1990; Azam and Long, 2001; Regaudie-de-Gioux and Duarte, 2012). However, the metabolic response of widespread phylogenetic groups of marine bacteria to ocean warming is largely unknown. In a companion paper assessing the temperature-dependence of heterotrophic bacteria growth rates from a community level perspective, a high temporal variability was found (Huette-Stauffer *et al.*, 2015). A plausible explanation for this finding was that distinct bacterial groups dominating different periods of the year exhibited different temperature-responses, an idea that, to our knowledge, has been never tested in a complete annual cycle. This hypothesis was addressed in the present work, in which we targeted four broad bacterioplankton groups widely distributed in marine waters in order to identify which taxa are potentially more sensitive to ocean warming. As our aim was to quantify the growth rates of different taxa under a range of temperature treatments, we used a single-cell approach (CARD-FISH), which allows a direct quantification of cell abundances of target phylogenetic groups (Amann and Fuchs, 2008) along the growth curves. It should be taken into account that the taxonomical resolution provided by CARD-FISH is restricted by the specificity and coverage of the phylogenetic probes used. In our case, we used specific probes for four abundant, major phylogenetic groups (SAR11, *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes*), yet, the growth rates measured were likely the result of a combined response of different taxa within each of them. On average, these four groups represented more than half of the initial bacterial abundance in our study site, but increased up to 90% of the cells ($71\% \pm 16\%$ on average) at the time of maximum abundance in the incubations. Therefore, we feel confident that we targeted the bacterial groups that were actively growing in the incubations and, most probably, also *in situ* (Campbell and Kirchman, 2013; Kirchman, 2016).

The bacterial groups analysed showed contrasting seasonal dynamics, likely related to their trophic strategies and specific adaptations to occupy different ecological niches (Gifford *et al.*, 2013). For instance, SAR11 dominated in summer, which is characterized by a stratified water column and oligotrophic conditions at the site of the study (Calvo-Díaz and Morán, 2006; Morán *et al.*, 2007). The seasonal dynamics of SAR11 is consistent with the well-known adaptation of this taxon to low nutrient concentrations (Giovannoni *et al.*, 2005; Sowell *et al.*, 2009; Giovannoni, 2017). The other analysed groups (*Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes*) showed opposite seasonal trends, with low abundance in summer stratified conditions and peaking in spring, coinciding with recurrent phytoplankton blooms in the area of study (Calvo-Díaz, 2008) due to high

inorganic nutrient concentration (Fig S1). These results agree with the general assumption that some members of the latter three taxonomic groups are predominately copiotrophs (Kirchman, 2002; López-Pérez *et al.*, 2012; Nelson and Carlson, 2012; Buchan *et al.*, 2014), and support the view that trophic strategies strongly determine the seasonal composition of bacterial communities in coastal environments (Pinhassi and Hagström, 2000; Bunse and Pinhassi, 2017).

The measured growth rates of specific taxa agree with the relatively few previous studies carried out in natural conditions (Teira *et al.*, 2009; Yokokawa *et al.*, 2004, Yokokawa and Nagata, 2005; Lankiewicz *et al.*, 2016). Thus, SAR11 consistently showed the minimum growth rates of the groups targeted (Fig. 2). However, it is remarkable that this group occasionally also had high growth rates (April and May), reaching values comparable to those of the other groups ($>1 \text{ d}^{-1}$). Similar high growth rates of SAR11 have been previously found only in predator-free as well as in virus-free incubations (Ferrera *et al.*, 2011), supporting the idea that at least some SAR11 lineages may be tightly controlled by top-down factors (Zhao *et al.*, 2013). The elevated growth rates of SAR11 cells measured in May were also accompanied by unusually large cell-sizes, in the higher end of the few previous estimates of SAR11 bacteria sizes ($0.05\text{-}0.12 \mu\text{m}^3$, Malmstrom *et al.*, 2004; Elifantz *et al.*, 2005; Schattenhofer *et al.*, 2009). These results suggest that a distinct SAR11 lineage, more responsive to high resource availability, was dominant in the post-bloom conditions of May. At the same site, according to Alonso-Sáez *et al.* (2015), two dominant SAR11 operational taxonomic units (OTU) are typically found, with one of them contributing on average 69% to total reads (OTU 1) while the second one was generally much less abundant (21%). Interestingly, although the OTU 1, affiliated with the broadly distributed surface 1 clade, was clearly dominant over most of 2012, a clear switch was found in May, when OTU 4, affiliated with the SAR11 surface 2 clade, contributed up to 83% of SAR11 reads (Fig. S4). While we cannot confirm which particular SAR11 phylotype grew in our incubations, these results suggest the presence of different SAR11 lineages with distinct ecophysiological properties, with the potential to attain high growth rates and large cell-size under resource-replete conditions, comparable to those of copiotrophic taxa. Interestingly, a higher activity of some SAR11 phylotypes during periods of high primary production and BP has been detected by metatranscriptomics (Gifford *et al.* 2014). Such elevated activity was related with a higher expression of genes associated to the use and transport of algal-derived low molecular weight compounds, suggesting that the activity of at least some SAR11 OTUs may respond to the large inputs of labile organic matter released after phytoplankton blooms.

Since temperature governs enzyme kinetics (Elias *et al.*, 2014), ocean warming is expected to have a generalized, direct impact on variables related to metabolism (Gillooly *et al.*, 2001; Kingsolver *et*

al., 2008; O'Connor *et al.*, 2009; Morán *et al.*, 2015; Huete-Stauffer *et al.*, 2015). However, we report here large discrepancies with the MTE predictions of four widespread phylogenetic groups of coastal bacterioplankton. While an overall increase in growth rates with increasing temperature (i.e. positive E values) was found in agreement with the MTE expectations (Gillooly *et al.*, 2001), a large variation in activation energies was observed (Fig. 2.3 e,f,g,h) and E values were often lower than that predicted by the MTE for heterotrophic organisms (0.65 eV, Brown *et al.*, 2004). On the other hand, the carrying capacity showed an opposite trend to MTE predictions (Brown *et al.*, 2004; Savage *et al.*, 2004), as the predicted decrease of K with temperature was only observed occasionally for some of the groups, and even then, values were close to zero. The pattern of increasing growth rates and maximum standing stocks with increasing temperature seems general for the broad taxonomic bacterial groups found in coastal temperate waters, which may ultimately lead to increasing bacterial biomass in a warmer ocean, as previously reported (Morán *et al.*, 2015). However, it should be taken into account that protistan grazing on bacteria, which was avoided in our experimental setup through pre-filtration of the samples, will also probably increase in warmer conditions (Vaqué *et al.*, 2009; Sarmiento *et al.*, 2010), ultimately limiting the observed increase of bacterial biomass with warming. Yet, increasing temperatures seem to have a larger impact on heterotrophic bacteria than on protistan grazers (Sarmiento *et al.*, 2010; Von Scheibner *et al.*, 2014), suggesting that the increase in bacterial biomass may exceed mortality caused by grazing under warmer ocean conditions.

Regardless of the potential effect of grazing, the observed enhancement of bacterial metabolism with temperature was mostly observed in spring, with E values for *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes* even exceeding the theoretical value predicted by the MTE (Brown *et al.*, 2004). The sBP values, used in this work as a proxy of carbon bioavailability (Fouilland *et al.*, 2014), were also maximum in spring, which suggests that the response to temperature of all phylogenetic groups was favored in periods of resource replete conditions. Our results agree with previous works reporting a tight relation between the temperature-dependence of bacterial metabolism and resource availability (López-Urrutia and Morán 2007; Berggren *et al.*, 2010). Yet, we cannot rule out that the observed higher temperature-response in spring may also be due to other processes, such as seasonal acclimation over the annual cycle. Accordingly, under the relatively low temperatures in spring, warming may have had a higher impact than in summer, when the temperature-sensitivity usually decreases after a gradual thermal adaptation (Hall *et al.*, 2010). However, the lack of statistical differences between summer and winter in the temperature-sensitivity of both growth rates and carrying capacities, suggests that the seasonal variation of *in*

situ temperature was not as relevant as resource availability in determining the bacterial response to temperature.

Among the groups targeted, *Rhodobacteraceae* showed the strongest response to temperature in spring, conferring this group with a potential competitive advantage under warming conditions. On the contrary, SAR11 showed the lowest temperature-sensitivity of all groups, possibly related to the limited ability of members of this clade to respond to environmental stimuli due to their streamlined genomes (Giovannoni *et al.*, 2005; Grote *et al.*, 2012). The other groups, presumably dominated by copiotrophic taxa with larger genomes (Yooseph *et al.*, 2010), may have a faster response to changes of environmental parameters (Lauro *et al.*, 2009) like temperature, as we found experimentally. While the reasons for the higher temperature-sensitivity of *Rhodobacteraceae* as compared with *Gammaproteobacteria* or *Bacteroidetes* are elusive, we hypothesize that the higher ability of *Rhodobacteraceae* to adapt to changing environmental conditions (Moran *et al.*, 2004; Polz *et al.*, 2006; Beier *et al.*, 2015), may lead the observed higher response to temperature. Another plausible explanation may be related to the composition of the substrates preferentially used by each taxa, which may be characterized by different energetic demands, therefore yielding distinct mean activation energies in response to temperature. Interestingly, differences in marine bacterial community activation energies with water column depth have been related to the quality of organic carbon compounds (Lønborg *et al.*, 2016), as less labile C would require a higher demand of energy for its breakdown and utilization according to the Carbon Quality Theory (CQT, Davidson *et al.*, 2000; Davidson and Janssens, 2006). Although C consumption by the copiotrophic groups in our short incubations would likely depend solely on labile C substrates, several works have shown that different taxa are specialized in the use of particular compounds (Cottrell and Kirchman, 2000; Alonso-Sáez and Gasol 2008, Teeling *et al.*, 2012), which may require different enzymes for their uptake and degradation. Evidence that some biochemical pathways are more sensitive to temperature than others was recently demonstrated for extracellular enzymes used for degrading specific DOM compounds in a global study in the tropical and subtropical ocean (Ayo *et al.* in press). We speculate that such differences in the patterns of substrate utilization by distinct bacterial groups may ultimately influence their growth activation energies, a hypothesis that will be interesting to test in future studies.

In summary, our work agrees with previous studies suggesting that warming may lead to important changes in bacterial community composition (Lindh *et al.*, 2013; Bergen *et al.*, 2016), and further illustrates that such alteration may be related to the different metabolic response to warming observed for distinct bacterial taxa. Thus, resource availability and taxonomic composition may be

key factors in the final response of bacterial communities to increasing temperatures. Although here we tested the effect of temperature on broad phylogenetic groups, the role of more specific taxa, including low abundance and rare phylotypes should also be considered in future works, as both can have significant roles in biogeochemical cycles (Morris *et al.*, 2005; Sauret *et al.*, 2014). On the other hand, predicting the effect of gradual ocean warming on the future composition of bacterial communities will require considering other factors such as long-term acclimation, interactions with other microorganisms and species evolution. Thus, in order to unveil which species substitution will be dominant in a future ocean scenario, sustained long-term observations on the evolution of bacterioplankton assemblages will be required. The differential temperature sensitivity of some of the main phylogenetic groups present in coastal, temperate waters along the productive season found in this study, clearly indicates that taxon-specific studies may be key to understand the future dynamics and biogeochemical function of marine bacterioplankton.

Experimental procedures

Sample collection and temperature incubations

The sampling site is located 13 km off the coast of Gijón/Xixón (Spain), in the Southern Bay of Biscay (43.675°N, 5.578°W). This station is part of a transect regularly monitored within the programme Radiales of the Spanish Institute of Oceanography (IEO). Seawater was sampled monthly between January and December 2012 from 5 m depth with 5 L Niskin bottles (Nalgene, Rochester, NY, USA). Seawater collections were carried out during the first hours of the morning, in order to avoid diurnal variations of the microbial community. After the collection, seawater samples were pre-filtered by 0.8 µm pore-size cartridges (PALL corporation, East Hills, NY, USA) to isolate the heterotrophic bacteria from predators, as used in many previous works (e.g. Williams, 1981; Malmstrom et al., 2007; Lami and Kirchman, 2014). *In situ* oceanographic conditions were measured by a SeaBird 25 CTD. Ambient chlorophyll *a* concentrations were determined in the lab prior to the pre-filtration steps by filtering 200 ml subsamples on 0.2 µm pore-size polycarbonate filters. Filters were frozen at -20°C and processed within 2 weeks as explained in Calvo-Díaz and Morán (2006). Samples for DNA sequencing were collected onto 0.2 µm filters as explained in Alonso-Sáez et al. (2015). DNA extraction was carried out using the PowerWater DNA isolation Kit (Mobio, Carlsbad, CA, USA). 16S rDNA genes were amplified using the general bacterial primers 341F and 805R (Herlemann *et al.*, 2011) following Alonso-Sáez et al. (2015). Amplicons were sequenced using a 454 FLX+ and the resulting sequences were analysed by the MOTHR platform (Schloss *et al.*, 2009).

Samples were transported to the laboratory in darkened 20 L polycarbonate transparent bottles (Nalgene, Rochester, NY, USA), avoiding direct sunlight exposure, within 6 hours of collection. Once in the laboratory, 6 polycarbonate bottles of 4 L (Nalgene, Rochester, NY, USA) were filled with 2 L of sampled water, and placed in duplicates in temperature-controlled incubators set at *in situ* temperature, 3°C above and 3°C below the *in situ* value of each month. The incubations were carried out with the natural photoperiod of the sampling date and under saturating photosynthetically active radiation (PAR) irradiance (ca. 150 µmol photons m⁻² s⁻¹). The experiments lasted between 3 and 7 days (Table S3) until total bacterial abundance declined after the stationary phase and the exponential growth phase was clearly distinguishable. We measured the abundances of heterotrophic bacteria (distinguishing between low and high nucleic acid content cells) and heterotrophic nanoflagellate abundance during the incubation with a FACSCalibur flow-cytometer (BD Biosciences, Heidelberg, Germany) using common protocols (Gasol & Morán, 2015), specifically following Calvo-Díaz and Morán (2006) for bacteria and Christaki et al. 2011

for HNFs. The efficiency of HNF removal by the 0.8 μm cartridge was assessed by counting before and after pre-filtration. During the incubations, we sampled once or twice per day, depending on the growth phase, to determine the abundance of target bacterial groups by CARD-FISH analysis.

Bacterial heterotrophic production

Bacterial heterotrophic production was measured based on [^3H]-leucine incorporation rates. Leucine was added at saturating concentration (40 nmol L^{-1}) to three experimental replicates (1.2 mL) and two controls, treated with 120 μL 50% TCA prior to isotope addition. Samples were incubated from 1 to 3 hours at *in situ* temperature in temperature-controlled incubators. The incorporation was stopped with the addition of 120 μL 50% TCA and the tubes were frozen until analysis following the centrifugation method (Smith and Azam, 1992). Finally, 1 mL of scintillation cocktail was added to the tubes and they were counted on a Beckman scintillation counter. Leucine incorporation rates were normalized by total bacterial abundance to estimate cell-specific bacterial production (sBP, $\text{pmol Leu cell}^{-1} \text{h}^{-1}$)

Catalysed Reporter Deposition Fluorescent In situ Hybridization (CARD-FISH)

CARD-FISH analysis (Pernthaler *et al.*, 2002) was carried out to estimate the abundance of specific phylogenetic groups of bacterioplankton. Samples were fixed with 4% formaldehyde solution (Sigma-Aldrich, St Louis, MO, USA) for 3 hours at room temperature, and filtered through 0.2 μm pore-size polycarbonate filters (GTTP type, 25 mm; Millipore). Filters were dipped in 0.1% (w/w) agarose and permeabilized in lysozyme (10 mg mL^{-1} , Sigma-Aldrich, St Louis, MO, USA) at 37°C for 1 h, and achromopeptidase (60 U mL^{-1} , 0.01 M NaCl, 0.01 M Tris-HCl, pH 7.6, Sigma-Aldrich), for 30 min at 37°C. Hybridizations were carried out overnight at 35°C using Horseradish Peroxidase (HRP) labeled probes (50 $\text{ng } \mu\text{L}^{-1}$) diluted in 900 μL of hybridization buffer, with different formamide concentrations. The probes and formamide concentrations used are described below; SAR11-441R (Morris *et al.*, 2002, 45% formamide) for SAR11, Ros537 (Eilers *et al.*, 2001, 55% formamide) for *Rhodobacteraceae*, Gam42a (Manz *et al.*, 1996) with its *Betaproteobacteria* competitor Bet42a (Manz *et al.*, 1992, 55% formamide) for *Gammaproteobacteria*, and CF319 (Manz *et al.*, 1996, 55% formamide) for *Bacteroidetes*. Hybridized samples were amplified (46°C, 30 min) using tyramide labeled with Alexa 488 dye (1 mg mL^{-1} , 46°C, 30 min, ThermoFisher, Germany), dried and stored at -80°C. Filters were transferred onto slides and stained with DAPI at 1 $\mu\text{g mL}^{-1}$. DAPI and CARD-FISH stained bacteria were quantified using an epifluorescence microscope Leica DM5500B, a monochromatic camera Leica DFC360 FX and the ACMETool (www.technobiology.ch) automatized image analysis software (Zeder *et al.*, 2009; Zeder *et al.*,

2011). Counts of hybridized bacteria are reported as percentage of total DAPI stained cells, calculated from 10 randomly chosen microscopic fields.

Growth rates, carrying capacity, cell-size and temperature dependence

We used the slope of ln-transformed abundance vs. time during the exponential growth phase to calculate the growth rates in duplicate seawater incubations. The carrying capacity (K) was calculated as the maximum bacterial abundance reached at stationary grow phase by each phylogenetic group and temperature treatment. Cell-sizes were calculated for the initial time of the incubations for the different phylogenetic groups. Microscopy images were transformed to binary images in order to minimize the brightness of DAPI signal in the surrounding area of the cell, as described in Massana *et al.* (1997). After transformation, biovolume was calculated by an automatic analysis system based in R programming language, which calculates the size of CARD-FISH positive cells for DAPI transformed images using the algorithm by Baldwin and Bankston (1988). Cells with volumes smaller than $0.0042 \mu\text{m}^3$ and larger than $0.344 \mu\text{m}^3$ were excluded from the analysis, as they were considered out of the range of typical bacterioplankton cells (Straza *et al.*, 2009). The image pre-processing method applied in this work was different from than that reported in Morán *et al.* (2015), producing some variations in the SAR11 cell-size reported for the same samples between the current work and the one by Morán *et al.*, (2015).

To calculate the temperature dependence of bacterial growth rates, we used the activation energy (E) as in the MTE basic equation:

$$I = I_0 M^b e^{-E/kT} \quad (1)$$

Where I is the individual metabolic rate, I_0 is the normalization constant, b is an allometric scaling exponent, M and T correspond to the body-size and temperature (in K), and k represents the Boltzmann's constant. According to Savage *et al.* (2004), the growth rate of an entire population is strictly dependent of individual metabolic rates. Thus, growth rates can be described as the sum of such individual rates with mass and temperature correction:

$$\mu = \sum \frac{I}{M} \simeq \frac{M^b}{M} e^{-E/kT} \quad (2)$$

where the activation energy is the slope of the ln transformed temperature-corrected growth rates against the temperature factor ($1/kT$). Regarding mass correction, the allometric scalling exponent (b) has been empirically estimated as $b=1/4$ for population growth (Slobodkin 1962, Blueweiss *et al.* 1978) and as $b=3/4$ for carrying capacity (Damuth, 1987; Belgrano *et al.*, 2002). However, such

universal values of the exponent have been questioned (Glazier 2015), and due to the small size variability of bacteria (usually less than $0.01 \mu\text{m}^3$) we did not perform any mass-correction as discussed by Huete-Stauffer *et al.* (2015).

The effect of temperature on the carrying capacity (K-TR) was measured as the linear slope between the maximum bacterial abundance (ln-transformed) and temperature (in °C). Mass-correction was omitted for calculating carrying capacity temperature dependence. Within this linear relationship, positive values indicate higher carrying capacity under warmer conditions, while negative values indicate a decrease in carrying capacity with increasing temperature.

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Tables

Supplementary table 1. Pearson correlation coefficients of the relationships between the initial abundance, carrying capacity (K), growth rate (μ), activation energy (E) and temperature effect on carrying capacity (K-RT) of the different groups, and the environmental variables temperature (T), initial cell-size and cell-specific bacterial heterotrophic production (LIR). Numbers in parentheses indicate p-values.

		T	Size	BHP _i
SAR11	Initial abund.	0.63 (0.017)		
Ros				0.59 (0.025)
Gamma				
CFB		-0.60 (0.023)		
SAR11	K	0.64 (0.015)	-0.53 (0.044)	-0.54 (0.040)
Ros		-0.58 (0.021)		0.58 (0.028)
Gamma		-0.66 (0.011)		
CFB			0.52 (0.047)	0.70 (<0.001)
SAR11	μ		0.74 (0.006)	0.83 (<0.001)
Ros				
Gamma				
CFB				
SAR11	E	-0.73 (0.004)		
Ros				
Gamma		-0.54(0.04)	0.60(0.023)	0.64 (0.015)
CFB			0.55(0.038)	
SAR11	K-RT		0.60(0.022)	0.75 (0.003)
Ros				0.59 (0.026)
Gamma				0.54 (0.042)
CFB				

Supplementary table 2. Summary statistics of selected parameters of the four phylogenetic groups considered.

		SAR11	<i>Rhodobacteraceae</i>	<i>Gammaproteobacteria</i>	<i>Bacteroidetes</i>
Growth rate (d ⁻¹)	Mean (+SD)	0.50 ± 0.38	0.9 ± 0.39	0.94 ± 0.56	1.00 ± 0.52
	Min	0.17	0.13	0	0
	Max	1.31	1.49	2.07	2.07
	Variance	0.14	0.15	0.32	0.27
<i>E</i> (eV)	Mean (+SD)	0.35 ± 0.35	0.62 ± 0.54	0.40 ± 0.34	0.44 ± 0.41
	Min	-0.3	-0.02	-0.26	-0.19
	Max	0.86	1.46	0.89	1.03
	Variance	0.12	0.29	0.11	0.17
K-RT (ln(cells mL ⁻¹) °C ⁻¹)	Mean (+SD)	0.01 ± 0.04	0.01 ± 0.08	0.02 ± 0.06	0.04 ± 0.07
	Min	-0.04	-0.12	-0.07	-0.04
	Max	0.09	0.17	0.12	0.21
	Variance	0.002	0.006	0.003	0.005

Supplementary table 3. Detailed conditions of each incubation experiment.

	Incubation period (days)	Temperature (°C)	chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	sBP (10^{-6} pmol Leu $\text{L}^{-1} \text{h}^{-1}$)	Bacteria abundance (10^5 cell mL^{-1})
January 2012	4	13.9	0.4	5.3	4.2
February 2012	4.7	13.8	0.6	5.2	4.0
March 2012	5.7	12.8	1.4	27.8	6.4
April 2012	3.7	13.3	1.4	72.7	7.7
May 2012	4.7	14	0.6	79.6	2.6
June 2012	3.7	16.4	0.4	5.5	5.9
July 2012	3.8	18.7	0.1	4.6	6.9
August 2012	6.7	21.2	0.4	5.1	9.2
September 2012	3	20.8	0.7	15.6	10.2
October 2012	3.7	18.3	1.0	26.9	4.0
November 2012	5.7	16.3	1.3	8.9	5.4
December 2012	7	13.3	1.8	7.9	7.1

Figures legends

Figure 1. *In situ* temperature, chlorophyll *a* concentration and cell-specific bacterial heterotrophic production (a), and DAPI-based abundances of total bacteria (line) and of each of the phylogenetic groups identified with CARD-FISH (b).

Figure 2. Initial and maximum cell abundance (a, b, c, d), growth rates at *in situ* temperature (e, f, g, h) and initial biovolume (i, j, k, l) of SAR11, *Rhodobacteraceae* (Rhodo), *Gammaproteobacteria* (Gamma) and *Bacteroidetes* (Bctd). Dashed line in Biovolume panels indicate year-round average cell-size.

Figure 3. Dependence of different variables on temperature for the four phylogenetic groups; (a, b, c, d) carrying capacity (K_{RT}) and (e, f, g, h) growth rates represented as activation energy (E). Error bars represent SD between two replicates. Black lines represent the absence of temperature dependence (coinciding with 0 value of E and K_{RT}). The dashed line represents the E value predicted by the MTE (0.65 eV).

Figure 4. Boxplot of the ratio between the specific growth rates in the +3°C and -3°C temperature treatments of each phylogenetic group in the different seasons.

Supplementary Figure 1. *In situ* concentrations of inorganic nutrients; dissolved inorganic nitrogen (DIN, orange), phosphate (PO_4^{3-} , green) and silicate (SiO_4^{2-} , black)

Supplementary Figure 2. Initial mean cell-size of the bacterial community during the 2012 annual cycle. Inset show correlation between bacterial biovolume and temperature.

Supplementary Figure 3. Abundance changes with time at three incubation temperatures (*in situ*, 3°C below and above *in situ*) for SAR11, *Rhodobacteraceae*, *Gammaproteobacteria* and *Bacteroidetes* for the experiment of April.

Supplementary Figure 4. Contribution of the two most abundant SAR11 OTUs (OTU1 and OTU4) to total SAR11 reads (in percentage) during the year 2012. Data were obtained by pyrosequencing as explained in Alonso-Sáez *et al.* (2015)

Figure 1. *In situ* temperature, chlorophyll a concentration and cell-specific bacterial heterotrophic production (a), and DAPI-based abundances of total bacteria (line) and of each of the phylogenetic groups identified with CARD-FISH (b).

243x277mm (300 x 300 DPI)

Figure 2. Initial and maximum cell abundance (a, b, c, d), growth rates at in situ temperature (e, f, g, h) and initial biovolume (i, j, k, l) of SAR11, *Rhodobacteraceae* (Rhodo), *Gammaproteobacteria* (Gamma) and *Bacteroidetes* (Bctd). Dashed line in Biovolume panels indicate year-round average cell-size.

220x231mm (300 x 300 DPI)

Figure 3. Dependence of different variables on temperature for the four phylogenetic groups; (a, b, c, d) carrying capacity (K-RT) and (e, f, g, h) growth rates represented as activation energy (E). Error bars represent SD between two replicates. Black lines represent the absence of temperature dependence (coinciding with 0 value of E and K-RT). The dashed line represents the E value predicted by the MTE (0.65 eV).

130x66mm (300 x 300 DPI)

Figure 4. Boxplot of the ratio between the specific growth rates in the +3°C and -3°C temperature treatments of each phylogenetic group in the different seasons.

121x89mm (300 x 300 DPI)