

Dear Julia Uitz,

We would like to sincerely thank the two reviewers for their valuable feedbacks and corrections. Based on the reviewer's comments, the manuscript has been strongly improved. It is my pleasure to provide the revised manuscript (with and without tracked changes).

5 Please find also attached the detailed answer (in blue) to reviewers' comments (in black) and the detailed modifications in the manuscript (in red). We also addressed your minor suggestions at the end (after referee 2).

The corrections are indicated in red (deletion) or blue (addition) in the marked-up manuscript version.

We hope that you will find the manuscript suitable for publication in Biogeosciences.

Jose Luis Otero-Ferrer, on behalf of all co-authors.

Referee 1

Overall comments

The manuscript investigates the relationship between nutrient supply estimated by measurements of turbulent mixing and nitrate gradients and the community structure of picophytoplankton (including both autotrophic and heterotrophic groups). The take-home message of the work is that studies that use ambient nutrient concentrations as a proxy of nutrient availability could be misleading as in many regions of the ocean the supply of nutrients by turbulent diffusion is not registered in bottle samples of nutrients. This is an important message that needs to be communicated to the wider marine science community.

The manuscript is well written and provides a nice overview of the ecological literature of marine picophytoplankton. The dataset of turbulent mixing, nitrate concentrations and picoplankton community structure is novel and covers a variety of hydrographic and trophic regimes.

I have a few questions and comments concerning both the estimation of nutrient supply using combined MSS and nutrient profiles and the choice of sampling stations used in the analysis which I hope the authors may be able to address.

Specific comments

1) NUTRIENT FLUX ESTIMATES

Although the authors correctly point out that concomitant datasets of turbulent mixing and picophytoplankton community structure are rare, this may be due in part to the lack of high-quality estimates of nutrient flux from microstructure profile measurements in the surface ocean with coincident depth-resolved nutrient profiles (required to obtain a robust estimate of the nutrient gradient near the nutricline). The vertical resolution of the nutrient profiles within the dataset is unclear (only a range between 3 and 9 depths is provided, but it could be tricky to use 3/4 depths to provide a good estimate of the nutrient gradient). Could the extent of the density gradient be over/underestimated in cases where the depth resolution is low and by how much? It would be good to have a frequency histogram for the dataset showing the number of depths per profile so the reader is aware of the vertical resolution of nutrient concentrations across the dataset.

We agree with the reviewer that the lower vertical resolution of nitrate concentration in comparison to turbulent data incorporates some uncertainty, which is very hard to evaluate, in the calculation of nitrate diffusive fluxes. Lower vertical resolution (5 ± 2 sampling depths) was used in the shallower stations sampled in the Galician coastal upwelling, whereas in the deeper NW Mediterranean and the tropical and subtropical regions we used 7 ± 1 and 11 ± 2 depths, respectively. The text has been modified and a new figure (Figure A1-C) has been included in the supplementary material, to represent the frequency histogram of sampling depths used to compute the nitrate vertical gradient in each region. The maximum sampling depth where the microstructure turbulence profiler was deployed is now indicated in Table 1.

Original: Page 5 – Line 11-13

Samples for the determination of nitrate (NO_3) + nitrite (NO_2) were collected from 3 to 9 depths in rinsed polyethylene tubes and stored frozen at -20°C until analysis on land, according to standard methods using the automated colorimetric technique (Grasshoff et al., 2007).

Change: Page 5 – Line 21-26

Samples for the determination of nitrate (NO_3) + nitrite (NO_2) were collected, on average, from 5 ± 2 (Galician coastal upwelling), 7 ± 1 (NW Mediterranean) and 11 ± 2 (tropical and subtropical Atlantic Ocean) depths in rinsed polyethylene tubes and stored frozen at -20°C until analysis on land, according to standard methods using the automated colorimetric technique (Grasshoff et al., 2007). The frequency histogram of sampling depths collected for the determination of nitrate concentration in each region is indicated in Figure A1-C in the Supplementary material, whereas the maximum sampling depth where the microstructure turbulence profiler was deployed is indicated in Table 1.

2) DENSITY AND NITRATE RELATIONSHIPS The authors also mention that for some of the stations nutrient data was not available, and instead of nutrient bottle data, a relationship between density and nitrate was used. Again it is not clear how robust the relationship between nitrate concentration and density was for the relevant stations. Could the authors provide supplementary plots of the nutrient versus density relationship that was used to estimate nitrate gradients, similar to that of Williams et al. (2013a GRL 40:5467-5472; 2013b Limnol. Oceanogr.: Fluids and Envs 3:61-73)?

Only in one station sampled in the Galician coastal upwelling ecosystem during the NICANOR project observations of nutrient concentration were not available. Instead nitrate concentration was computed from a nitrate-density (σ_t) relationship built by using all samples ($n=52$) collected during the NICANOR sampling period. A new figure (Figure A1-D) has been

included in the supplementary material to show this relationship. The relationship showed a linear behavior ($NO_3 = 9.7788 * \sigma_t - 256.38$; Adj- $r^2=0.87$; $p < 0.001$) for density ranging between 26.1 and 27.1 $kg\ m^{-3}$ (Figure A1-D)

Original: Page 5 – Line 13-14

5 In those stations carried out during the NICANOR cruises, where nitrate concentrations were not available, they were obtained by using nitrate-density relationships (Moreira-Coello et al., 2017)

Change: Page 5 – Line 26-29

10 In one station carried out during the NICANOR cruise, where nitrate concentrations were not available, these were obtained by using a nitrate-density relationship built by using all samples ($n=52$) collected during the NICANOR sampling period. The relationship showed a linear behavior ($NO_3 = 9.7788 * \sigma_t - 256.38$; Adj- $r^2=0.87$; $p < 0.001$) for density ranging between 26.1 and 27.1 $kg\ m^{-3}$ (Figure A1-D).

15 3) EPISODIC NATURE OF MIXING Mixing events in some regions can be episodic, yet short-term vertical pulses of nutrients can trigger significant shifts in community structure. In some oceanic regions tidal mixing can also be important. The authors mention that 3-10 profiles were taken, but it would also be helpful to know the time interval over which these profiles were made and how this varied between the three study regions (again a frequency histogram documenting this would be helpful). Could it be that for some regions the flux of nutrients could be significantly underestimated given that such short-term events may not be captured in MSS profile data? Given the general audience of the journal, both the strengths and shortcomings of this method of estimating nutrient supply should be provided.

20 We completely agree with the reviewer that episodic bursts of turbulence can induce episodic inputs of nutrient supply which can be easily missed in sets of 2-10 profiles. The microstructure turbulence profiles used for computing nitrate fluxes at each station were always deployed successively. Sets include 2-11 in the tropical and subtropical Atlantic (37 ± 18 min), 6-7 in the NW Mediterranean (76 ± 22 min) and 3-402 in the Galician coastal upwelling (65 ± 246 min). Our dataset includes two high-frequency samplings carried out in the outer part of Ría de Vigo (Galician upwelling ecosystem) in August 2013 (CHAOS cruises). During these cruises two 25-hour series of turbulent microstructure and currents observations were carried out during spring and neap tides. Turbulent kinetic energy dissipation at the interface between upwelled and surface waters was enhanced by two orders of magnitude during the ebbs, as the result of the interplay of the bi-directional upwelling circulation and the tidal currents shear (Fernández-Castro et al., 2018). This mechanism could have important implications for the functioning of biological processes, as it can act as a pathway for nutrient supply from upwelled nutrient-rich deep waters into the sunlit surface waters. In fact, diffusive nitrate fluxes due to the enhanced dissipation observed during CHAOS-springs, could be responsible for about half of the phytoplankton primary production estimated in this system during periods of upwelling relaxation-stratification (Villamaña et al., 2017).

30 A new figure included in the supplementary material (Figure A1-B) indicates the number of turbulent profiles deployed at each station for each region. The duration of the microstructure turbulent profiler operation is indicated in Table 1 and the text has been modified to:

Original: Page 4 Line 28-29

35 Measurements of dissipation rates of turbulent kinetic energy (ϵ) were conducted at 3-10 profiles for each station to the bottom, or 300 m over deep waters.

Change:Page 4 Line 26- Page 5 Line 5

40 Measurements of dissipation rates of turbulent kinetic energy (ϵ) were conducted to the bottom, or to 137-323 m over deep waters (see Table 1). The microstructure turbulence profiles used for computing nitrate fluxes at each station were always deployed successively. Sets include 2-11 in the tropical and subtropical Atlantic (operation time 37 ± 18 min), 6-7 in the NW Mediterranean (76 ± 22 min) and 3-402 in the Galician coastal upwelling (65 ± 246 min) (Figure A1-B).

45 Bursts of turbulence can induce episodic inputs of nutrient supply which can be easily missed in sets of low number of profiles. In coastal regions where short-term variability of mixing processes is expected to be higher, our dataset includes two high-frequency samplings carried out in the outer part of Ría de Vigo (Galician upwelling ecosystem) in August 2013 (CHAOS cruises). During these cruises two 25-hour series of turbulent microstructure and currents observations were carried out during spring and neap tides. Turbulent kinetic energy dissipation at the interface between upwelled and surface waters was enhanced by two orders of magnitude during the ebbs, as the result of the interplay of the bi-directional upwelling circulation and the tidal currents shear (Fernández-Castro et al., 2018).

4) PICOPHYTOPLANKTON BIOMASS The authors report the estimates of picophytoplankton biomass and ratios of biomass, but I was unable to find how the authors convert from cell abundance to carbon per unit volume. This is quite important, as there are several group-specific carbon conversion factors in the literature and for the larger eukaryotic cells it is likely a biovolume conversion factor may provide a better estimate, as the size range within this subgroup can be significant.

5 We thank the reviewer for pointing out that this information was missing. In order to estimate biovolume (BV), we used an empirical calibration between Size Scatter (SSC) and cell diameter (Calvo-Díaz and Morán, 2006), assuming spherical shape for all groups. The following volume-to-carbon conversion factors were used for picoautotrophic groups: 230*fg C*BV for *Synechococcus*, 240*fg C*BV for *Prochlorococcus* and 237*fg C*BV for picoeukaryotes (Worden et al., 2004). For bacteria BV was converted into carbon biomass by using the allometric relationship: 108.8*fg C*BV^{0.898} (Gundersen et al., 2002).

10 Original: Page 6 Line 19-24

Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and one group of small picoeukaryotes, based on their fluorescence and light scatter signals (SSC), as explained in Calvo-Díaz et al. (2006). Two groups of heterotrophic prokaryotes (LNA and HNA) were distinguished based on their relative green fluorescence, which was used as a proxy for nucleic acid content (Gasol and del Giorgio, 2000; Bouvier et al., 2007).

15 Change: Page 7 Line 4-15

Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and one group of small picoeukaryotes, based on their fluorescence and light scatter signals (SSC), as explained in Calvo-Díaz et al. (2006). Two groups of heterotrophic prokaryotes (LNA and HNA) were distinguished based on their relative green fluorescence, which was used as a proxy for nucleic acid content (Gasol and del Giorgio, 2000; Bouvier et al., 2007).

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25 5) EXTRAPOLATION TO THE GLOBAL OCEAN I was surprised to see that over half of the stations used were from coastal embayments. One could argue from many points of view that these regions may not be representative of the open-ocean eutrophic environments (likely different taxonomic diversity within these gross cytometric groupings, potential supply of nutrients from terrigenous sources, different light environment caused by attenuation by CDOM and sediments, need to correct for advective flux). Perhaps the authors have supporting literature/data that would help convince the reader that these embayments broadly reflect the environmental conditions of offshore stations, but even with such supporting information they should also highlight the need for data from open-ocean meso- and eutrophic environments that would help further resolve the global relationship between mixing and picoplankton community structure.

35 We agree with this reviewer that a larger data set including a wider range of conditions will be desirable. However, please note that the Galician Rías, despite being in general longer and narrower than many open bays in upwelling areas (e.g., Monterey Bay in California, False Bay in South Africa, Antofagasta Bay in Chile, and Todos Santos Bay in Mexico), they resemble them in that its primary hydrographic and circulation features are determined by the extension of wind-driven flow on the external continental shelf throughout the bay (Alvarez-Salgado et al., 2010). Fertilization in the Rías occurs essentially by coastal upwelling, being fresh and rain water inputs residual (2%) (Fernández et al., 2016).

40 Original: Page 5 Line 21-25

Most stations carried out in the Galician coastal upwelling were conducted inside three different Rías (Ría de Vigo, Ría de Pontevedra and Ría de A Coruña). The Rías are coastal embayments affected by seasonal wind-driven coastal upwelling of cold, nutrient-rich North Atlantic Central water (Wooster et al., 1976; Fraga, 1981; Álvarez-Salgado et al., 1993). The total nitrate supply in the Galician Rías was computed as the sum of nitrate vertical diffusion plus nitrate vertical advection due to coastal upwelling.

45 Change: Page 6 Line 5 - 12

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The dataset is largely confined to a specific geographic region (40N-30S, covering a limited number of biochemical provinces in the Atlantic Basin), yet the authors use relationships from this study to predict future changes in picoplankton community structure across the entire globe. Would the authors consider limiting their predictions of future community structure to the geographic regions/ latitudinal gradients that are used to develop the predictive models? Given that the global ocean covers a variety of biogeochemical regimes, some of which may not be limited by nitrate, restricting the geographical scope of the future predictions may be advisable, even though the overall patterns tend to broadly resemble those from other global studies.

Following the comments by both reviewers this section has been deleted from the manuscript.

6) USE OF MOREL MODEL TO ESTIMATE DEPTH OF PHOTIC LAYER Is the light attenuation observed in the Galician coastal stations largely a result of phytoplankton or other optically-active substances? I mention this because the model of Morel used to estimate euphotic depth is restricted to Case-1 (open ocean) waters where light attenuation is dominated by phytoplankton.

As mentioned above primary hydrographic and circulation features in the Galician Rías are determined by the extension of wind-driven flow on the external continental shelf throughout the bay. Note that light attenuation is exclusively used in the manuscript to get an estimate of the base of the euphotic layer, to be used as the lower limit to compute depth-integrated biomass. Using water type 2 equations would result in a shallower limit for the euphotic layer, which could miss phytoplankton biomass sometimes located deeper than this limit in this system (see Figure 1 in Cermeño et al., (2016)).

In our study the Morel equation was only used to compute light attenuation coefficients during the NICANOR, ASIMUTH and CHAOS cruises, which sampled stations in the outer part of the Rias. This information is now clarified in the methods section. A new figure (Figure A1-A) has been also included in the supplementary material to compare the base of the euphotic zone, as derived from PAR data and the Morel equation, by using data collected during the HERCULES cruises.

Original: Pag 4 Line 20-24

For those cruises where PAR profiles were not available (ASIMUTH, CHAOS and NICANOR), the depth of the photic layer was calculated by considering light attenuation coefficients derived from surface chlorophyll-a data estimated from the space, following the algorithms proposed by Morel et al. (2007) (<http://globcolour.info>).

Change: Pag 4 Line 17-22

For those cruises where PAR profiles were not available (ASIMUTH, CHAOS and NICANOR), which sampled stations in the outer part of the Rias, the depth of the photic layer was calculated by considering light attenuation coefficients derived from surface chlorophyll-a data, following the algorithms proposed by Morel et al. (2007) for Case-1 waters ($\log_{10}Z_{eu} = 1.524 - 0.460[Chl]_{surf} - 0.00051[Chl]_{surf}^2 + 0.0282[Chl]_{surf}^3$). A comparison of the estimation of the base of the euphotic zone by using the Morel et al equation and the data collected by a radiometer during the HERCULES cruise is shown in Figure A1-A.

Referee 2

The manuscript by Otero-Ferrer et al reports the relationship between nitrate supply and temperature in the structure of picoplankton groups determined by flow cytometer. The manuscript is really well written, and the literature seems extensively covered. The strength of this work resides in the use of nitrate diffuse flux as a proxy of nutrient availability and depth integrate biomass for the different picoplankton groups to predict the niche of the groups analyzed. Commonly, these measurements are treated as discrete rather than continuous variables. The data and results presented by Otero-Ferrer et al have lot of potential and I am confident that the community will benefit from its publication. However, I believe there are very few points that can be amended in a way to improve clarity of the main message of this work.

1) Plots with the vertical distribution of the main variables considered (nitrate, cell abundance of picoplankton groups and temperature) could be provided and would certainly help the reader to have a better assessment of the conditions in the sampled stations.

5 A new figure (Figure A3) has been include in the supplementary material to show the vertical distribution of temperature, nitrate concentration and picoplankton biomass of autotrophic and heterotrophic groups. References to the new figure have been included in the manuscript.

Original:

Page 13 Line 7-10

10 It is also important to note that surface abundance of picoplankton subgroups reported in our study, which are consistent with previous observations Zubkov et al. (2000); Frojan et al. (2014); Teira et al. (2015), did show higher surface abundance of picoeukaryotes in the Galicia coastal upwelling and the NW Mediterranean compared to the tropical and subtropical Atlantic (Table 2).

Page 9 Lines 8-9

15 Finally, HNA (55%) and LNA (21%) prokaryotes dominated in the Galician coastal upwelling system, followed by pi-coeukaryotes (11%), *Synechococcus* (6%) and *Prochlorococcus* (1%).

Change:

Page 13 Lines 8-12

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Page 9 Lines 10-14

25 Finally, HNA (55%) and LNA (21%) prokaryotes dominated in the Galician coastal upwelling system, followed by pi-coeukaryotes (11%), *Synechococcus* (6%) and *Prochlorococcus*(1%). Vertical distributions of temperature, nitrate concentra-tion and the biomass of autotrophic and heterotrophic picoplankton groups are shown in Figure A3.

2) Which values of cell-to-carbon conversion factors were used to transform abundance into biomass of the different groups of picoplankton analyzed?

30 We thank the reviewer for pointing out that this information was missing. In order to estimate biovolume (BV), we used an empirical calibration between Size SCatter (SSC) and cell diameter (Calvo-Díaz and Morán, 2006), assuming spherical shape for all groups. The following volume-to-carbon conversion factors were used for picoautotrophic groups: 230*fg C*BV for *Synechococcus*, 240*fg C*BV for *Prochlorococcus* and 237*fg C*BV for picoeukaryotes (Worden et al., 2004). For bacteria BV was converted into carbon biomass by using the allometric relationship: 108.8*fg C*BV^{0.898} (Gundersen et al., 2002).

Original: Page 6 Line 19-24

35 Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and one group of small picoeukaryotes, based on their fluorescence and light scatter signals (SSC), as explained in Calvo-Díaz et al. (2006). Two groups of heterotrophic prokaryotes (LNA and HNA) were distinguished based on their relative green fluorescence, which was used as a proxy for nucleic acid content (Gasol and del Giorgio, 2000; Bouvier et al., 2007).

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NICANOR). Abundance data obtained at different depths for each station were combined to compute depth-integrated biomass for the photic layer.

3) The depth integrated biomass of picoeuks was not linearly correlated with temperature, PAR nor nitrate flux when the authors used the simple linear model. However, with the additive model, picoeuks showed a negative trend with temperature and unimodal distribution with nitrate. Could the authors elaborate a bit more in the discussion about this contradiction between methods?

There is no contradiction between the results from the Generalized Linear and Generalized Additive Models. Generalized Additive Models are a form of regression analysis in which observational data are modeled by a function which is a nonlinear combination of the model parameters, and depends on one or more independent variables. By contrast, Generalized Linear Models generally include a nonlinear relationship between response and predictors, but the link-transformed mean response is linear in the parameters. If parameters have a linear behavior, partial effects will show a linear relationship.

This was indicated in the previous draft in the following sections:

Page 6, Lines 26 - Page 7 Line 9:

"A Generalized Additive Model (GAM) approach was used to predict depth-integrated biomass of each picoplankton subgroup, the contribution of LNA prokaryotes to heterotrophic picoplankton, the cyanobacteria to picoeukaryotes ratio, and the autotrophic to heterotrophic ratio based on observations and estimates of three environmental factors: sea surface temperature (SST), daily surface PAR, and the transport of nitrate into the euphotic zone (NO_3 Flux), including both diffusive and advective processes. GAMs assume that the effect of each predictor on the response variable can be described by smoothed functions whose effects are additive. Due to the large number of zero observations, data overdispersion, and the need for a single parsimonious model to make predictions for a large number of groups, we assumed that the depth-integrated biomass of each picoplankton subgroup, relative contribution values and biomass ratios all followed negative binomial distributions. Those niche descriptors that did not follow normal distributions were log transformed. The complete model structure for the biomass of each picoplankton subgroup was:

$$y_j = I + s(SST) + s(PAR) + s(\log(NO_3 Flux)) + Error \quad (1)$$

where y_j represents the depth-integrated biomass for each picoplankton subgroup j , and s a cubic regression spline used for fitting the observations to the model (Wood, 2006).

Generalized models include a function linking the mean value of y_j and the predictors. For those response variables that followed a negative binomial distribution the used link function was the natural logarithm. The LNA contribution to total heterotrophic prokaryotes was adjusted using a gaussian distribution and an identify link (Wood et al., 2016)."

Page 9, Lines 24-25:

"In order to exclude cross-correlation between the three environmental factors and consider the possibility of non-linear relationships, we subsequently fitted the data to Generalized Additive Models (Figure 4 and Table 3).

4) The dataset of this manuscript was originated from coastal waters rather than oceanic, from two oceanic regions (Atlantic and Med Sea) and it is confined to a narrow latitude range. Thus It does not support extrapolations to worldwide oceans. I recommend the authors to be more caution and remove figure A2 and the lines 23 to 27 of the last paragraph.

Attending to the referee advise we remove this part of the text.

Specific comments

INTRO Line 26 – missing a space between the word communities and the reference.

Done

MM Section 2, Line 5 – please keep one abbreviation for the Med sea to avoid confusion by writing the only once north-western. No need to repeat every time since for the Atlantic the same was done.

Done

Section 2, line 14 – Diaz et al 18 does not seem to be on bioRxiv or any other repository, thus the info is not available. I would not cite unless the paper has been already released.

This citation has been deleted

Section 2.6, line 6 – add Generalized Linear Models before GLM abbreviation since it is the 1st time that appears.

We remove section 2.6.

RESULTS Section 4.1, line 15, please add the average temperature value for picoeuk, especially because it seems very close to the one found for syn.

Synechococcus and picoeukaryotes niches overlap when temperature is considered. For this reason it does not seem necessary to report temperature value for these groups.

5 Original: Page 11 Line 13-15

Synechococcus and HNA prokaryotes prevailed mainly in cooler (below 20°C) marine environments characterized by intermediate and high levels of nitrate supply, and finally, the niche for picoeukaryotes was characterized by lower temperatures and high nitrate supply.

Change:Page 11 Line 13-15

10 *Synechococcus* and HNA prokaryotes prevailed mainly in cooler (below 20°C) marine environments characterized by intermediate and high levels of nitrate supply, and finally, the niche for picoeukaryotes was characterized by low temperatures and high nitrate supply.

DISCUSSION The figures and tables still can be cited in the discussion section. It facilitates a lot the follow up of the points discussed.

15 Cites referring key figures in the discussion have been included.

Julia Uitz

Minor suggestions

I consider you and your co-authors have satisfactorily addressed both Reviewers' comments and therefore invite you to revise your manuscript accordingly. Then your revised manuscript should be uploaded online.

20 Please also consider the following minor suggestions: - Caption of Fig. A2 "measured used a radiometer" : Replace "used" by "using"

Done

Unless justified otherwise, I think that plotting the vertical distribution of nitrates, picoplankton and bacteria in linear scale (instead of log10 scale) in Fig. A3 would be a lot more informative.

25 The range of these variables over the dataset is too wide to represent them using a linear scale. For this reason we prefer to use logarithm scale. Logarithm scale allows us to represent the three different domains in one scale. However we have the x-axis in order to provide an untransformed value to the reader.

Following both Reviewers' suggestions you removed the very last part of Section 5. Yet it is unclear in the Responses to Reviewers document which part precisely will be deleted. Although I agree that predicting future changes in picophytoplankton community is premature, I encourage you to keep a couple of sentences related to the implications of your results in the context of global climate change.

30 See below for a complete point-by-point changes in the manuscript. We deleted section 2.6, a small part in the abstract and the last part of the outlook where the extrapolation was done. However we maintained a few sentences of the global implications of our results in the context of global change.

35 Original: Page 14 Line 19-27

According to our results, in a future ocean where global change scenarios predict an increase in surface temperature and stratification (Howes et al. 2015), the resulting decrease in nitrate supply into the euphotic zone (Lewandowska et al. (2014) would lead to the dominance of autotrophic picoplankton by cyanobacteria, whereas the picoeukaryotes would decrease their contribution. In order to get a rough estimation of this effect, we computed the cyanobacteria to picoeukaryotic biomass ratio for the present and a future global change scenario (See supplementary Figure A1 and Methods). According to this analysis, the projected decrease in averaged global nitrate supply (ca. 20%) would lead to an average increase of 9% in the ratio of cyanobacteria to picoeukaryotes. Due to the smaller contribution of cyanobacteria to the transfer of carbon to the deep ocean compared to picoeukaryotes, this pattern could have important implications regarding the contribution of the smaller cells to the biological carbon pump (Corno et al., 2007).

45 Change: Page 14 Line 18-24

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coeukaryotes, this pattern could have important implications in the efficiency of the biological carbon pump (Corno et al., 2007).

Factors controlling the community structure of picoplankton in contrasting marine environments

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Abstract. The effect of inorganic nutrients on planktonic assemblages has been traditionally relied on concentrations rather than estimates of nutrient supply. We combined a novel dataset of hydrographic properties, turbulent mixing, nutrient concentration and picoplankton community composition with the aim of: i) quantifying the role of temperature, light and nitrate fluxes as factors controlling the distribution of autotrophic and heterotrophic picoplankton subgroups, as determined by flow cytometry; and ii) describing the ecological niches of the various components of the picoplankton community. Data were collected in 97 stations in the Atlantic Ocean, including tropical and subtropical open ocean waters, the northwestern Mediterranean Sea, and the Galician coastal upwelling system of the Northwest Iberian Peninsula. A Generalized Additive Model (GAM) approach was used to predict depth-integrated biomass of each picoplankton subgroup based on three niche predictors: sea surface temperature, averaged daily surface irradiance, and the transport of nitrate into the euphotic zone, through both diffusion and advection. In addition, niche overlap between different picoplankton subgroups was computed using non-parametric kernel density functions. Temperature and nitrate supply were more relevant than light in predicting the biomass of most picoplankton subgroups, except for *Prochlorococcus* and low nucleic acid (LNA) prokaryotes, for which irradiance also played a significant role. Nitrate supply was the only factor that allowed the distinction between the ecological niches of all autotrophic and heterotrophic picoplankton subgroups. *Prochlorococcus* and LNA prokaryotes were more abundant in warmer waters (> 20°C) where the nitrate fluxes were low, whereas *Synechococcus* and high nucleic acid (HNA) prokaryotes prevailed mainly in cooler environments characterized by intermediate or high levels of nitrate supply. Finally, the niche of picoeukaryotes was defined by lower low temperatures and high nitrate supply. ~~We estimated that with the predicted decrease in nitrate supply as the result of global change, a 9% increase in the ratio cyanobacteria to pieoeukaryotes would occur in a future ocean, something that would have important implications, given the contribution of smallest cells to the biological carbon pump.~~ These results support the key role of nitrate supply, as it not only promotes the growth of large phytoplankton, but it also controls the structure of marine picoplankton communities.

1 Introduction

Picoplankton, including archaea, bacteria and picoeukaryotes are the smallest (cell diameter $<2\ \mu\text{m}$) and most abundant organisms in marine ecosystems. Photosynthetic picoplankton often dominate marine phytoplankton biomass and primary production in oligotrophic tropical and subtropical regions (Chisholm, 1992), whereas they are typically a minor component in nutrient-replete coastal environments, usually dominated by large-sized plankton species (Finkel et al., 2010; Marañón, 2015). However, due to the large temporal and spatial variability in the structure and composition of the microbial community in shelf seas (Sherr et al., 2005), picoplankton, together with nanoplankton, can dominate the microbial community under certain conditions (Morán, 2007; Espinoza-González et al., 2012). In addition, picoplankton contributes overwhelmingly to the recycling of organic matter (Azam et al., 1983; Fenchel, 2008), and previous studies suggest that photosynthetic picoplankton could also play a role in the export of carbon to the deep ocean (Richardson and Jackson, 2007; Lomas and Moran, 2011; Guidi et al., 2015). As a result, picoplankton is considered a key component of the current carbon cycle and likely more important in future climate warming scenarios (Laufkötter et al., 2016).

When analyzed by flow cytometric techniques, two genera of picocyanobacteria (*Prochlorococcus* and *Synechococcus*), one or two subgroups of autotrophic picoeukaryotes of different size (small and large), and two subgroups of heterotrophic prokaryotes, based on their high (HNA) or low nucleic acid (LNA) content, can be distinguished (Gasol and del Giorgio, 2000; Marie and Partensky, 2006). Although closely related phylogenetically, *Synechococcus* and *Prochlorococcus* exhibit distinct physiological traits (Moore et al., 1995), divergent evolutionary strategies (Scanlan and West, 2002) and disparate geographic distributions (Zubkov et al., 2000). *Prochlorococcus* tend to be restricted to relatively warm (above 15°C) and nutrient-poor waters, extending from the surface down to 150 m, along the 40°N – 40°S latitudinal band (Partensky et al., 1999b; Johnson et al., 2006). *Synechococcus* exhibit a wider geographic and thermal distribution, including high-nutrient waters and occasionally reaching polar latitudes (Paulsen et al., 2016), their vertical distribution being shallower than that of *Prochlorococcus* (Partensky et al., 1999a; Li, 2002). The contribution of picoeukaryotes to picoplankton biomass is generally smaller than the contribution of picocyanobacteria (Zubkov et al., 2000; Buitenhuis et al., 2012), except in coastal regions where their contribution usually increases (Grob et al., 2007). In general, LNA prokaryotes dominate heterotrophic prokaryotic biomass in the oligotrophic open ocean, whereas HNA cells dominate in coastal regions (Li et al., 1995; Bouvier et al., 2007). These contrasting spatial distributions suggest that the picoplankton subgroups occupy differential ecological niches or, according to the classical definition proposed by Hutchinson (1957), distinct multidimensional hypervolumes of environmental factors in which viable populations develop. By describing the overlaps of environmental factors, realized niche partitioning can be defined, and the factors controlling the distribution of picoplankton subgroups can be identified. However, despite decades of experimental and field observations, the relative importance of the factors driving the variability of the growth and the spatial distribution of picoplankton subgroups remain largely unknown.

Aside from the effect of trophic controls, the distribution of microbial plankton is primarily determined by seawater temperature, light and nutrients (Li, 2009, 2007; Barton et al., 2015). Quantifying their relative influence on the spatial and temporal distribution of the different picoplankton subgroups is complicated by the fact that the above mentioned factors are often correlated in the ocean (Finkel et al., 2010). This shortcoming can be circumvented by using experimental approaches in the laboratory, where the influence of each independent factor is isolated. Alternatively, it can be approached by combining large data sets of hydrographic and biological observations collected from contrasting marine environments, which allow us to characterize the suite of variables that best define the organism's ecological niches.

In order to study the significance of temperature and nutrient concentrations in determining the contribution of picophytoplankton to total phytoplankton biomass and production, Agawin et al. (2000) reviewed the available literature from oceanic and coastal estuarine areas. Although the number of observations for which both temperature and nutrient concentration were available was too small to statistically separate their effects, these authors hypothesized that the dominance of picoplankton in warm, oligotrophic waters was due to differences between picophytoplankton and larger cells in the capacity to use nutrients and in their intrinsic growth rate. Bouman et al. (2011) investigated how vertical stratification controls the community structure of picophytoplankton in subtropical regions. According to their results, photosynthetic picoeukaryotes dominate in weakly-stratified, whereas in strongly stratified waters, *Prochlorococcus* cyanobacteria are prevalent. More recently, Flombaum et al. (2013), using a compilation of flow cytometry data from all major ocean regions, concluded that *Prochlorococcus* and *Synechococcus* abundance distributions were controlled by temperature and photosynthetically active radiation (PAR, 400-700 nm), discarding the role of nitrate concentration. However, in tropical and subtropical domains, the best represented regions in Flombaum et al.'s study, surface nitrate is almost depleted and the variability of its concentration can be widely disconnected from changes in its availability for phytoplankton, which depends more on the supply from deeper waters by turbulent diffusion (Mouriño-Carballido et al., 2016). It is also believed that fine-scale turbulence can enhance the nutrient uptake and subsequent growth of larger phytoplankton (Lazier and Mann, 1989; Karp-Boss et al., 1996; Guasto et al., 2012), especially in regions with low nutrient levels and strong grazing pressure (Barton et al., 2014).

As far as we know, only one study has previously used estimates of nitrate availability, derived from observations of microstructure turbulence, to investigate the role of nutrient availability in controlling the composition of picoplankton communities (Mouriño-Carballido et al., 2016). These authors, using local data from the northwestern Mediterranean Sea, found that different autotrophic picophytoplankton subgroups exhibit contrasting responses to nitrate supply, and that as a result the ratio of prokaryotic to picoeukaryotic photoautotrophic biomass decreased with increasing nitrate supply. However, whether these patterns are general and widespread in the ocean remains largely uncertain, given that no concomitant datasets including measurements of turbulent diffusion nutrient flux and picoplankton subgroup structure have been available to date.

Here we extend the analysis described in Mouriño-Carballido et al. (2016) by combining a dataset of picoplankton community composition, hydrographic properties, turbulent mixing and inorganic nutrient concentrations collected in a total of 97 stations. Observations were made in contrasting environments of the Atlantic Ocean, in order to quantify the role of temperature, light and nitrate availability in the composition of the picoplankton community, and to describe the ecological niches of each picoplankton subgroup.

2 Material and Methods

This study includes data collected in 97 stations from three contrasting environments covering the tropical and subtropical Atlantic Ocean (T), the northwestern ~~(NW)~~-Mediterranean Sea (M), and the Galician coastal upwelling ecosystem (G), between October 2006 and December 2015 (~~see~~-Table 1 and Figure 1). Two cruises (CARPOS Oct-Nov 2006 and TRYNITROP 5 Apr-May 2008) sampled 26 stations located in the tropical and subtropical Atlantic Ocean. Three other cruises carried out in the ~~NW~~-Mediterranean Sea (FAMOSO1 Mar 2009, FAMOSO2 Apr-May 2009, and FAMOSO3 Sep 2009) sampled 19 stations during three contrasting hydrographic conditions, covering from winter mixing to summer stratification. Finally, 52 stations were sampled in the Galician coastal upwelling ecosystem during HERCULES1 Jul 2010, HERCULES2 Sep 2011, HERCULES3 Jul 2012, DISTRAL Feb-Nov 2012, ASIMUTH Jun 2013, CHAOS Aug 2013, and NICANOR Feb 2014-Dec 2015 10 cruises. Additional information about the sampling design of these cruises is presented in Aranguren-Gassis et al. (2011, CARPOS), Mouriño-Carballido et al. (2011, TRYNITROP), Mouriño-Carballido et al. (2016, FAMOSO), Cermeño et al. (2016, DISTRAL), Villamaña et al. (2017, CHAOS) ~~;~~ ~~Díaz et al. (under revision 2018, ASIMUTH)~~ and Moreira-Coello et al. (2017, NICANOR).

At each station, information about hydrographic properties, turbulent mixing, nitrate concentration and picoplankton com- 15 munity composition were collected. Light conditions for each sampling station were considered as the 5-day averaged daily surface photosynthetic active radiation (PAR) obtained from satellite data (<http://globcolour.info>). Light attenuation coefficients were obtained from vertical profiles of PAR estimated by Licor sensors using the Beer-Lambert Law equation (Kirk, 1994). Depth of the euphotic layer was calculated as the depth where PAR was 1% of its surface value. For those cruises where PAR profiles were not available (ASIMUTH, CHAOS and NICANOR), ~~the~~ which sampled stations in the outer part of the 20 Galician Rías, the depth of the photic layer was calculated by considering light attenuation coefficients derived from surface chlorophyll-*a* data ~~estimated from the space~~, following the algorithms proposed by Morel et al. (2007) ~~(~~ for Case-1 waters ($\log_{10} Z_{eu} = 1.524 - 0.460[Chl]_{surf} - 0.00051[Chl]_{surf}^2 + 0.0282[Chl]_{surf}^3$). A comparison of the estimation of the base of the euphotic zone by using the Morel et al. (2007) equation and the data collected by a radiometer during the HERCULES cruise is shown in Figure A1-A.

25 2.1 Hydrography and turbulent mixing

Hydrographic properties and turbulent mixing were derived from a microstructure turbulent profiler (Prandke and Stips, 1998, MSS) equipped with a high-precision Conductivity-Temperature-Depth (CTD) probe, two microstructure shear sensors (type PNS06), and also a sensor to measure the horizontal acceleration of the profiler. Measurements of dissipation rates of turbulent kinetic energy (ε) were conducted ~~at 3-10 profiles for each station~~ to the bottom, or ~~300~~ to 137-323 m over deep waters ~~;~~ 30 (Table 1). The number of microstructure turbulence profiles used for computing nitrate fluxes at each station were always deployed successively. Sets include 2-11 in the tropical and subtropical Atlantic (37 ± 18 min), 6-7 in the Mediterranean (76 ± 22 min) and 3-402 in the Galician coastal upwelling (65 ± 246 min) (Figure A1-B). Episodic bursts of turbulence can induce episodic inputs of nutrient supply which can be easily missed in sets of low number of profiles. In coastal regions

where short-term variability of mixing processes is expected to be higher, our dataset includes two high-frequency samplings carried out in the outer part of Ría de Vigo (Galician upwelling ecosystem) in August 2013 (CHAOS cruises). During these cruises two 25-hour series of turbulent microstructure and currents observations were carried out during spring and neap tides. Turbulent kinetic energy dissipation at the interface between upwelled and surface waters was enhanced by two orders of magnitude during the ebbs, as the result of the interplay of the bi-directional upwelling circulation and the tidal currents shear (Fernández-Castro et al., 2018). Diffusive nitrate fluxes due to the enhanced dissipation observed during spring tide, could be responsible for about half of the phytoplankton primary production estimated in this system during periods of upwelling relaxation-stratification (Villamaña et al., 2017).

The profiler was balanced to have negative buoyancy and a sinking velocity of 0.4 to 0.7 m s⁻¹. The frequency of data sampling was 1024 Hz. The sensitivity of the shear sensors was checked after each use. Due to significant turbulence generation close to the ship, only the data below 5 (HERCULES1, HERCULES2, HERCULES3, DISTRAL, ASIMUTH, CHAOS, and NICANOR) and 10 m (CARPOS, TRYNITROP, FAMOSO1, FAMOSO2, FAMOSO3) were considered reliable. Data processing and calculation of dissipation rates of (ε) was carried out with the commercial software MSSpro. The squared Brunt Väisälä frequency (N^2) was computed from the CTD profiles according to the equation:

$$N^2 = - \left(\frac{g}{\rho_w} \right) \left(\frac{\partial \rho}{\partial z} \right) (s^{-2}) \quad (2)$$

where g is the acceleration due to gravity (9.8 m s⁻²), ρ_w is seawater density (1025 kg m⁻³), and $\partial \rho / \partial z$ is the vertical potential density gradient. Vertical diffusivity (K_z) was estimated as:

$$K_z = \Gamma \frac{\varepsilon}{N^2} (m^2 s^{-1}) \quad (3)$$

where Γ is the mixing efficiency, here considered as 0.2 (Osborn, 1980).

2.2 Nutrient supply

Samples for the determination of nitrate (NO₃) + nitrite (NO₂) were collected from 3 to 9.5±2 (Galician coastal upwelling), 7±1 (Mediterranean) and 11±2 (tropical and subtropical Atlantic Ocean) depths in rinsed polyethylene tubes and stored frozen at -20 °C until analysis on land, according to standard methods using the automated colorimetric technique (Grasshoff et al., 2007). In those stations The frequency histogram of sampling depths collected for nitrate concentration in each region is indicated in Figure A1-C, whereas the maximum sampling depth where the microstructure turbulence profiler was deployed is indicated in Table 1. In one station carried out during the NICANOR cruises, where nitrate concentrations were not available, they these were obtained by using a nitrate-density relationships (Moreira-Coelho et al., 2017). relationship built by using all samples (n=52) collected during the NICANOR sampling period. The relationship showed a linear behavior ($NO_3 = 9.7788 * \sigma_t - 256.38$; Adj-r²=0.87; p < 0.001) for density ranging between 26.1 and 27.1 kg m⁻³ (Figure A1-D).

Vertical diffusive fluxes of nitrate into the euphotic zone were calculated following the Fick's law as:

$$FluxNO_3 = \bar{K}_z \Delta NO_3 \quad (4)$$

where ΔNO_3 is the nitrate vertical gradient obtained by linear fitting of nitrate concentrations in the nitracline, determined as a region of approximately maximum and constant gradient, and (\bar{K}_z) is the averaged turbulent mixing over the same depth interval. In the Galician coastal upwelling, nitrate diffusive fluxes were estimated over a fixed depth interval using the same procedure (20-40 m) except in the shallowest stations where we compute the surface-bottom flux.

5 Most stations carried out in the Galician coastal upwelling were conducted inside three different Rías (Ría de Vigo, Ría de Pontevedra and Ría de A Coruña). The Rías are coastal embayments affected by seasonal wind-driven coastal upwelling of cold, nutrient-rich North Atlantic Central water (Wooster et al., 1976; Fraga, 1981; Álvarez-Salgado et al., 1993). The Galician Rías, despite being in general longer and narrower than many open bays in upwelling areas, they resemble them in that its primary hydrographic and circulation features are determined by the extension of wind-driven flow on the external continental shelf throughout the bay (Alvarez-Salgado et al., 2010). Fertilization in the Rías occurs essentially by coastal upwelling, being fresh and rain water inputs residual (2%) (Fernández et al., 2016). The total nitrate supply in the Galician Rías was computed as the sum of nitrate vertical diffusion plus nitrate vertical advection due to coastal upwelling. A simplified estimate of nitrate supply through vertical advection due to upwelling was computed considering the Galician Rías as single boxes divided into two layers (Álvarez-Salgado et al., 1993), the deeper one influenced by upwelled inflowing waters and the surface layer dominated by the outgoing flow. Assuming that the bottom layer volume is conservative and stationary, the vertical advective flux ($Q_Z, m^3 s^{-1}$), would be equivalent to the incoming bottom flux ($Q_B, m^3 s^{-1}$), computed as the product of the upwelling index ($I_W, m^3 s^{-1} km^{-1}$) and the lengths of the mouth of the Rías (ca. 10-11.5 km). I_W was averaged over the three-day period before each cruise from wind data recorded by meteorological buoys located in Cabo Vilano (HERCULES, NICANOR) and Cabo Silleiro (DISTRAL, ASIMUTH, CHAOS, ASIMUTH), or modeled by the Fleet Numerical Meteorology and Oceanography Center (FNMOC) model when buoy data were not available (<http://www.indicedeafloramamiento.ieo.es>). Finally, the transport of nitrate into the euphotic zone through vertical advection was computed as:

$$NO_3 \text{ Advective flux} = \frac{Q_z}{A_{basin}} [NO_3]_D \quad (5)$$

where A_{basin} is the surface area of the Galician Rías, Q_Z is the vertical advective flux, and $[NO_3]_D$ is the averaged nitrate concentration at the base of the euphotic layer. A_{basin} is 141 km² for Ría de Pontevedra (ASIMUTH), 174 km² for Ría de Vigo (CHAOS, ASIMUTH, DISTRAL), and 145 km² for Ría de A Coruña (HERCULES, NICANOR) (see Villamaña et al. 2017 and Moreira-Coello et al. 2017, for details).

2.3 Flow cytometry

Picoplankton samples (1.8 ml) for the determination of picoplankton abundance and cell properties were taken from 3-9 depths and measured immediately on board (TRYNITROP), or preserved with paraformaldehyde plus glutaraldehyde (P+G) and frozen at $-80^\circ C$ until analysis in the laboratory (the other cruises). Unfortunately, due to problems with sample preservation, only heterotrophic or autotrophic picoplankton subgroup data were available for the DISTRAL and ASIMUTH cruises, respectively. Two aliquots from the same sample were used for the study of picophytoplankton (0.6 ml) and heterotrophic prokaryotes (0.4 ml), analyzed at high (mean 60 $\mu l \text{ min}^{-1}$) and low (mean 18 $\mu l \text{ min}^{-1}$) flow rates during 4 and 2 min, re-

spectively. Before the analysis, the DNA of heterotrophic prokaryotes was stained with nucleic acid-specific fluorescent dye (SYTO-13 or SYBR1). A FACSCalibur flow cytometer (Becton-Dickinson) equipped with a laser emitting at 488 nm was used to measure and count picoplankton. Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and one group of small picoeukaryotes, based on their fluorescence and light scatter signals (SSC), as explained in (Calvo-Díaz and Morán, 2006). Two groups of heterotrophic prokaryotes (LNA and HNA) were distinguished based on their relative green fluorescence, which was used as a proxy for nucleic acid content (Gasol and del Giorgio, 2000; Bouvier et al., 2007).

In order to estimate biovolume (BV), we used an empirical calibration between Size SCatter (SSC) and cell diameter (Calvo-Díaz and Morán, 2006), assuming spherical shape for all groups. The following volume-to-carbon conversion factors were used for picoautotrophic groups: 230 fg C BV for *Synechococcus*, 240 fg C BV for *Prochlorococcus* and 237 fg C BV for picoeukaryotes (Worden et al., 2004). For bacteria BV was converted into carbon biomass by using the allometric relationship: 108.8 fg C BV^{0.898} (Gundersen et al., 2002). More details about the processing and analysis of flow cytometry samples are provided in Calvo-Díaz and Morán (2006, TRYNITROP), Gomes et al. (2015, FAMOSO), Villamaña et al. (2017, CHAOS) and Moreira-Coello et al. (2017, NICANOR). Abundance data obtained at different depths for each station were combined to compute depth-integrated biomass for the photic layer.

2.4 Generalized Additive Models

A Generalized Additive Model (GAM) approach was used to predict depth-integrated biomass of each picoplankton subgroup, the contribution of LNA prokaryotes to heterotrophic picoplankton, the cyanobacteria to picoeukaryotes ratio, and the autotrophic to heterotrophic ratio based on observations and estimates of three environmental factors: sea surface temperature (SST), daily surface PAR, and the transport of nitrate into the euphotic zone (NO₃ Flux), including both diffusive and advective processes. GAMs assume that the effect of each predictor on the response variable can be described by smoothed functions whose effects are additive. Due to the large number of zero observations, data overdispersion, and the need for a single parsimonious model to make predictions for a large number of groups, we assumed that the depth-integrated biomass of each picoplankton subgroup, relative contribution values and biomass ratios all followed negative binomial distributions. Those niche descriptors that did not follow normal distributions were log transformed. The complete model structure for the biomass of each picoplankton subgroup was:

$$y_j = I + s(SST) + s(PAR) + s(\log(NO_3 Flux)) + Error \quad (6)$$

where y_j represents the depth-integrated biomass for each picoplankton subgroup j , and s a cubic regression spline used for fitting the observations to the model (Wood, 2006).

Generalized models include a function linking the mean value of y_j and the predictors. For those response variables that followed a negative binomial distribution the used link function was the natural logarithm. The LNA contribution to total heterotrophic prokaryotes was adjusted using a gaussian distribution and an identify link (Wood et al., 2016). The inclusion of the different predictors to explain the response variable (the biomass of each picoplankton subgroup, its relative contribution

and biomass ratios) was assessed via stepwise model selection using the minimum Akaike Information Criterion (Hastie and Tibshirani, 1993).

Smooth terms were tested using a Bayesian test (Marra and Wood, 2012) to prevent overfitting. GAMs were evaluated based on explanatory power (explained variance) and goodness-of-fit (GOF). GOF was assessed via quantile–quantile (QQ) plots of Pearson residuals (provided in SM Figure A2). All calculations were done using the mgcv package (Wood, 2011) in R (R Core Team, 2015).

2.5 Niche overlap analysis

The estimation of niche overlap between different picoplankton subgroups based on non-parametric kernel density functions (NO_K) was calculated following Mouillot et al. (2005):

$$NO_{K_{i,j,t}} = 1 - \frac{1}{2} \int |f_{it}(x) - f_{jt}(x)| dx \quad (7)$$

where $NO_{K_{i,j,t}}$ is the niche overlap between picoplankton subgroups i and j for the environmental factor t , and f_{it} and f_{jt} are the kernel population density functions of factor t for species i and j , respectively. In order to correct the correlation between niche predictors, we used the estimator in a dependent sample (EDS) proposed by Kark et al. (2002).

To assess the statistical niche differences between subgroups, null model permutation tests were performed to verify whether the niche overlaps were significantly lower than 100% (Geange et al., 2011). When the contribution of depth-integrated biomass for each picoplankton subgroup exceeded that expected by chance (1/3 for autotrophic and 1/2 for heterotrophic picoplankton), niche predictors for each station were selected. Statistical null distributions (the distribution of the statistic test under the null hypothesis of no niche differentiation) were generated by calculating pseudo-values through randomly permuting group labels in the corresponding data set over 10000 runs. The distributions of the average niche overlaps for the null model were then computed. Niche overlap calculations and associated null model tests were performed using the density function and the source code provided as supporting information in Geange et al. (2011). All calculations were done using R (R Core Team, 2015).

2.6 Present and future spatial distributions of the cyanobacteria to picoeukaryote biomass ratio

~~A rough estimation of the spatial distribution of the cyanobacteria to picoeukaryotic depth-integrated biomass ratio was calculated by first estimating a proxy for nitrate diffusive flux based on SST, the vertical gradient of nitrate across the nitracline and surface chlorophyll- a applying a GLM model (Adj- $R^2=0.31$, $p<0.01$, $n=147$). This relationship was determined with data from all stations included in our dataset plus 50 additional stations visited during the Malaspina circumnavigation expedition (Fernández-Castro et al., 2015). We then used this relationship to compute the present large-scale spatial distribution of nitrate diffusive supply based on sea surface temperature and chlorophyll- a provided by NASA (National Aeronautics and Space Administration,) and nitrate gradient derived from the World Ocean Atlas (WOA09) provided by NOAA (National Oceanographic and Atmospheric Administration). Finally, these estimates of nitrate diffusive supply were used as input variables in the GAM model derived from our study to compute the spatial distribution of the cyanobacteria to picoeukaryotic biomass ratio for the present scenario. Only nitrate supply was used as a predictor, as this factor explained 51% (Adj- $R^2=0.35$,~~

~~p < 0.001, n = 97) of the variability. To compute the same ratio in a future global change scenario (year 2100), we followed the same procedure but used the averaged global decrease in dissolved nitrogen flux into the euphotic zone estimated by Lewandowska et al. (2014) between years 2000 and 2100 by using global ocean model simulations (ca. 20%). The percentage change in the cyanobacteria to picoeukaryote biomass ratio was calculated based on the difference between the annual averages of future and present estimates.~~

3 Results

3.1 Environmental variables and picoplankton biomass

Our database covered a wide environmental gradient from oligotrophic to eutrophic conditions. Stations sampled in the tropical and subtropical Atlantic oceans (T) were, on average, characterized by warm surface waters (26 ± 2 °C, mean \pm SD) where the supply of nitrate through vertical diffusion from deeper waters ~~was low~~ (0.7 ± 1.6 mmol N m⁻² d⁻¹), and surface chlorophyll-*a* ~~was were~~ low (0.1 ± 0.1 mg m⁻³) (Table 2 and Figure 2). The ~~NW~~ Mediterranean Sea (M), sampled from March to September, was characterized by cooler surface waters (16 ± 4 °C), and intermediate values of both nitrate vertical diffusive supply (41 ± 113 mmolN m⁻² d⁻¹) and also surface chlorophyll-*a* (0.9 ± 0.9 mg m⁻³). Finally, the stations sampled in the Galician coastal upwelling system (G), which included year-round samples, were characterized by relatively cold surface waters (16 ± 2 °C), enhanced rates of nitrate supply (30 ± 46 mmolN m⁻² d⁻¹) and high values of surface chlorophyll-*a* (2.2 ± 2.5 mg m⁻³). No statistically significant differences were observed in averaged surface photosynthetic active radiation between the three regions.

Differences in picoplankton biomass and composition were also observed between the three domains. Averaged photic layer depth-integrated total picoplankton biomass (including both LNA and HNA prokaryotes, *Prochlorococcus*, *Synechococcus* and picoeukaryotes) was higher in the tropical and subtropical Atlantic (1052 ± 215 mg C m⁻²) and ~~NW~~ the Mediterranean (1038 ± 485 mg C m⁻²), compared to the Galician coastal upwelling (216 ± 36 mg C m⁻²) (Table 2). In the tropical and subtropical Atlantic, the contribution of *Prochlorococcus* to total picoplankton biomass was 41%, followed by LNA (27%) and HNA (22%) prokaryotes, with smaller contributions of *Synechococcus* and picoeukaryotes (<5% each). In the ~~NW~~ Mediterranean, picoplankton biomass was on average dominated by *Synechococcus* (50%), followed by LNA and HNA prokaryotes (~17% for each group), picoeukaryotes (10%) and *Prochlorococcus* (5%). Finally, HNA (55%) and LNA (21%) prokaryotes dominated in the Galician coastal upwelling system, followed by picoeukaryotes (11%), *Synechococcus* (6%) and *Prochlorococcus* (1%).

Vertical distributions of temperature, nitrate concentration and the biomass of autotrophic and heterotrophic picoplankton groups for each domain are shown in Figure A3.

3.2 The role of environmental factors in picoplankton composition

In order to explore the role of temperature, light and nitrate supply in the composition of the picoplankton community, we first used Generalized Linear Models (~~GLM~~) to investigate simple linear relationships between each of these factors and the

depth-integrated biomass of each picoplankton subgroup, the contribution of LNA prokaryotes to heterotrophic picoplankton biomass, the cyanobacteria (*Prochlorococcus* and *Synechococcus*) to picoeukaryotes ratio, and the autotrophic to heterotrophic picoplankton ratio (Figure 3 and Table 3). All picoplankton groups except picoeukaryotes exhibited statistically significant relationships with sea surface temperature. This relationship was negative for *Synechococcus*, and positive for all the other subgroups (Figure 3). Only LNA and HNA prokaryotes and *Synechococcus* exhibited significant, positive relationships with surface radiation. All groups except the picoeukaryotes were negatively correlated with nitrate fluxes. The contribution of LNA prokaryotes to heterotrophic picoplankton biomass only exhibited a significant negative relationship with nitrate fluxes, whereas the cyanobacteria to picoeukaryotes ratio was positively correlated with surface temperature and negatively with nitrate fluxes. Finally, the ratio of autotrophic to heterotrophic biomass was not linearly correlated with any of the studied environmental factors. In order to exclude cross-correlation between the three environmental factors and consider the possibility of non-linear relationships, we subsequently fitted the data to Generalized Additive Models (Figure 4 and Table 3). Temperature was the only factor included in the models built for predicting the depth-integrated biomass of all picoplankton subgroups. HNA prokaryotes exhibited a positive relationship with temperature above 19°C, whereas *Prochlorococcus* and LNA prokaryotes showed a nearly sigmoid curve relationship with a transition between ca. 16 °C and 25 °C (Figure 4). The relationship between the biomass of both *Synechococcus* and picoeukaryotes and temperature showed a negative trend until ~20°C, and remained relatively constant above this temperature. PAR was included in the models of all picoplankton groups except picoeukaryotes. Whereas the biomass of *Prochlorococcus* exhibited a saturation type relationship with PAR, heterotrophic prokaryotes and *Synechococcus* showed a linear positive relationship. Finally, only LNA prokaryotes, *Synechococcus* and picoeukaryotes exhibited statistically significant relationships with nitrate supply. This relationship was negative for LNA prokaryotes and *Synechococcus*, whereas picoeukaryotes showed a unimodal function, peaking at ~1 mmol NO₃ m⁻² d⁻¹. Nitrate flux was the only factor selected in the models to predict the contribution of LNA prokaryotes to heterotrophic picoplankton biomass, and both the cyanobacteria to picoeukaryotes biomass ratio and the autotrophic to heterotrophic biomass ratio. This relationship was negative in the three models. Temperature was also negatively correlated with the contribution of LNA prokaryotes to heterotrophic biomass, and the ratio of autotrophic to heterotrophic biomass. Finally, PAR showed a positive correlation with the contribution of LNA prokaryotes to heterotrophic biomass, and the cyanobacteria to picoeukaryotes biomass ratio.

3.3 Ecological niches for picoplankton groups

By using non-parametric kernel density functions, we investigated the overlapping in the ecological niches of the autotrophic and heterotrophic picoplankton subgroups defined by using the three variables previously considered together with surface nitrate concentration (Figure 5). Photic layer depth-integrated biomass of each picoplankton group was used for this analysis. These results revealed three ecological niches in the distribution of picoplankton subgroups. *Prochlorococcus* and LNA prokaryotes were more abundant in warm waters, where nitrate supply was low. HNA prokaryotes and *Synechococcus* dominated in cooler regions with medium to high nitrate supply, and picoeukaryotes were more abundant in cold waters with high nitrate supply. A large degree of overlapping of the ecological niches for all picoplankton subgroups was observed when only surface light was considered. For each picoplankton subgroup Table 4 shows the partial weighted overlap of the ecological

niches defined by the four factors: SST, PAR, nitrate flux and surface nitrate concentration. According to these data only nitrate supply enabled a statistically significant separation of the niches of both heterotrophic (HNA and LNA prokaryotes), and autotrophic (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) picoplankton subgroups. Although the minimum overlap between *Prochlorococcus* and the other autotrophic picoplankton subgroups was also well defined by temperature, only nitrate supply could statistically distinguish the niche partitioning between the two groups of heterotrophic prokaryotes ($p < 0.05$) and between *Synechococcus* and picoeukaryotes ($p < 0.1$).

4 Discussion

4.1 Environmental factors and ecological niches

Picoplankton community composition and concurrent estimates of nitrate supply into the euphotic zone from highly contrasting marine environments allowed us to conclude that sea surface temperature and nitrate supply are the main factors controlling the variability in the biomass of different subgroups, whereas surface light generally played a minor role. As far as we know, only one study had previously investigated the role of these environmental factors in the distribution of, in this case, the two major groups of cyanobacteria. By using a large flow cytometry dataset from all major ocean regions, Flombaum et al. (2013) concluded that temperature and light were the most important predictors of the abundances of *Prochlorococcus* and *Synechococcus*, with nitrate availability exerting a negligible effect. Although this conclusion seems to be contradictory with the results presented here, some important differences between these studies should be noted. Firstly, Flombaum et al. (2013) used bulk estimates of seawater nitrate concentration as a proxy for nitrate availability in the euphotic zone. However, in near steady-state systems such as the subtropical gyres, where diffusive nutrient supply into the euphotic zone is slow, nitrate concentrations are kept close to the detection limit due to phytoplankton uptake. For this reason, nitrate concentrations and actual nitrate supply into the euphotic zone in the vast oligotrophic regions are often largely disconnected Mouriño-Carballido et al. (2011, 2016). Moreover, whereas Flombaum et al. (2013) used *Prochlorococcus* and *Synechococcus* abundances determined at several depths in the upper 200 m, we used depth-integrated biomass of both autotrophic and heterotrophic picoplankton subgroups in the photic layer.

Although our results point to both temperature and nitrate supply as important factors controlling the distribution of the picoplankton subgroups (Figure 4 and Table 3), nitrate supply was the only factor that allowed the distinction between the ecological niches of autotrophic and heterotrophic picoplankton subgroups (Figure 5 and Table 4). Our attempt to sort out the ecological niches of picoplankton subgroups gave rise to three distinct categories. *Prochlorococcus* and LNA prokaryotes were more abundant in warmer waters (above 20°C) where the availability of nitrate was low. *Synechococcus* and HNA prokaryotes prevailed mainly in cooler (below 20°C) marine environments characterized by intermediate and high levels of nitrate supply, and finally, the niche for picoeukaryotes was characterized by lower-low temperatures and high nitrate supply. These results underline the physiological and ecological features of the distinct picoplankton functional subgroups. Our results confirm the previously reported ecological differences between the two major groups of unicellular cyanobacteria (Scanlan and West, 2002; Partensky and Garczarek, 2010; Li, 2009).

Moreover, the ecological niche alignment of the two cyanobacteria genera with the two heterotrophic prokaryotes subgroups is consistent with taxa that prevail in oligotrophic regions (e.g. SAR11) being included in the LNA prokaryotes, whereas copiotrophic and more diverse taxa (Gammaproteobacteria, Bacteroidetes/Flavobacteria, etc) are generally grouped under HNA (Vila-Costa et al., 2012; Schattner et al., 2011). Although the relationship between stratification, mixing and nutrient supply is not obvious (Mouriño-Carballido et al., 2016), our results are in general consistent with the patterns described by Bouman et al. (2011). These authors, by using vertical density stratification as a proxy for the three main environmental factors influencing phytoplankton growth (temperature, light and nutrients) in subtropical regions of the Pacific, Atlantic and Indian Oceans, described the dominance of photosynthetic picoeukaryotes in well-mixed waters, and the prevalence of cyanobacteria in strongly stratified conditions.

10 4.2 Physiological traits of picoplankton subgroups

Although previous studies have revealed that *Prochlorococcus* may have acquired the ability to use nitrate by horizontal gene transfer, their photosynthetic activity primarily relies on regenerated forms of nitrogen (Moore et al., 2002; Malmstrom et al., 2013). Our results support this view and substantiate that, after controlling for the concurrent effects of light and seawater temperature, *Prochlorococcus* biomass was uncorrelated with nitrate fluxes. However, it is important to note that we could not discriminate between high-light (HL) and low-light (LL) ecotypes, and that the presence of nitrate reductase seems to be more relevant in LL (Martiny et al., 2009; Berube et al., 2014). Evolutionary adaptation to light limiting conditions makes *Prochlorococcus* the most efficient light harvesters among Earth's photosynthetic organisms (Morel et al., 1993). Their competitive ability under light limiting conditions could explain the negative effect of light as a predictor for *Prochlorococcus* biomass. Ultimately, the photo-physiological strategy of *Prochlorococcus* leads to i) thermal sensitivity of photosystem II (Mackey et al., 2013), which limits its fundamental niche to temperatures greater than 15 °C (Moore et al., 1995), and ii) high sensitivity to ultraviolet (UV) radiation in surface waters (Llabrés et al., 2010; Mackey et al., 2013; Sommaruga et al., 2005). This could explain that, after removing the effect of light, our data analysis revealed that the effect of temperature on *Prochlorococcus* biomass showed a sigmoid relationship as temperature increased.

Synechococcus is able to use both new and regenerated forms of nitrogen (Moore et al., 2002; Mulholland and Lomas, 2008), which largely explains its wider geographical distribution range (Flombaum et al., 2013). The fact that it is more abundant at intermediate levels of nitrate supply is consistent with the lower intracellular nitrogen quota of *Synechococcus* relative to *Prochlorococcus*, and hence their higher growth rate under saturating nutrient conditions (Marañón et al., 2013). On the other hand, the large affinity of *Prochlorococcus* to acquire nutrients (Partensky and Garczarek, 2010) and absorb light under severe nutrient- and light-limiting conditions (Mella-Flores et al., 2012), preclude the supremacy of *Synechococcus* in warm and stratified oligotrophic systems (Moore et al., 2007). Although *Prochlorococcus* and *Synechococcus* are not very different in cell size and they usually coexist in oligotrophic regions, differences in adaptation to light conditions and UV-stress lead to segregate their maximal distributions across space (vertical segregation) and through time (Chisholm, 1992; Mella-Flores et al., 2012).

Picoeukaryotes, like *Synechococcus*, also exhibited a negative relationship with seawater temperature, again reflecting the superior competitive ability of *Prochlorococcus* under severe nutrient-limiting conditions (Moore et al., 2007). The relative dominance of cyanobacteria in oligotrophic systems results from the fact that cyanobacteria are less negatively affected by nutrient diffusion limitation than picoeukaryotes (Chisholm, 1992). It is widely accepted that small cells are at an advantage
5 over large cells in stratified open ocean environments, where nutrient recycling dominates biogeochemical fluxes (Raven, 1998). First, the surface-to-volume ratio increases with decreasing cell size, which narrows the nutrient diffusion boundary layer around the cell and facilitates the acquisition of nutrients in nutrient impoverished environments. Second, small-sized cells have lower sinking rates than their larger counterparts, which allow them to extend their chances of survival in the euphotic layer (Smayda, 1980; Chisholm, 1992; Kiørboe, 1993).

10 Our analysis indicates that among the picophytoplankton, picoeukaryotes were the most responsive to nutrient fluxes. This is consistent with experimental observations under laboratory-controlled conditions revealing that, within the picoplankton size range, the maximum attainable growth rate increases with increasing cell size (Raven, 1994; Marañón et al., 2013). This positive relationship between maximum growth rate and cell size in the pico- to small nano-phytoplankton size range has been explained as a trade-off between intracellular nitrogen quotas (N requirements) and mass-specific nitrate uptake rates (N uptake)
15 (Marañón et al., 2013). Whereas nitrogen uptake rate exhibits an isometric relationship with cell size, smaller picoplankton cells have substantially larger intracellular nitrogen quotas, which reduce their capability to maximize carbon-specific growth rates. On the other hand high maximum growth rates represent an advantage for picoeukaryotes, as to any other organism, as they attenuate the effect of loss processes such as predation or the wash-out of plankton communities in highly dynamic, turbulent systems (Sherr et al., 2005; Echevarría et al., 2009). For instance, the microzooplankton is thought to maintain the
20 biomass of their prey under tight control, and thus slight variations in picophytoplankton growth rate may substantially alter the resulting biomass of the different picophytoplankton subgroups (Chen et al., 2009).

The unimodal relationship observed between the biomass of picoeukaryotes and nitrate supply ([Figure 4](#)) could seem at first contradictory with the rising hypothesis proposed by Barber and Hiscock (2006), which describes that improved growth conditions benefit all phytoplankton size-classes, including picoplankton. In this regard, [Brewin et al. \(2014\)](#) [Brewin et al. \(2014\)](#)
25 by using data collected along the Atlantic Meridional Transect cruises showed that $<2 \mu\text{m}$ size-fractionated chlorophyll was positively correlated with total chlorophyll only until a value of 1 mg m^{-3} , and then it did not show any positive relationship with total chlorophyll. It is also important to note that surface abundance of picoplankton subgroups reported in our study, which are consistent with previous observations [Zubkov et al. \(2000\)](#); [Frojan et al. \(2014\)](#); [Teira et al. \(2015\)](#)
[\(Zubkov et al., 2000; Frojan et al., 2014; Teira et al., 2015\)](#), did show higher surface abundance of picoeukaryotes in the Galicia coastal upwelling and the NW-Mediterranean compared to the tropical and subtropical Atlantic (Table 2 [and Figure A3](#)).
30 However, this pattern was diluted when depth-integrated biomasses were computed as the lower limit for the integration (the base of the photic zone) was much shallower in the coastal upwelling domain (ca. 37 m), compared to the NW-Mediterranean (ca. 62 m) and the tropical and subtropical regions (ca. 109 m).

Heterotrophic prokaryotes also use dissolved inorganic nutrients, including nitrate, for growth (Kirchman, 2000). Consistent
35 with this, Gasol et al. (2009) showed a positive relationship between prokaryotic abundance and a proxy for nutrient supply

in a latitudinal gradient across the Atlantic. They did not partition this effect on the two subgroups that can universally be differentiated among bacteria and archaea. Our results suggest that LNA prokaryotes respond less markedly to nutrient fluxes than HNA prokaryotes. The effect of nitrate supply on the biomass of LNA prokaryotes showed a linear negative relationship as nitrate supply increases, perhaps associated with their ability to survive under nutrient starving conditions (~~Mary et al. 2008~~) (Mary et al., 2008). Under such conditions, proteorhodopsin-containing LNA prokaryotes (e.g. example SAR11) can use energy from light (~~Mary et al. 2008; Pinhassi et al. 2016~~) (Mary et al., 2008; Pinhassi et al., 2016) improving their competitiveness against non-proteorhodopsin-containing prokaryotes. Consistent with this idea, our results showed a positive relationship between the biomass of LNA prokaryotes and PAR. Li et al. (2004) already proposed the ubiquity of this bottom-up control of prokaryotic abundance in oligotrophic environments ($<1 \text{ mg Chl m}^3$). Therefore, we believe that it is more the difference in the suite of genes (Schattenhofer et al., 2011) than in cell size (? (Morán et al., 2015)) the underlying cause for the clear niche difference between LNA and HNA prokaryotes.

5 Outlook

Picoplankton often dominate marine phytoplankton biomass and primary production in oligotrophic regions (~~Chisholm 1992; Agawin et al. 2000~~) (Chisholm, 1992; Agawin et al., 2000), contribute overwhelmingly to the recycling of organic matter (Azam et al., 1983; Fenchel, 2008), and could have a substantial contribution to the export of carbon to the deep ocean (Richardson and Jackson, 2007). However, our limited understanding about the factors that control picoplankton community composition constrains our ability to include them in ocean biogeochemical models, and predict the consequences of future global change scenarios. For the first time, by combining observations which allowed us to estimate vertical nutrient fluxes, instead of nitrate concentrations, we investigated the role of temperature, light and nitrate supply in the distribution of the major autotrophic and heterotrophic picoplankton subgroups. Our results highlight the role of nitrate supply in the distribution of picoplankton subgroups, as it was the only factor that allowed the statistically significant distinction of the ecological niches between the autotrophic and heterotrophic picoplankton subgroups. In general, autotrophic picoplankton biomass was dominated by *Prochlorococcus* in warmer waters where the availability of nitrate was low, and by *Synechococcus* and picoeukaryotes in cooler waters with medium to high nitrate availability. Similarly, LNA prokaryotes dominated heterotrophic picoplankton biomass in regions of weak nitrate supply, whereas HNA prokaryotes dominated the heterotrophic community in regions of enhanced nutrient supply. Although our study included 97 stations sampled in contrasting environments, a larger dataset, including a broader range of environmental conditions, will be needed to accurately discern the role of temperature and nitrate supply in the field, as both factors are strongly correlated in the ocean. In this regard, by growing three phytoplankton species (the diatom *Skeletonema costatum*, the coccolithophore *Emiliania huxleyi* and the picocyanobacteria *Synechococcus* spp.) in the lab, Marañón et al. (2018) showed a reduced sensitivity of metabolic rates to temperature variability under nutrient-depleted conditions, suggesting that nutrient availability controls the temperature dependence of metabolism. Consistent with these results, our statistical analyses stress the relevance of nitrate supply in the distinction of the ecological niches of heterotrophic and autotrophic picoplankton subgroups. Other mechanisms of nutrient supply, such as mesoscale and sub-mesoscale turbulence,

atmospheric deposition, nitrogen fixation and more complex 3-dimensional dynamics Jenkins and Doney (2003); Bonnet et al. (2005); Estrada et al. (2014); Fernández-Castro et al. (2015), as well as the influence of trophic interactions Van Mooy et al. (2006); Baudoux et al. (2007); Chen et al. (2009); Rusch et al. (2010) deserve further investigation.

5 According to our results, in a future ocean where global change scenarios predict an increase in surface temperature and stratification (~~Howes et al. (2015)~~[\(Howes et al., 2015\)](#)), the resulting decrease in nitrate supply into the euphotic zone ~~Lewandowska et al. (2014)~~[\(Lewandowska et al., 2014\)](#) would lead to the dominance of autotrophic picoplankton by cyanobacteria, whereas the picoeukaryotes would decrease their contribution. ~~In order to get a rough estimation of this effect, we computed the cyanobacteria to picoeukaryotic biomass ratio for the present and a future global change scenario (See supplementary Figure A1 and Methods). According to this analysis, the projected decrease in averaged global nitrate supply (ca. 20%) would~~
10 ~~lead to an average increase of 9% in the ratio of cyanobacteria to picoeukaryotes.~~ Due to the smaller contribution of cyanobacteria to the transfer of carbon to the deep ocean compared to picoeukaryotes, this pattern could have important implications ~~regarding the contribution of the smaller cells to the~~[in the efficiency of the](#) biological carbon pump (Corno et al., 2007).

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15 *Competing interests.* The authors declare no conflict of interest.

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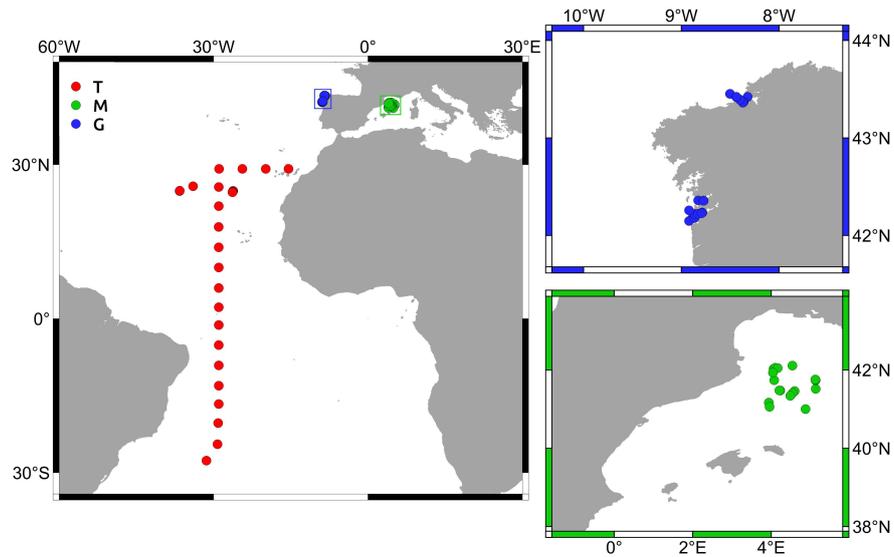


Figure 1. Location of the stations sampled in the tropical and subtropical Atlantic ocean (T), the ~~northwestern~~ Mediterranean Sea (M), and the Galician coastal upwelling (G). Small panels provide details about those stations sampled in M (green color) and G (blue color).

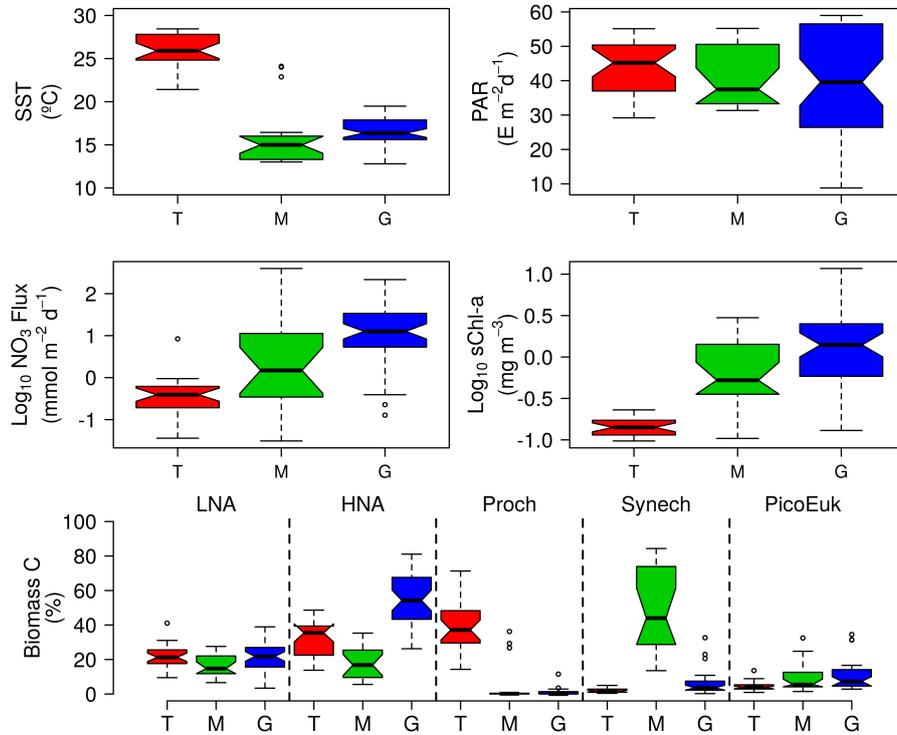


Figure 2. Box-and-whiskers plots of Sea Surface Temperature (SST), surface photosynthetic active radiation (PAR), nitrate supply (NO_3 Flux), surface chlorophyll-*a* concentration (sChl-*a*), and contribution to total picoplankton biomass of low (LNA) and high (HNA) nucleic acid content bacteria, *Prochlorococcus* (Proch), *Synechococcus* (Synech), and small picoeukaryotes (PicoEuk) computed for the tropical and subtropical Atlantic ocean (T), the Northwestern-Mediterranean (M) and the Galician coastal upwelling (G). On each box, the central mark indicates the median, the notches the 95% confidence interval for the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers, and the outliers are plotted individually using white circles.

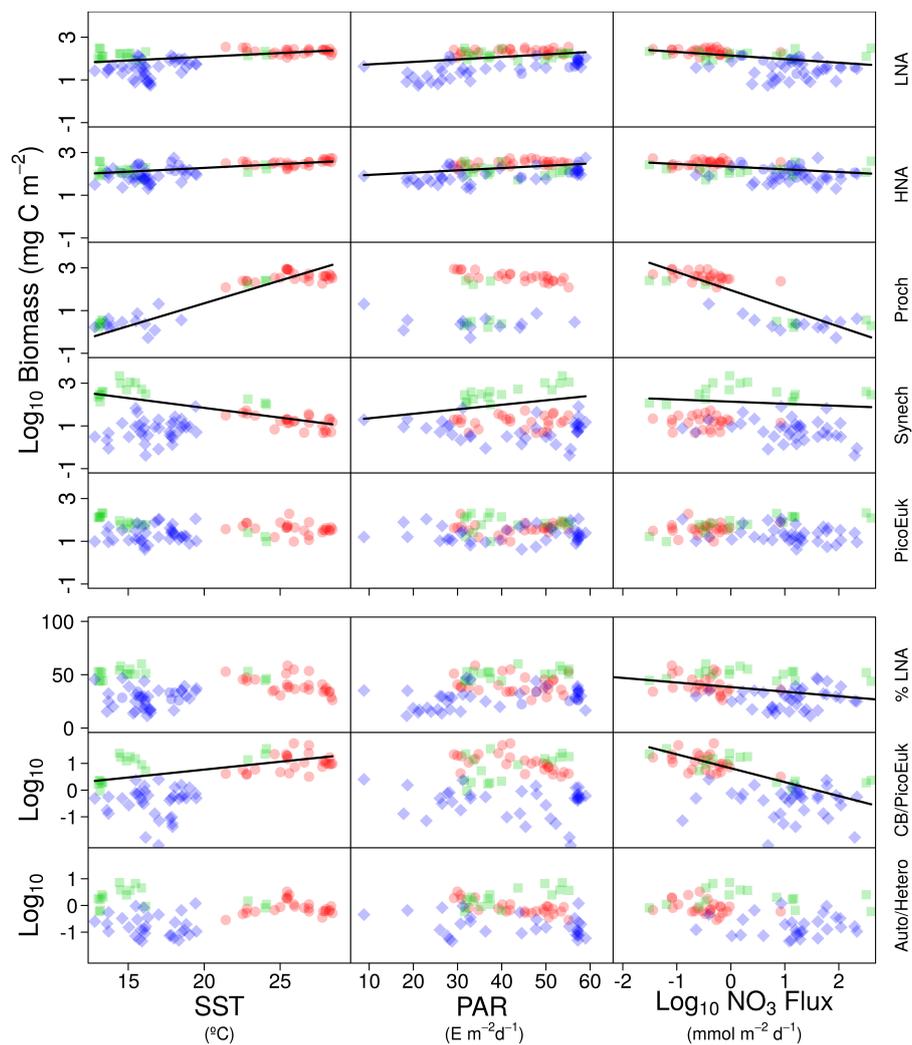


Figure 3. Pair scatter plots representing the relationship between log-transformed depth-integrated biomass for each picoplankton subgroup, the contribution of low nucleic acid content bacteria to heterotrophic picoplankton biomass (%LNA), the ratio of cyanobacteria (*Prochlorococcus* + *Synechococcus*) to picoeukaryotes depth-integrated biomass (CB/PicoEuk), and the ratio autotrophic to heterotrophic picoplankton biomass (Auto/Hetero) versus sea surface temperature (SST), surface photosynthetically active radiation (PAR) and nitrate flux ($\text{NO}_3\text{-NO}_3$ flux). Significant linear relationships are indicated as solid (p-value < 0.01) black lines. Samples collected at different regions are indicated as red dots (tropical and subtropical Atlantic ocean), green squares (NW-Mediterranean) and blue diamonds (Galician coastal upwelling).

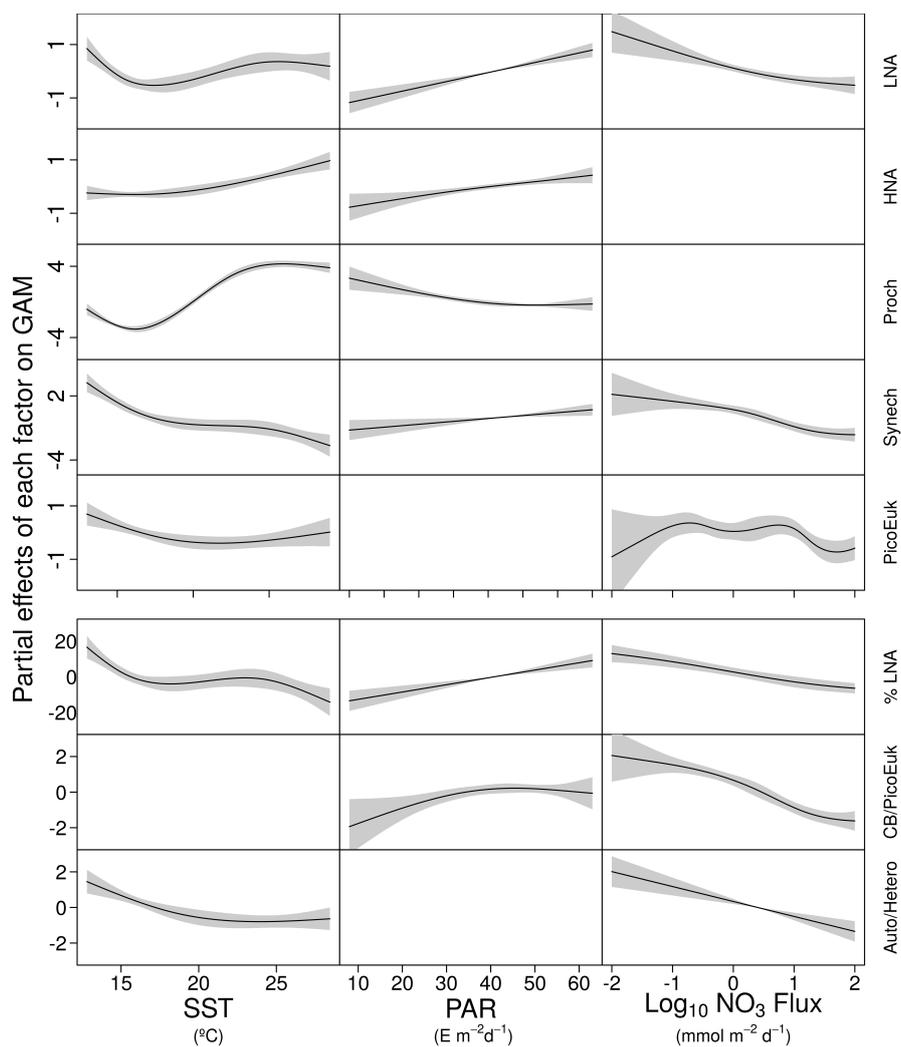


Figure 4. GAM-predicted effects of the response variables (biomass and contribution of picoplankton subgroups) as a smooth function of surface temperature (SST), photosynthetically active radiation (PAR) and nitrate flux (NO_3 flux). All terms were centered in zero. Significant linear relationships are indicated as solid (p -value < 0.01) black lines. Shaded regions represent the 95% confidence intervals of the smooth spline functions. Intercept values were 4.6 (LNA), 5.1 (HNA), 2.1 (*Prochlorococcus*), 3.6 (*Synechococcus*), 3.7 (picoeukaryotes), 36.4 (contribution of LNA to heterotrophic picoplankton, %LNA), 1.4 (cyanobacteria to picoeukaryotes ratio, CB/PicoEuk), and -0.1 (autotrophic to heterotrophic biomass ratio, Auto/Hetero).

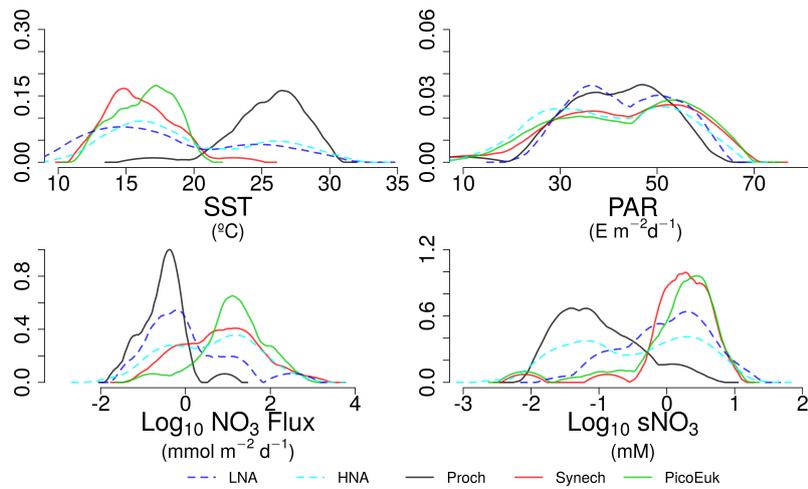


Figure 5. Kernel density estimates of LNA and HNA bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes based on the considered niche descriptors: sea surface temperature (SST), surface photosynthetically active radiation (PAR), nitrate flux (NO_3 flux) and surface concentration (sNO_3).

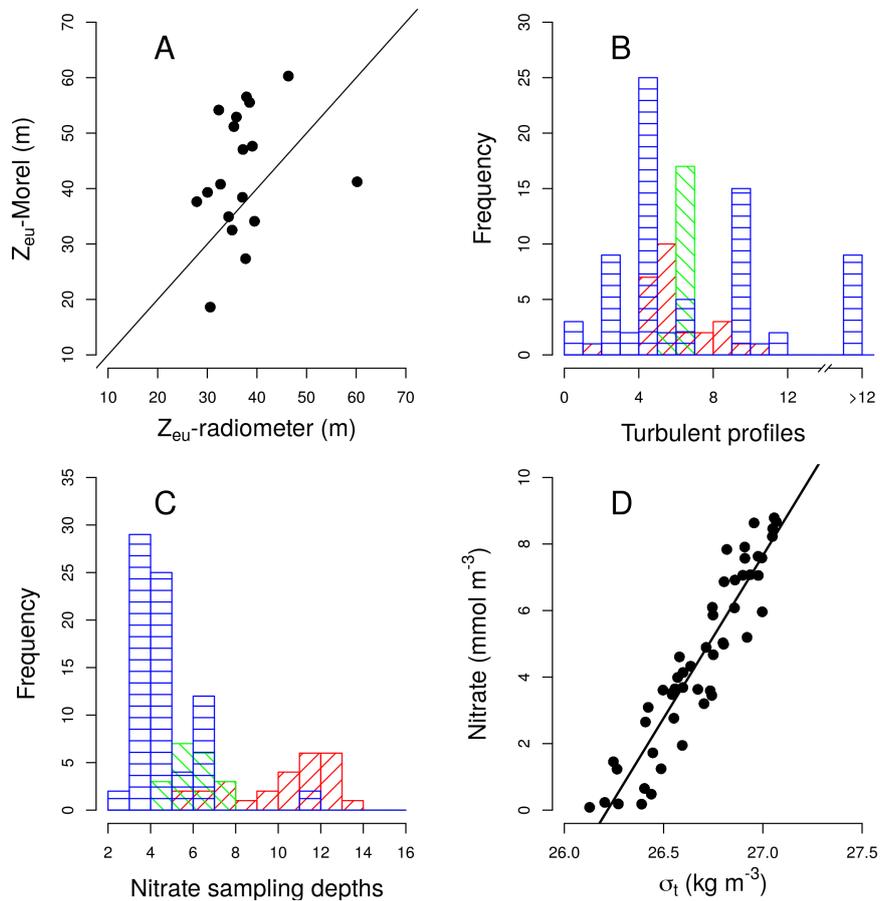


Figure A1. A) Pair scatter plot representing the relationship between the euphotic zone depth (Z_{eu}) computed using the Morel et al. (2007) equation and the data collected by a radiometer during the HERCULES cruises, the solid line represents 1:1 relationship. B) Frequency histogram of the number of turbulence profiles deployed at each station and domain. C) Frequency histograms of the number of samples collected for the determination of nitrate concentration at each station and domain: tropical and subtropical Atlantic ocean (red), the Mediterranean (green) and the Galician coastal upwelling (blue). D) Pair scatter plot representing the relationship between nitrate concentration and density built by using all samples collected during the NICANOR sampling period.

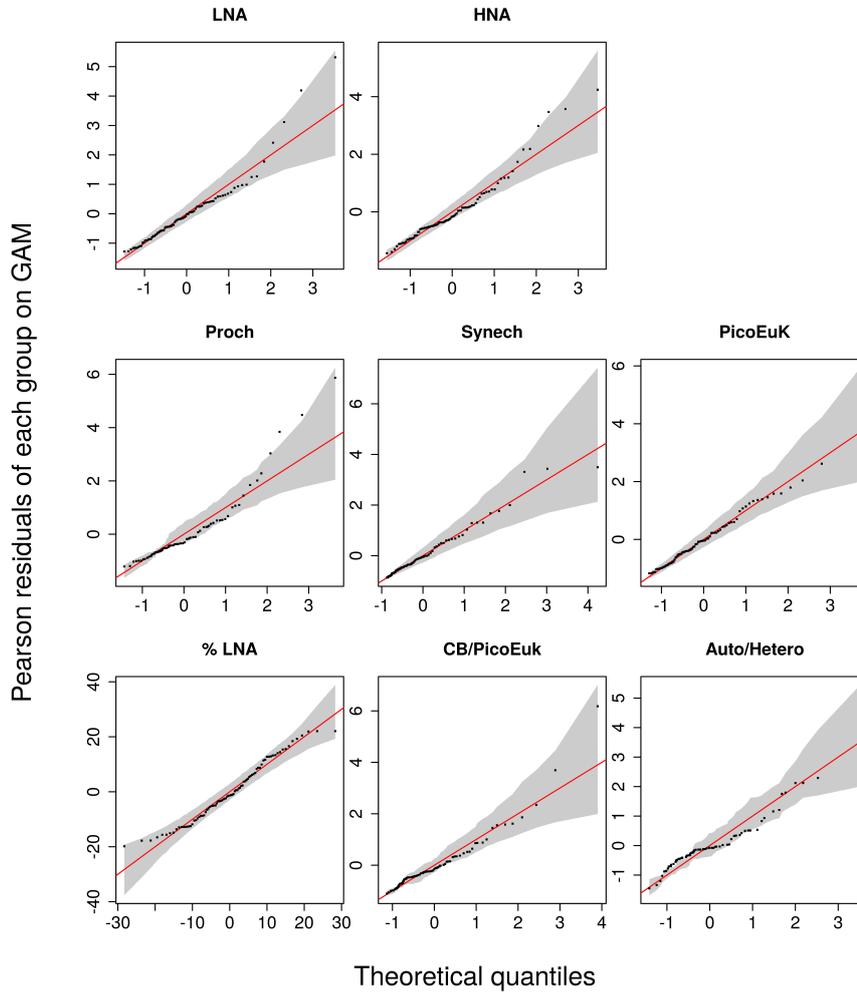


Figure A2. Quantile-Quantile (QQ) plots between the observations and the selected GAM models for each picoplankton subgroup, the contribution of LNA to heterotrophic picoplankton (%LNA), the cyanobacteria to picoeukaryotes ratio (CB/PicoEuk), and the autotrophic to heterotrophic biomass ratio (Auto/Hetero). The y-axes represent the Pearson residuals and the x-axes the negative binomial theoretical quantiles. Solid red lines indicate the theoretical quantile of the models and grey shadows the 95% confidence intervals.

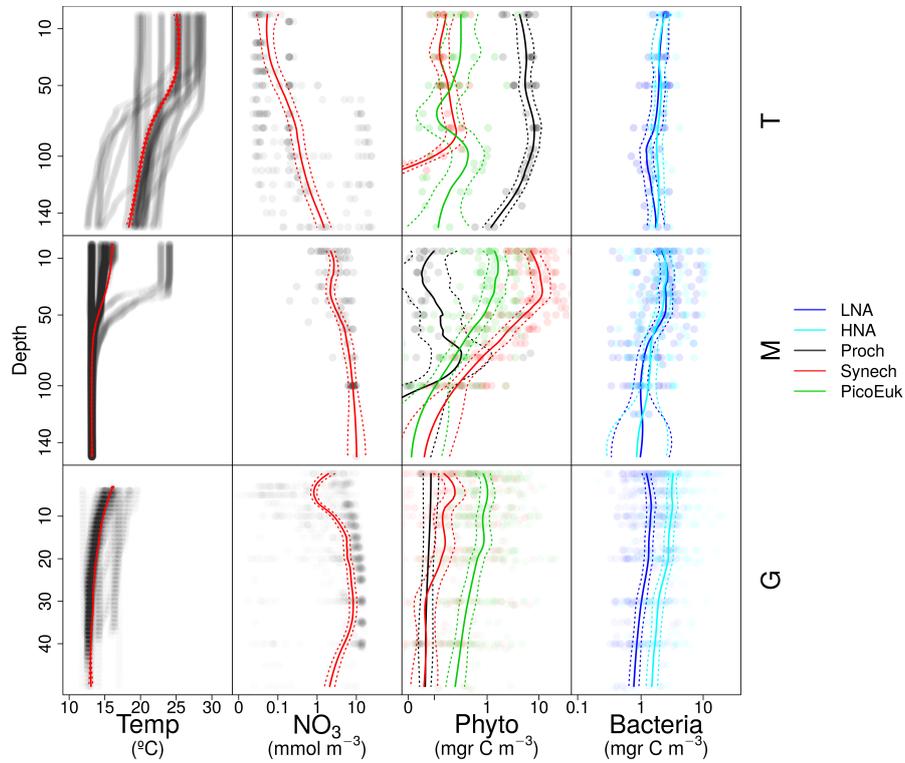


Figure A3. Vertical distribution of temperature (Temp), nitrate (NO₃) and picoplankton biomass of autotrophic (Phyto) and heterotrophic (Bacteria) groups for each domain: tropical and subtropical Atlantic ocean (T), the Mediterranean (M), and the Galician coastal upwelling (G). Points represent raw data and the solid line the locally weighted scatterplot smoothing (LOESS). Dashed lines indicate 95% confidence intervals. Dot and line color intensity indicates the number of overlapping observations.

Table 1. Details of the data included in this study. Domain referred to the tropical and subtropical Atlantic ocean (T), the **Northwestern** Mediterranean Sea (M), and the Galician coastal upwelling (G). N indicates the number of stations sampled at each cruise. Duration (mean \pm standard deviation, in minutes) is the time used for the turbulence profiler deployment in each station. Depth (mean \pm standard deviation, in meters) is the maximum depth reached by the microstructure profiler.

Domain	Region	N	Cruise	Vessel	Date	Duration	Depth
T	NE Atlantic	8	CARPOS	Hespérides	14/10/06- 22/11/06	<u>57 \pm 24</u>	<u>137 \pm 15</u>
T	Atlantic	18	TRYNITROP	Hespérides	14/04/08 - 02/05/08	<u>45 \pm 12</u>	<u>219 \pm 19</u>
M	Liguro-Provençal Basin	6	FAMOSO I	Sarmiento de Gamboa	14/3/09 - 22/3/09	<u>66 \pm 5</u>	<u>259 \pm 38</u>
M	Liguro-Provençal Basin	10	FAMOSO II	Sarmiento de Gamboa	30/4/09 - 13/05/09	<u>94 \pm 4</u>	<u>273 \pm 2</u>
M	Liguro-Provençal Basin	3	FAMOSO III	Sarmiento de Gamboa	16/09/09 - 20/09/09	<u>133 \pm 3</u>	<u>323 \pm 24</u>
G	Ría de A Coruña	1	HERCULES I	Lura	07/06/10	<u>20 \pm 4</u>	<u>35 \pm 2</u>
G	Ría de A Coruña	5	HERCULES II	Lura	28/09/11 - 29/09/11	<u>11 \pm 8</u>	<u>33 \pm 26</u>
G	Ría de A Coruña	13	HERCULES III	Lura	16/07/12 - 20/07/12	<u>8 \pm 5</u>	<u>41 \pm 29</u>
G	Ría de Vigo	9	DISTRAL	Mytilus	14/02/12 - 06/11/12	<u>110 \pm 76</u>	<u>38 \pm 1</u>
G	Ría de Vigo	2	CHAOS	Mytilus	20/08/13 - 27/08/13	<u>1515 \pm 6</u>	<u>41 \pm 29</u>
G	Ría de A Coruña	12	NICANOR	Lura	27/02/14 - 17/12/15	<u>33 \pm 5</u>	<u>62 \pm 3</u>
G	Rías de Vigo & Pontevedra	10	ASIMUTH	Ramón Margalef	17/06/13 - 21/06/13	<u>10 \pm 4</u>	<u>28 \pm 10</u>

Table 2. Mean \pm standard deviation of Sea Surface Temperature (SST), surface Photosynthetic Active Radiation (PAR), Mixed Layer Depth (MLD), Photic Layer Depth (1% PAR), surface nitrate concentration (sNO₃), nitrate gradient, vertical diffusivity (K), nitrate supply (NO₃ flux), surface chlorophyll (sChl-*a*), photic layer depth-integrated chlorophyll-*a* (Chl-*a*), biomass (B), abundance (A), and contribution (C) to total picoplankton biomass (Total Pico B), and surface abundance (s) of LNA and HNA bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes computed for the Tropical and subtropical Atlantic ocean (T), the ~~Northwestern~~ Mediterranean (M), and the Galician coastal upwelling (G). MLD was estimated from an increase in water column density of 0.125 Kg m⁻³ relative to surface values. A nonparametric 1-way ANOVA (Kruskal-Wallis) was performed to test the null hypothesis that independent groups come from same distribution. The Bonferroni multiple comparison test was applied a posteriori to analyze the differences between every pair of groups. *p < 0.05; **p < 0.01; ***p < 0.001.

Variables (Units)	T	M	G	KW p-value	Post hoc Bonferroni
SST (°C)	26 ± 2	16 ± 4	16 ± 2	<0.001***	T>G>M
PAR (E m ⁻² d ⁻¹)	43 ± 23	42 ± 13	39 ± 17	0.69	
MLD (m)	61 ± 30	61 ± 71	12 ± 10	<0.001**	T,M>G
1% PAR (m)	109 ± 23	62 ± 13	37 ± 17	<0.001**	T>M>G
sNO ₃ (μmol m ⁻³)	90 ± 77	2414± 1635	1601 ± 1604	<0.001***	T<M,G
NO ₃ gradient (μmol m ⁻⁴)	146 ± 158	90 ± 40	105 ± 100	0.71	
K (m ² s ⁻¹) x10 ⁻³	0.2 ± 0.3	5.3 ± 13.9	0.5 ± 0.8	0.24	
NO ₃ flux (mmol m ⁻² d ⁻¹)	0.7 ± 1.6	41 ± 113	30 ± 46	<0.001***	T<M<G
sChl- <i>a</i> (mg m ⁻³)	0.1 ± 0.1	0.9 ± 0.9	2.2 ± 2.5	<0.01**	T<M<G
Chl- <i>a</i> (mg m ⁻²)	31 ± 6	70 ± 99	81 ± 66	<0.001***	T<G
sLNA A (cell ml ⁻¹) x10 ⁵	4.4±2.4	3.7±2.5	2.1±1.1	<0.001***	T>M>G
sHNA A (cell ml ⁻¹) x10 ⁵	3.0±1.8	4.0±4.5	3.6±2.3	0.13	
sProchl A (cell ml ⁻¹) x10 ³	144±132	2.2±4.4	1.0±2.8	<0.001***	T>M,G
sSynech A (cell ml ⁻¹) x10 ³	18±66	75±81	5.7±6.9	<0.001***	T<M>G
sPicoEuk A (cell ml ⁻¹) x10 ³	2.5±9.4	6.8±8.4	5.7±6.9	<0.001***	T<M,G
LNA A (cell m ⁻²) x10 ¹²	40 ± 20	22 ± 8	6.4 ± 4	<0.001***	T,M>G
HNA A (cell m ⁻²) x10 ¹²	27 ± 1	22 ± 1	9.4 ± 0.8	<0.001***	T>M>G
Prochl A (cell m ⁻²) x10 ¹¹	156 ± 121	10 ± 23	0.5 ± 1	<0.001***	T>M,G
Synech A (cell m ⁻²) x10 ¹¹	7 ± 15	50 ± 49	2 ± 2	<0.001***	T<M>G
PicoEuk A (cell m ⁻²) x10 ¹¹	1.7 ± 3	2.8 ± 2	1 ± 2	<0.001***	T<M>G
LNA B (mg C m ⁻²)	253 ± 105	170 ± 97	43 ± 34	<0.001***	T>M>G
HNA B (mg C m ⁻²)	216 ± 127	168 ± 105	108 ± 73	0.02*	T>M>G
Prochl B (mg C m ⁻²)	482 ± 516	36 ± 84	1.3 ± 4	<0.001***	T>M,G
Synech B (mg C m ⁻²)	43 ± 83	576 ± 530	19 ± 26	<0.001***	T,M>G
PicoEuk B (mg C m ⁻²)	59 ± 102	86 ± 59	43 ± 59	<0.001***	T<M>G
Total Pico B (mg C m ⁻²)	1052 ± 215	1038 ± 485	216 ± 36	<0.001***	T,M>G
LNA C (%)	27 ± 10	18 ± 8	21 ± 9	<0.001***	T>M,G
HNA C (%)	22 ± 12	17 ± 10	55 ± 15	<0.001***	T,M<G
Prochl C (%)	41 ± 16	5 ± 12	1 ± 2	<0.001***	T,M<G
Synech C (%)	4 ± 5	50 ± 24	6 ± 7	<0.001***	T,G>M
PicoEuk C (%)	5 ± 5	10 ± 9	11 ± 9	<0.001***	T<M,G

Table 3. Simple (R^2) and adjusted squared correlation coefficients (Adj- R^2) for simple linear regression and multiple Generalized Additive Models (GAM) built to predict depth-integrated biomass for each picoplankton subgroup, the contribution of LNA bacteria to total heterotrophic picoplankton biomass (% LNA), the ratio cyanobacteria (*Prochlorococcus* + *Synechococcus*) to picoeukaryotes depth-integrated biomass (CB/PicoEuk) and the ratio autotrophic (CB+PicoEuk) to heterotrophic bacteria (LNA + HNA) biomass based on Sea Surface Temperature (SST), surface photosynthetically active radiation (PAR) and nitrate supply (NO_3 flux). Negative binomial distribution was assumed. Multiple model selection was based on stepwise regression and the Akaike Information Criterion (see Methods). Only significant (p -value < 0.05) results are shown. Percentage of total effects represent the contribution of each environmental factor to the variability explained by each GAM model (see methods).

Group	R^2 simple linear			Adj- R^2 multiple regression	Percentage of total effects		
	SST	PAR	NO_3 flux		SST	PAR	NO_3 flux
LNA	0.39	<0.01	0.34	0.55	0.30	0.35	0.35
HNA	0.47	0.05	0.18	0.53	0.52	0.48	
Proch	<0.01		<0.01	0.86	0.72	0.28	
Synech	0.11	<0.01		0.52	0.53	0.16	0.31
PicoEuk				0.23	0.51		0.49
% LNA		0.05	0.12	0.49	0.39	0.26	0.35
CB/PicoEuk	0.25		0.28	0.40		0.38	0.62
Auto/Hetero				0.29	0.39		0.61

Table 4. Partial weighted niche overlap (%) for each environmental factor and picoplankton subgroup. sNO₃ represent surface nitrate concentration. Asterisks denote the existence of significant differences between niches (^ p<0.1, * p<0.05, ** p<0.01, *** p<0.001).

		LNA	HNA	Proch	Synech	PicoEuk
SST	LNA	100				
	HNA	85	100			
	Proch			100		
	Synech			9***	100	
	PicoEuk			5***	84	100
PAR	LNA	100				
	HNA	80	100			
	Proch			100		
	Synech			80	100	
	PicoEuk			74*	94	100
NO₃ Flux	LNA	100				
	HNA	69*	100			
	Proch			100		
	Synech			31***	100	
	PicoEuk			14***	77 ^	100
sNO₃	LNA	100				
	HNA	73^	100			
	Proch			100		
	Synech			22***	100	
	PicoEuk			29***	89	100