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Interactive comment on "Factors controlling the community structure of picoplankton in contrasting marine environments" *by* Jose Luis Otero-Ferrer et al.

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Referee comments shown in black, Author replies shown in blue, Changes to manuscript in red

Overall comments

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

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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.

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

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

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

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Page 13 Lines 8-12

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Page 9 Lines 10-14

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 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?

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*BV0.898 (Gundersen et al., 2002).

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).

Change: Page 7 Line 4-15

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In order to estimate biovolume (BV), we used an empirical calibration between Size SCatter (SSC) and cell diameter (Calvo-Díaz et al., 2006), assuming spherical shape for all groups. The following volume-to-carbon conversion factors were used for picoautotrophic groups: 230 fg C for *Synechococcus*, 240 fg C for *Prochlorococcus* and 237 fg C for picoeukaryotes (Worden et al., 2004). For bacteria BV was converted into carbon biomass by using the allometric relationship: 108.8 fg C*BV0.898 (Gundersen et al., 2002). More details about the processing and analysis of flow cytometry samples are provided in Calvo-Díaz et al. (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.

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 Gener-

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alized 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_3Flux), 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:

$$y_j = I + s(SST) + s(PAR) + s(log(NO_3Flux)) + Error$$

where y 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 yj and the predictors. For those response variables that followed a negative binomial distribution the used

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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 northwestern. No need to repeat every time since for the Atlantic the same was done.

Done

Section 2, line 14 – Diaz et al 2018 does not seem to be on bioxriv 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.

Done

RESULTS Section 4.1, line 15, please add the average temperature value for picoeuks, 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.

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

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.

Cites referring key figures in the discussion have been included.

Bibliography

Calvo-Díaz, A. and Morán, X. A. G.: Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay, Aquat. Microb. Ecol., 42(2), 159–174,

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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 ± standard deviation) is the time duration in minutes of the turbulence profiler deployment in each station. Duration (mean ± standard deviation, in minutes) is the time used for the microstructure turbulence operation at each station). Depth (mean ± 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	57 ± 24	137 ± 15
T	Atlantic	18	TRYNITROP	Hespérides	14/04/08 - 02/05/08	45 ± 12	219 ± 19
M	Liguro-Provençal Basin	6	FAMOSO I	Sarmiento de Gamboa	14/3/09 - 22/3/09	66 ± 5	259 ± 38
M	Liguro-Provençal Basin	10	FAMOSO II	Sarmiento de Gamboa	30/4/09 - 13/05/09	94 ± 4	273 ± 2
M	Liguro-Provençal Basin	3	FAMOSO III	Sarmiento de Gamboa	16/09/09 - 20/09/09	133 ± 3	323 ± 24
G	Ría de A Coruña	1	HERCULES I	Lura	07/06/10	20 ± 4	35 ± 2
G	Ría de A Coruña	5	HERCULES II	Lura	28/09/11 - 29/09/11	11 ± 8	33 ± 26
G	Ría de A Coruña	13	HERCULES III	Lura	16/07/12 - 20/07/12	8 ± 5	41 ± 29
G	Ría de Vigo	9	DISTRAL	Mytilus	14/02/12 - 06/11/12	110 ± 76	38 ± 1
G	Ría de Vigo	2	CHAOS	Mytilus	20/08/13 - 27/08/13	1515 ± 6	41 ± 29
G	Ría de A Coruña	12	NICANOR	Lura	27/02/14 - 17/12/15	33 ± 5	62 ± 3
G	Rías de Vigo & Pontevedra	10	ASIMUTH	Ramón Margalef	17/06/13 - 21/06/13	10 ± 4	28 ± 10

Fig. 1. Table 1

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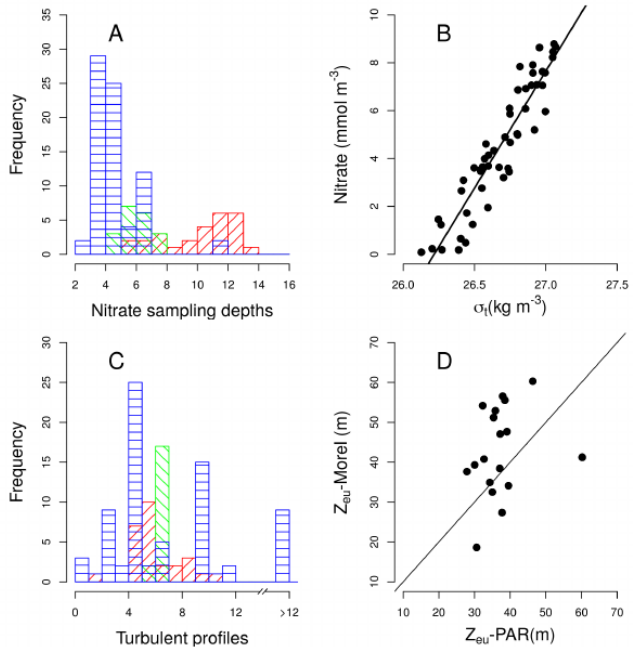

Figure A2. A) Frequency histograms of the number of nutrient where samples for nitrate concentration were collected at each station and domain: tropical and subtropical Atlantic ocean (red), the Northwestern Mediterranean (green) and Galician coastal upwelling (blue). B) Pair scatter plot representing the relationship between nitrate concentration and density built by using all samples collected during the NICANOR sampling period. C) Frequency histogram of the number of turbulence profiles deployed at each station and domain. D) 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 cruise measured used a radiometer and predicted using the relationship with surface chlorophyll Morel et al. (2007), the solid line represents 1:1 relationship.

Fig. 2. Figure A2

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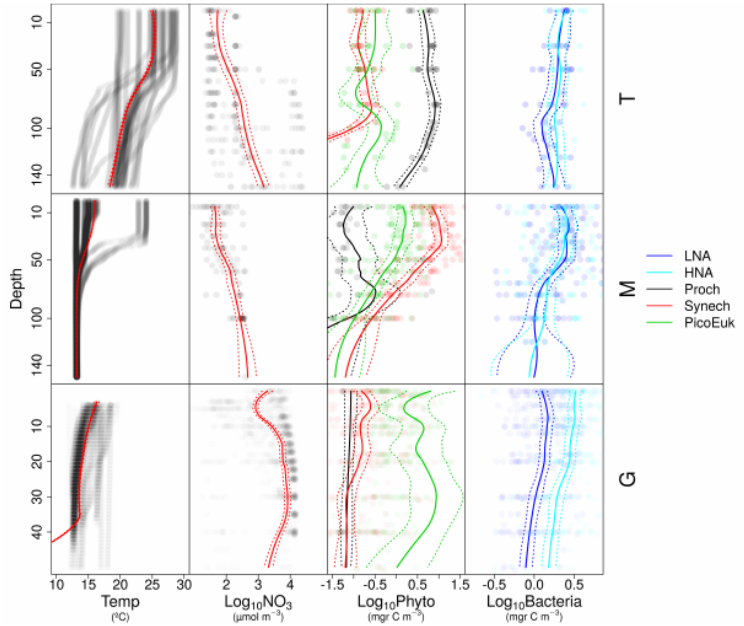

Figure A3. Vertical distribution of temperature (Temp), nitrate (NO_3) and picoplankton biomass of autotrophic (Phyto) and heterotrophic (Bacteria) groups for each domain: tropical and subtropical Atlantic ocean (T), the Northwestern Mediterranean (M), and Galician coastal upwelling (G). Points represent 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.

Fig. 3. Figure A3

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