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**PHYTOPLANKTON BIOVOLUME IS INDEPENDENT FROM THE SLOPE OF THE SIZE
SPECTRUM IN THE OLIGOTROPHIC ATLANTIC OCEAN**

Enrique Moreno-Ostos^{a,*}, José María Blanco^a, Susana Agustí^{b,c}, Luis M. Lubián^d,
Valeriano Rodríguez^a, Roberto L. Palomino^a, Moira Llabrés^b and Jaime Rodríguez^a

^a Universidad de Málaga. Department of Ecology. Campus Teatinos S/N. 29071 Málaga. SPAIN.

^b Department of Global Change Research. IMEDEA Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB). Miquel Marqués 21. 07190 Esporles, Mallorca. SPAIN

^c Red Sea Research Center. KAUST. Thuwal 23955-6900. SAUDI ARABIA.

^d Instituto de Ciencias Marinas de Andalucía (CSIC). Campus Universitario Río San Pedro, s/n. Puerto Real. Cádiz. 11519. SPAIN.

*** Corresponding author at:** Enrique Moreno-Ostos. Marine Ecology and Limnology Research Group. Department of Ecology. University of Málaga. Campus Universitario de Teatinos S/N. 29071 Málaga. SPAIN. Phone +34952136649. E-mail address: quique@uma.es

ABSTRACT

Modelling the size-abundance spectrum of phytoplankton has proven to be a very useful tool for the analysis of physical-biological coupling and the vertical flux of carbon in oceanic ecosystems at different scales. A frequent observation relates high phytoplankton biovolume in productive regions with flatter spectrum slope and the opposite in oligotrophic ecosystems. Rather than this, the relationship between high biovolume phytoplankton assemblages and flatter size-abundance spectra does not correspond with measurements of the phytoplankton community in the Atlantic Ocean open waters. As part of the Malaspina Circumnavigation Expedition, sixty seven sampling stations within the Atlantic Ocean covering six oceanographic provinces, at different seasons, produced a complete set of phytoplankton size-spectra whose slope and biovolume did not show any obvious interrelation. In these oligotrophic sites, small (prokaryotes) and medium-size (nanoplankton) cells are responsible for the most part of biovolume, and their response to environmental conditions does not apply to changes in the size-abundance spectrum slope as expected in richer, large-cell dominated ecosystems.

Keywords: Phytoplankton, size-abundance spectrum, biovolume, Atlantic Ocean, Malaspina Circumnavigation Expedition.

1. Introduction

Phytoplankton size structure is a key property strongly determining food-web organisation, energy flow and matter cycling in marine ecosystems. Phytoplankton physiological rates and ecological function, including metabolic rates, light and nutrients dynamics, sinking rates, trophic organization and the efficiency of organic carbon exportation into the deep ocean are directly related to the size structure of phytoplankton assemblages (Legendre and Le-Fèvre, 1989; Chisholm, 1992; Legendre and Rassoulzadegan, 1996; Rodríguez et al., 2001; Platt et al., 2005; Falkowski and Oliver, 2007; Marañón, 2009; Finkel et al., 2010; Ward et al., 2012; Marañón et al., 2013; Marañón, 2015).

Studies on the phytoplankton size structure are usually based on size-abundance spectra, a valuable taxonomic and integrative approach to analyze the structure of the complex and highly diverse phytoplankton community (Rodríguez and Mullin, 1986; Dickie et al., 1987; Gaedke, 1992; Blanco et al., 1994; Marañón, 2009). Mathematically, it consists in a two-dimensional plot showing the phytoplankton abundance (e.g., cells mL⁻¹) distribution along a scale of cell volume (e.g., µm³). A simple least-squares linear regression on the log-transformed values of abundance and cell size provides the two parameters that characterize the size spectrum, slope (b) and y-intercept (a). The integral of the mathematical function describing the size-abundance spectrum is the total phytoplankton biovolume (e.g. µm³ mL⁻¹) (Platt and Denman, 1977; Blanco et al., 1994).

Both a and b parameters have been proposed as quantitative measures of aquatic ecosystem structure (Sprules and Munawar, 1986; Rodríguez et al., 1998; Marañón et al., 2007). The intercept a seems to be related to total phytoplankton biovolume (Sprules and Munawar, 1986), while the slope of the linear model (b) is an indicator of the proportion of biovolumes between consecutive size classes (Blanco et al., 1994) providing information on the transfer efficiency along the spectrum (Gaedke, 1993). Phytoplankton size-abundance spectrum slope is also related to the ecosystem productivity. Thus, oligotrophic regions typically show steeper slopes (−1.3 to −1.1),

while the slope become less negative (-0.8 to -0.6) in highly productive, coastal ecosystems (Rodríguez et al., 1998; Cavender-Bares et al., 2001; Reul et al., 2005; Cermeño et al., 2006; Cermeño and Figueiras, 2008; Marañón et al., 2007; Huete-Ortega et al., 2010, 2011, 2014).

This pattern has led to the common assumption that there is a relation between the value of the size-abundance spectrum slope and the phytoplankton community biovolume. Thus, communities with high phytoplankton biovolume (found in productive regions) usually exhibit size-abundance spectra with a less-negative (flatter) slope, this being due to the comparatively higher contribution of large-size cells to total biovolume. By contrast, low biovolume communities (usually found in low productivity regions) depict more-negative (steeper) slopes due to the comparatively higher contribution of small pico- and nanophytoplanktonic cells. As a consequence of this paradigm, b could be considered as an adequate ecosystem indicator itself.

However, on theoretical, mathematical grounds, Gómez-Canchong et al. (2013) have demonstrated that total phytoplankton biovolume is, at the same time, directly related to a and inversely related to the absolute value of b . Consequently, total biovolume could remain constant for different spectrum shapes if changes in one of the parameters were compensated by opposite changes in the other. They concluded that to characterize any aquatic ecosystem from its phytoplankton size structure at least two elements of the size-abundance spectrum triad (biovolume or abundance, a and b) are indeed necessary. On the other side, the assumed relation between total biovolume and spectrum slopes derives from the comparison of phytoplankton communities from highly contrasting ecological conditions, such as coastal *versus* open ocean ecosystems. Does this relation between spectrum slope and total phytoplankton biovolume hold for less-contrasting ecosystems, such as those occupying open ocean waters?

In this article we examine the size-abundance spectra of phytoplankton along six biogeographical provinces (Longhurst, 2007) in the Atlantic Ocean. Our spectra cover a cell size (volume scale)

range of eight orders of magnitude (from phototrophic picoplankton to microplankton). The main goal of this study is to analyze the relationship between size-abundance spectrum parameters and total phytoplankton biovolume in the open ocean. Our results will show that, in spite of sharp changes in cell abundance, total phytoplankton biovolume and spectrum slope are uncorrelated in the oligotrophic ocean.

2. Material and Methods

2.1 Study area and general hydrography

Three oceanographic cruises were conducted in the Atlantic Ocean on board R/V *Hespérides* as part of the Malaspina Circumnavigation Expedition (Fig. 1). The cruises took place in December 2010-January 2011 (Malaspina Leg 1; Atlantic latitudinal cruise), January-February 2011 (Malaspina Leg 2; South Atlantic longitudinal cruise) and June-July 2011 (Malaspina Leg 7; North Atlantic longitudinal cruise). A total of 67 sites were visited, covering the following biogeographical provinces (Longhurst, 2007): Caribbean (CARB), North Atlantic Subtropical Gyral (NASE), North Atlantic Tropical Gyral (NATR), Western Tropical Atlantic (WTRA), South Atlantic Gyral (SATL) and Benguela Current Coastal (BENG). Some of them (NASE, NATR and SATL) were partially surveyed in two of the three cruises (NASE and NATR during Malaspina Legs 1 and 7; SATL during Malaspina Legs 1 and 2).

At each sampling station, vertical profiles of water temperature and fluorescence were obtained from the surface to 300 m depth using a Conductivity-Temperature-Depth probe (CTDSea-Bird 911) attached to a rosette sampler equipped with 24 Niskin bottles. The CTD fluorometer was calibrated against chlorophyll *a* (chl *a*) concentration extracted from water samples and determined by fluorometric analysis. The upper mixed layer depth (UML) was defined as the depth at which the thermal gradient was higher than $0.1^{\circ}\text{C m}^{-1}$ (Pérez et al., 2006; Leppäranta and Myrberg, 2009;

Ortega-Retuerta et al., 2010).

2.2 Nutrient concentrations

Concentrations of nitrate were determined on-board on fresh samples using a Skalar autoanalyzer following the methods described in Moreno-Ostos (2012). The nitracline was defined as the depth at which nitrate concentration reached $1 \mu\text{mol L}^{-1}$ (Robinson et al., 2006).

2.3 Phytoplankton size-abundance spectra

Water samples for phytoplankton size and abundance determinations were collected at the Deep Chlorophyll Maximum (DCM) depth after visual inspection of the vertical fluorescence profiles obtained with the CTD system. The DCM nutrient and light conditions allow the sustainment of phytoplankton communities developing a wide range of cell size.

Cell size was described as “cell volume” (μm^3). Some of our first phytoplankton size-abundance spectra (e.g. Rodríguez and Mullin, 1986) used “cell-carbon content” as a more precise descriptor of single-cell biomass. However, the study of size-abundance spectra in large collections of field samples covering the whole spectrum of phytoplankton cell size is unlikely to be effective or feasible if cell size is to be measured as carbon or any other element cell content. As a matter of fact, the origin of plankton size-abundance spectrum empirical research started with the application of the “particle-counter” technology where cell volume is the reference descriptor for cell size (Sheldon et al., 1972). Today this kind of analysis can be improved through technologies like flow-cytometry and image analysis that, in addition, overcome the problem of counting and measuring non living particles as the old particle counters do. These approaches permit to efficiently collect information related with morphological properties that can be transformed in estimations of cell volume, as summarized below (more detailed methodological descriptions can be found in Rodríguez and Li, 1994, Rodríguez et al., 1998, Rodríguez et al., 2001, Huete-Ortega et al., 2010, Huete-Ortega et al., 2012, Marañón et al., 2013, Huete-Ortega et al. 2014, among others).

Flow cytometry analysis

Picophytoplankton ($< 2\mu\text{m}$ equivalent spherical diameter, ESD) and nanophytoplankton ($2\text{--}20\mu\text{m}$ ESD) cell abundance were determined on-board on fresh samples using a FACSCalibur flow cytometer (Becton Dickinson) with a laser emitting at 448 nm. To obtain an estimation of cell size we carried out a calibration of the flow cytometer signals with 7 phytoplankton cultures with cell sizes ranging from 1 to $22\mu\text{m}$ ESD (*Synechococcus oceanicus*, *Nannochloropsis gaditana*, *Chaetoceros calcitrans*, *Isochrysis galbana*, *Tetraselmis chuii*, *Rhodomonas salina* and *Gyrodinium dorsum*). Although expected that “forward scatter” (FSC) signal should be directly related with cell size, we found a better relationship between the Side Scatter (SSC) signal and cell volume (μm^3) measured by microscopy (see below) in samples of 100 cells/species. We thus decided to use the regression model “log cell volume (μm^3) = 0.0056 SSC- 1.61” ($r^2=0.95$, $p<0.0005$, $n=7$) to predict cell volume from SSC flow cytometer signal.

Microscopy-Image analysis

Phytoplankton $>20\mu\text{m}$ ESD was concentrated on-board from 5 L water samples through a $15\mu\text{m}$ pore mesh by gravity. This is a volume large enough to have an unbiased representation of large phytoplankton cells with abundances as low as 1 cell mL^{-1} in these oligotrophic environments. The concentrate was fixed with 1% Lugol's iodine and stored in the dark. Once in the laboratory, samples were settled following Utermöhl method (Lund et al., 1958) and phytoplankton cells were counted and measured using an inverted Leica DMIL microscope. Photomicrographs were taken with an Allied Pike F145C-IRF16 digital camera. Images were processed using specific and public software developed by one of the authors (J.M. Blanco) at the University of Málaga (Fot-O-matón II). At least 100 microscope measurements of cells/colonies length and width were made for the different phytoplankton geometric shapes (Hillebrand et al., 1999) present in the samples. Cell volumes were calculated from the obtained morphological data through numerical computation.

Size-abundance spectra

Finally, complete phytoplankton size-abundance spectra were elaborated by a combination of the size-abundance distributions derived from flow cytometry and microscopy image analysis measurements (Fig. 2). First, measured abundance data were arranged on size classes established on an octave ($\log_2$) scale of cell volume, where size amplitude is 2x the value of cell nominal volume. The ($\log_2$) permits to work with a rather “fine-scale” but still wide enough to absorb the potential errors derived from the techniques employed to measure cell volume (use of the SSC flow cytometer signal, boundary and cell-shape effects of the image analysis, etc). Classes with zero observations were excluded, as $\log(0)$ is mathematically undefined (Gómez-Canchong et al., 2013). Then, a regression model was applied to the log-transformed values of cell abundance (y axis, cells mL^{-1}) and cell volume (x axis, μm^3), using the lower limit of the size class as its nominal size. This regression provided a definite value of the size-abundance spectra slope (b) and an estimated first value of the y-axis intercept parameter (a). The intercept was corrected to compensate the bias caused by choosing the nominal value for each size class (see mathematical details in Blanco et al. 1994).

Simultaneously, total phytoplankton biovolume ($\mu\text{m}^3 \text{ mL}^{-1}$) for each sample was calculated as the simple sum of all the individual cell volumes obtained from both flow cytometry and microscopy methods.

This method to build size-spectra remains as the most widely used, despite some issues caused by the loss of information when individual sizes are filed in just one class. The way the data is obtained generates crowded classes (often for small organisms) and almost-empty classes (often for the bigger ones) and this feature translates in different standard errors for each size class (Blanco et al., 1994; Vidondo et al., 1997). Several alternative methods have been proposed to overcome this,

as a cumulative method based on Pareto's distribution (Vidondo et al., 1997) that keeps all the countings with equal weight in the distribution, or a sequential reiterated sub-sampling of the same data set (bootstrapping) to estimate the confidence limits of the size distribution (Schartau et al., 2010). Also, a number of alternatives to describe size diversity instead of size distribution have been proposed (Ruiz et al., 1994; Quintana et al., 2008). These alternatives, although well known, are less popular due to the increase in mathematical complexity with the data arrangement and parameter interpretation. Fortunately, modern automatic particle counters (as flow cytometer) avoid in great manner this problem. Furthermore, to lessen the effect in microscopy analysis, a 5 liter water sample was concentrated and the largest size class with less than 4 counts was eliminated.

3. Results

3.1 General oceanographic conditions

The thermal structure recorded on the three cruises is shown in Fig. 3. During the North Atlantic longitudinal cruise (Malaspina leg 7), the warmest sea surface temperature (SST) and the more intense thermal stratification were recorded at longitudes higher than 60°W within the CARB province ($28.5 \pm 0.2^\circ\text{C}$), where UML was located at $114 \pm 30\text{m}$ depth. Within NATR, SST remained high ($27.2 \pm 1.2^\circ\text{C}$) while the UML was wider ($150 \pm 28\text{m}$). NASE province was characterized by lower SST ($21.9 \pm 1^\circ\text{C}$), less intense thermal stratification and a narrower UML ($96 \pm 40\text{m}$).

During the latitudinal transect Malaspina Leg 1, the lowest SST ($20.1 \pm 1.3^\circ\text{C}$) was recorded at latitudes higher than 25°N within the NASE province and became warmer ($25.4 \pm 1.2^\circ\text{C}$) as the cruise proceed throughout the NATR province. The UML depth observed was similar in both provinces ($75 \pm 12\text{m}$ in NASE and $64 \pm 22\text{m}$ in NATR). The warmest SST ($27.4 \pm 0.4^\circ\text{C}$), the narrowest UML depth ($59 \pm 13\text{m}$) and the most intense thermal stratification were recorded within the WTRA province as a result of the upwelling of cold subsurface waters. Within SATL province

SST remained high ($27.1 \pm 0.3^\circ\text{C}$) meanwhile the UML depth significantly deepened ($141 \pm 40\text{m}$).

Most of the South Atlantic longitudinal cruise (Malaspina Leg 2) was carried out throughout the SATL province. Maximum SST ($26.1 \pm 0.7^\circ\text{C}$) and UML ($144 \pm 11\text{m}$) were recorded at longitudes higher than 27°W . Surface water became colder and UML shallower as the cruise proceeded towards the African continent, depicting minimum temperature ($20.7 \pm 0.2^\circ\text{C}$) and UML ($50 \pm 10\text{m}$) at the easternmost station, near the BENG province.

Nitracline depth was positively correlated with the thermocline depth ($r^2 = 0.63$, $p < 0.005$, $n = 46$). In agreement, the shallowest nitraclines were found within the equatorial upwelling WTRA province (40 m) and within BENG province (50 m), while the deepest nitracline (210 m) was recorded in NASE province.

DCM depth was positively correlated with UML depth ($r^2 = 0.73$, $p < 0.001$, $n = 59$) and nitracline depth ($r^2 = 0.62$, $p < 0.001$, $n = 46$), whereas chl *a* concentration at DCM and nitracline depth were negatively correlated throughout the cruises ($r^2 = 0.42$, $p < 0.001$, $n = 47$). As expected, the highest levels of chl *a* concentration at the DCM depth were found at some points within WTRA ($1.04 \mu\text{g L}^{-1}$) and BENG ($1.06 \mu\text{g L}^{-1}$) provinces because of the influence of upwelled waters (nitrate concentrations between 4 and $9 \mu\text{M}$). Chl *a* concentration at the DCM was generally lower than $0.5 \mu\text{g L}^{-1}$ at the rest of sampling stations, the lowest concentration being found within NASE

($0.15 \mu\text{g L}^{-1}$).

3.2 Phytoplankton biovolume and size-abundance spectrum

As expected in these mostly oligotrophic waters, phytoplankton biovolume was very low, with a mean under $50000 \mu\text{m}^3 \text{mL}^{-1}$. The stations with highest biovolume were located within the BENG and WTRA provinces, in coincidence with areas with high chlorophyll concentrations. On the contrary, the North Atlantic longitudinal cruise, carried out along the CARB, NATR and NASE provinces, included the lowest biovolume stations of all the surveyed Atlantic (Fig. 4).

In consonance, phytoplankton size-abundance spectra exhibited steep slopes, most of them showing values more negative than -1 (the slope of a spectrum with biovolume evenly shared among log size classes). At the whole surveyed Atlantic Ocean, the average size-abundance spectrum can be described as linear, with a mean slope of -1.08 ± 0.01 , significantly different than -1 .

Most of the values of the size-abundance spectrum slope in all the surveyed oceanic regions were more negative than -1 . The steepest slopes ($b \sim -1.2$) were found at sites with shallow thermoclines at both sides of the equator during the latitudinal cruise (WTRA province). Contrary to expectations, size-abundance spectra within the productive BENG province (UML: 40m; chl *a*: $0.8 \mu\text{g L}^{-1}$; biovolume: $116409 \mu\text{m}^3 \text{ mL}^{-1}$) also showed some of the steepest slope values ($b \sim -1.2$). In fact, these spectra exhibited a slight curved shape, with a relative but significant dominance of medium- size cells.

The flattest spectrum slopes ($b > -1$) were found at the northernmost stations of Malaspina Leg 1, in coincidence with minimum thermal stratification, but not with the highest chlorophyll concentrations or biovolume. The flattest slopes during the South Atlantic longitudinal cruise were also found in stations with shallower thermoclines, but with exceptions near Benguela upwelling.

At the whole surveyed Atlantic Ocean, the size-spectrum intercept (the predicted abundance of organisms in class 0 (size $\approx 1 \mu\text{m}^3$) was, as expected, significantly correlated with total phytoplankton biovolume (Fig. 5). The North Atlantic longitudinal cruise samples, with very low phytoplankton biovolume, showed a particular correlation when treated apart, with higher intercept values for equivalent biomass of other legs. This indicated strong changes in the abundance of picoplanktonic cells with a small impact on total phytoplankton biovolume.

Thus, unlike the *a*-intercept, the size-spectrum slope *b* did not show significant correlation with total biovolume for the whole Atlantic Ocean (Fig. 5). In other words, the spectra with the highest

integrated biovolume were not the flattest size-abundance spectrum slopes, neither the steepest slopes coincided with the lowest biovolume phytoplankton assemblages. Only the picoplankton sub-spectra of the South Atlantic longitudinal cruise showed correlation with their biovolume when the size range was reduced below 20 μm ESD.

4. Discussion

Our size-abundance spectrum models for phytoplankton are coherent with previous findings for the Atlantic Ocean (Huete-Ortega et al., 2010, 2012). The ensemble spectrum slope, -1.08 ± 0.01 , is similar to previously reported values. Huete-Ortega et al (2012) described a mean slope of -1.15 ± 0.09 for the tropical and subtropical Atlantic Ocean. Cavender-Bares et al (2001) found slope values ranging between -1.4 and -1 for the size range of pico-nanoplankton (“flow cytometry” microbial size spectra). Similarly, Quinones et al (2003) obtained a slope not significantly different from -1 when extending the size spectrum from microbial to mesozooplankton organisms. Our “flow cytometry”, global size spectrum (without heterotrophic bacteria) produces a slope value of -1.04 ± 0.01 , significantly different from -1 and less negative than that obtained by Cavender-Bares et al. (2001).

The linearity of the size spectrum of phytoplankton cannot be extended to every province of the global ocean. Using the same methodological procedures, Rodríguez et al. (2002) describe clearly non-linear size spectra in Antarctic waters during summer. In that case, non-linearity was due to the very low density of picoplanktonic prokaryota as well as to the presence of dense blooms of ultra- and nanoflagellates like *Cryptomonas*. We have also described clear non-linear spectra in the Celtic Sea, under conditions that favour the bloom of the large microplanktonic diatom *Coscinodiscus wailesii* (Reul et al., 2006). In this context, we can conclude that our results support that the linearity of the size spectrum of phytoplankton and its global slope value are conservative properties

of the Atlantic oligotrophic ecosystem.

In addition, our results illustrate empirically for the first time the theoretical predictions of Gómez-Canchong et al. (2013). Through numerical modelling, these authors showed that changes in a and b parameters may have opposite effects on global properties (as biomass) that can be mutually cancelled. That is, under natural conditions it should be possible to find steeped size-abundance spectra with high phytoplankton biomass as well as very flat spectra with low phytoplankton biomass. This is in contradiction with the frequent association between high productivity ecosystems, high phytoplankton biovolume and flat size-abundance spectra due to the comparatively higher contribution of microplankton (cells $> 20 \mu\text{m}$) to total phytoplankton biovolume (e.g. Rodríguez et al., 1998, 2002; Huete-Ortega et al., 2010, 2012, 2014). On the other side, open ocean ecosystems are typically dominated by low biovolume communities and steeped size-abundance spectra where picophytoplankton is the dominant component. There are, however, some previous observations about the uncoupling between biomass and the size structure of phytoplankton. Regarding just the biggest organisms (without picoplankton) San Martín et al. (2006) described an inverse relation between slope and phytoplankton biomass that could lead to a nonsignificant relation if picoplankton were included. Marañón et al. (2001) found that the equatorial upwelling causes an increase in phytoplankton biomass and productivity without altering the typical size structure (described through size-fractionated chlorophyll concentration) found in less productive regions such as the subtropical gyres. Huete-Ortega et al. (2011) achieved similar conclusions regarding size-fractionated carbon fixation rates in the equatorial upwelling. They found that picophytoplankton and small nanophytoplankton were the most responsive size fractions to the enhanced nitrate supply into the euphotic layer within the equatorial region. These authors argue that, in the oligotrophic ocean, significant changes in carbon fixation rates take place without accompanying variations in the magnitude of the phytoplankton standing stocks or the size structure

of the microbial community. In a previous paper, Barber and Hiscock (2006) emphasized that small-sized phytoplankton constitute a very dynamic component of the community that responds rapid and dramatically to nutrient entrainment and environmental variability.

Our results show that changes in biovolume do affect the a -parameter of the size-abundance spectrum in an expected way, but the effect on spectrum slope seems to be highly variable. Thus, there is a positive relation between total biovolume and the a -parameter but there is no correlation between total biovolume and the slope (b) of the size-abundance spectrum. Changes in a and b can have cancelling effects on total biovolume and the relationship between slope and biovolume is lost when many of these cases dominate the system. A symptom of this phenomenon is the correlation of both parameters a and b that appears theoretically when organisms are randomly sampled from the same set of biomass (Gómez-Canchong et al., 2013). We found this correlation in the Atlantic Ocean (Fig. 6). Nevertheless, in our samples, phytoplankton biovolume builds up following a increases or b decreases, as shown in Fig. 7. These changes could be just in parameter a (transition from St.23 to St.17) or changes in slope b (from St.23 to St.11), or both of them (Fig. 7). An increase of a implies an equitable effect over all size classes, conversely a decrease of b implies changes that only medium and big cells experience.

As conclusion of the above, the biovolume of a phytoplankton assemblage may increase as a result of an adequate combination of a and b changes, just until the biological maximum limit of abundance for small cells is reached (Agustí et al., 1987; Duarte et al., 1987; Chisholm, 1992). Beyond this limit, greater values of biovolume can only be achieved through reducing the slope, that is, through increasing the relative contribution of large sized cells. In the surveyed open ocean, the biovolume does not exhibit the dependence on size-abundance spectrum slope because phytoplankton assemblages do not reach the level of biovolume at which the contribution of big cells is needed and the size-abundance spectrum may swing around similar biovolumes with

different combinations of slopes and intercepts. This emphasizes the importance of pico and nanoplankton in the oligotrophic ocean (Agawin et al., 2000), where nutrient concentration become extremely low and small-sized cells are selected according to size-dependent physiological traits (see Falkowski and Oliver, 2007; Litchman et al., 2007) and dominate the community. By contrast, in richer and more fluctuating systems (i.e. coastal upwelling areas, Rodríguez et al., 1998) the biggest part of biovolume resides in large eukaryotic cells (microphytoplankton), whereas the smaller size ranges just top to their biomass limit and remain almost invariable among the different hydrodynamical regimes.

Variations in the combination of intercept and slope in the oligotrophic ocean could be randomly justified (as Gómez-Canchong et al., 2013 numerically demonstrated), although the very steep slopes found at biomass rich equatorial samples could also be related with the efficiency of the energy transfer along the size-spectrum (Gaedke, 1993; Gaedke and Straile, 1994). In this region, stationary hydrodynamics results in modest and persistent increases in nutrient supply and provide a steady environment where organisms develop tight ecological relations, extending the trophic cascade beyond the limited steps of more changing and productive systems such as coastal upwellings. The more steps has the energy to travel, the steeper should be the size-abundance spectra slope (Jennings and Mackinson, 2003).

On the other hand, the increase of biovolume throughout a poor-to-rich gradient does not always respond to linear changes, neither in spectra slope nor in intercept. Thus, the maximum biovolume values found at the southeasternmost extreme of the South Atlantic longitudinal cruise (just before Benguela upwelling) were achieved through dome-like form spectra. In this area, the medium-sized cells become dominant, likely due to a better trade-off between nutrient uptake and growth rate (Marañón et al., 2012) that dynamically develops in non-stationary dome-like spectra. However, this interesting situation could only reflect that nanoplankton is near to the pivot-point of the

phytoplankton spectrum analysed: extreme classes are more sensitive to forcing factors as nutrient or hydrology and could depart from the global linear spectrum with a secondary slope (Dickie et al. 1987). So, the contribution of picoplanktonic cells to the total biovolume may remain almost constant in spite of its secondary scale, in contrast with a direct, proportional relation between total biovolume and microplankton biomass in coastal waters (not the case here). Therefore, if biovolume is independent of the slope of size spectrum, the relative relevance of nanoplankton biovolume changes in the range of size spectra slopes from -1 to -1.2 (the most common in the oligotrophic ocean) could be just a numerical consequence of how we build the nominal classes (pico-, nano-, micro-). Great advances were achieved with this broad class approach, but more realistic conclusions on the structure and function of planktonic communities should be based on the size-abundance spectrum per se, without trying to discretize it into arbitrary or methodological phytoplankton size ranges

5. Conclusions

Our results demonstrate that in the open waters of the Atlantic Ocean phytoplankton size-abundance spectrum slope is independent of the total phytoplankton biovolume, and consequently it cannot be considered as an ecosystem indicator itself. In these oligotrophic waters, phytoplankton biovolume is always below its theoretical maximum value (from the best combination of slope and intercept), and shifts in biovolume are not followed by an increase in microphytoplankton cells and flattening of the spectrum slope. This provides multiple potential combinations of size-abundance spectra parameters, many of them with cancelling effects. Even more, the smallest cells showed the strongest response to nutrient supply throughout the surveyed Atlantic waters, so biovolume increments in productive waters (such as the equatorial divergence or areas near the Benguela province) resulted in a more negative size-abundance spectrum slope. In agreement, we strongly

encourage the study of the complete size-abundance spectrum, considering both a and b parameters and total biovolume or abundance, if community and ecosystem properties are to be inferred from phytoplankton size structure in the open ocean waters. In addition, we advice caution against ecological conclusions derived from studies where phytoplankton counts were discretized and sorted into arbitrary or methodological size classes. We reclaim the focus on the whole size-abundance spectrum and on the insight in new analysis procedures that would profit the new technologies with new mathematical tools.

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FIGURE CAPTIONS

Figure 1. Cruise tracks and location of the sampling stations. Circles, Malaspina Leg 1; Squares, Malaspina Leg 2; Triangles, Malaspina Leg 7. CARB: Caribbean province; NATR: North Atlantic Tropical Gyre province; NASE: North Atlantic Subtropical Gyral province; WTRA: Western Tropical Atlantic province; SATL: South Atlantic Gyral province; BENG: Benguela Current Coastal province.

Figure 2. Phytoplankton abundance in octave size classes. N (abundance) is a good estimator of biovolume density function β , with the same slope b and corrected a intercept (Blanco et al., 1994). The example size-spectrum corresponds to a station sampled within SATL province in this study.

Figure 3. Chlorophyll a concentration at DCM depth and thermal vertical structure (contours) during Malaspina Leg 7 (a), Leg 1 (b) and Leg 2 (c). Dots in contours show the nitracline depth.

Figure 4. Total phytoplankton biovolume ($\mu\text{m}^3 \text{ mL}^{-1}$, squares) and size-abundance spectrum slope (b , dots) during Malaspina Leg 7 (a), Leg 1 (b) and Leg 2 (c).

Figure 5. (Left) Non-significant relation between size-spectrum slope (b) and total phytoplankton biovolume. (Right) Significant relation between size-abundance spectrum intercept (a) and total phytoplankton biovolume.

Figure 6. Correlation between size-abundance spectrum intercept (a) and slope (b). Each survey

has different symbol.

Figure 7. (Left) Different ways to achieve the same biovolume depending on slope or intercept.

(Right) a flatter slope does not imply higher biovolume.

ACCEPTED MANUSCRIPT

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

HIGHLIGHTS

- A survey of 67 sampling stations at 6 biogeographic provinces in the Atlantic Ocean
- The study focus on the size structure of phytoplankton from pico- to microplankton
- Pico- and nanophytoplankton were responsible for the most part of the biovolume
- Phytoplankton size-spectrum slope and biovolume were not related