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Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer *Trichodesmium* along the subtropical North Atlantic

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Abstract

The spatial variability of biomass and stable isotopes in plankton size fractions in the upper 200 m was studied in a high spatial resolution transect along 24ºN from Canary Islands to Florida to determine nitrogen and carbon sources. Vertical advection of waters predominated in lateral zones while the central Atlantic (30-70º W) was characterised by a strong stratification and oligotrophic surface waters. Plankton biomass was low in the central zone and high in both eastern and western sides, with most of the variability due to either large (>2000 µm) and small plankton (<500 µm). Carbon isotopes reflected mainly the advection the deep water in lateral zones. Stable nitrogen isotopes showed a nearly symmetrical spatial distribution in all fractions, with the lowest values ($\delta^{15}N$<1‰) in the central zone, and were inversely correlated to carbon stable isotopes ($\delta^{13}C$) and to the abundance of the nitrogen-fixer *Trichodesmium*. Diazotrophy was estimated to account for >50% of organic nitrogen in the central zone, and even >30% in eastern and western zones. The impact of diazotrophy increased with the size of the organisms, supporting the wide participation of all trophic levels in the processing of recently fixed nitrogen. These results indicate that atmospheric sources of

carbon and nitrogen prevail over deep water sources in the subtropical North Atlantic and that the zone influenced by diazotrophy is much larger than reported in previous studies.

Keywords: Stable isotopes, plankton, Subtropical North Atlantic, *Trichodesmium*

INTRODUCTION

Large regions of the ocean at subtropical latitudes are characterised by gyres of ocean currents rotating clockwise in the Northern Hemisphere. Biological production in the central regions of these gyres is generally low because of low nutrient inputs while production is enhanced at their borders (e.g. Behrenfeld et al., 2006). For instance, the supply of nitrogen from deep waters to the photic zone is lowest in the middle oceanic gyres, where a deep thermocline and smooth nutrient gradients determine slow rates of nutrient supply by diffusion (Mouriño-Carballido et al., 2011). Notwithstanding their low production, these gyres contribute a large fraction of global biogenic carbon export into the deep ocean because of their size (Emerson et al., 1997; Karl et al., 2008).

A variety of physical mechanisms are known to contribute to nitrogen inputs in oligotrophic gyres, including mesoscale and submesoscale turbulence (Oschlies and Garçon, 1998), lateral transport from other regions (Williams and Follows, 1998; Torres-Valdes et al., 2009), and atmospheric deposition (Duce et al., 2008). However, biological fixation of atmospheric N_2 (diazotrophy) can be also a major input of nitrogen in the oligotrophic ocean (Gruber and Sarmiento, 1997; Capone et al., 2005; Moore et al., 2009). In the North Atlantic, diazotrophy contributed to a large fraction of new production, even exceeding the contributions by nitrate diffusion across the pycnocline (Capone et al., 2005; Fernández et al. 2010; Mouriño-Carballido et al., 2010). Nitrogen fixation is controlled by temperature (Breitbarth et al., 2007), CO_2 (Barcelos e Ramos et al., 2007) and the availability of other nutrients, notably phosphorus and iron, the latter provided by atmospheric dust inputs (Moore et al., 2009; Sohm et al., 2011). Nitrogen of diazotrophic origin is made available to the pelagic food web through excretion and mortality of cyanobacteria (Glibert and Bronk, 1994) and further processing by microbes and planktonic metazoa (Montoya et al., 2002).

The colonial cyanobacteria of the genus *Trichodesmium* is the best known diazotroph, with a widespread distribution across tropical and subtropical regions of the ocean (Capone et al., 1997; Luo et al., 2012) where surface water temperature exceeds 20°C (Breitbarth et al., 2007). In the North Atlantic *Trichodesmium* is more abundant between 20°N and 20°S (Tyrrell et al., 2003; Davis and McGillicudy, 2006; Fernández et al., 2010, 2012) but most studies on N_2 fixation have been focused in the subtropical and tropical regions where blooms are frequent (Voss et al., 2004; Capone et al., 2005; Mulholland et al., 2006; Montoya et al., 2007). Only a few studies have measured concurrently *Trichodesmium* abundance and nitrogen fixation over large spatial scales in the Atlantic, as reviewed by Luo et al. (Luo et al., 2012). However, further evidence of the impact of diazotrophy at regional scales was provided by measurements of the natural abundance of stable nitrogen isotopes in seston and plankton (Waser et al., 2000; Mino et al., 2002; Montoya et al., 2002; Reynolds et al., 2007; Landrum et al., 2011).

Stable isotopes can trace N_2 inputs because atmospheric nitrogen is relatively depleted in heavy (^{15}N) isotopes compared to marine nitrate (Owens, 1987). Assimilation of this light N_2 by diazotrophs produces organic matter with a characteristic isotopic signature that can be traced along the food web. Because of the different turnover time of planktonic organisms (hours to days in bacteria and phytoplankton, and up to several months in large zooplankton) the isotopic signature of organic matter in various compartments provides an integrative, *in situ* tracer of the movement and transformation of nitrogen in the water column beyond the instantaneous effects reported during N_2-uptake measurements. Nitrogen isotopes in seston reflect the uptake of atmospheric N_2 by cyanobacteria (e.g. Montoya et al., 2002) while those in zooplankton show the assimilation of organic matter initially produced by diazotrophs (McClelland et al., 2003). This feature allows an estimation of the contribution of diazotrophy to net nitrogen assimilation in different components of the food web (Mino et al., 2002; Montoya et al., 2002; Reynolds et al., 2007; Landrum et al., 2011). Previous estimates using measurements of natural abundance of nitrogen isotopes in seston and zooplankton revealed a large contribution of diazotrophic nitrogen (up to 100%) in the north-eastern tropical and subtropical Atlantic (Montoya et al., 2002). Landrum et al. (Landrum et al., 2011) reported lower contributions in the central and eastern subtropical Atlantic compared to those in the eastern region, however this study was made in waters near 30ºN, where *Trichodesmium* abundances were lower than in southern waters (Tyrrell et al., 2003; Davis and McGillicuddy, 2006; Fernández et al., 2010). Direct measurements revealed significant N_2 fixation also in the eastern subtropical Atlantic (Fernández et al., 2010; Wannicke et al., 2010; Benavides et al., 2011; Fernández et al., 2012), although there are few measurements of either abundance or N_2 fixation in the central region of the gyre (Luo et al., 2012).

The objective of this study is to characterize spatial patterns of plankton in the oligotrophic subtropical North Atlantic by means of the analysis of size-fractionated plankton biomass and natural abundance of stable carbon and nitrogen isotopes. The patterns are related to the abundance of *Trichodesmium* and indicate a large influence of diazotrophy across plankton size classes over most of the subtropical northern Atlantic.

MATERIAL AND METHODS

Samples and water column measurements were obtained during Leg 8 of Malaspina-2010 expedition (http://www.expedicionmalaspina.es) on R/V Sarmiento de Gamboa (January-March 2011) in a transect mostly along 24 °N between Canary Islands and Florida (Fig. 1). The transect was arbitrarily divided in eastern, central, and western zones to summarize its oceanographic and plankton characteristics.

Plankton samples were collected by vertical tows of a microplankton net (40 µm mesh size) and a mesoplankton net (200 µm mesh size) through the upper 200 m of the water column. Sampling was made between 10:00 and 16:00 h GMT. Plankton was separated into five size fractions (40-200, 200-500, 500-1000, 1000-2000 and >2000 µm) by gentle filtration of the samples by a graded series of nylon sieves (2000, 1000, 500, 200 and 40 µm). Large gelatinous organisms were removed before filtration. Aliquots for each size-fraction were collected on pre-weighted glass-fibre filters, dried (60ºC, 48 h) and stored in a dessicator before determination of biomass (dry weight), carbon and nitrogen content and natural abundance of stable carbon and nitrogen isotopes ashore.

After determination of dry weight, finely ground aliquots of each size fraction were packed in tin capsules for elemental and stable isotope analysis by conversion into CO_2 and N_2 in an elemental analyser (Carlo Erba CHNSO 1108) coupled to an isotope-ratio mass-spectrometer (Finnigan Mat Delta Plus). Samples were not acidified to remove carbonates because other studies showed that the acidification may not cause substantial modification in carbon isotope results, but it may affect nitrogen determinations (Bunn et *al*., 1995; Bode at *al*., 2003). Similarly no corrections were made for lipid content potentially affecting carbon isotope composition (Symantec et al., 2007). In this case, the average (±se) C:N molar ratio of all samples was 4.8±0.0 (n=218) and showed little variations among size fractions, suggesting low influence of lipids. Carbon and nitrogen stable isotope abundance was expressed as $\delta^{13}C$ and $\delta^{15}N$ relative to VPDB (Vienna PeeDee Belemnite carbonate) and atmospheric N_2 isotope standards. Precision (± standard error) of replicate determinations of both C and N stable isotopes was <0.03‰.

Water properties were estimated from CTD casts (SBE-911 Plus) in the upper 300 m. In absence of more detailed observations, sea surface temperature (SST 0-10 m) was used as a surrogate of nutrient supply to the surface by advection from deeper layers, and *in vivo* fluorescence (SFluor) as an estimate of phytoplankton biomass. Total nitrate (NO_3^- + NO_2^-) and phosphate were analysed colorimetrically (Grashoff et al., 1983) on frozen samples collected by Niskin bottles at standard depths.

Abundance of the diazotroph *Trichodesmium* sp. was estimated by counts of 50 ml aliquots of the sample from the microplankton net preserved in glutaraldehyde (25% final concentration) using a FlowCAM® system (Fluid Imaging Technologies). Prior to analysis the samples were screened by a 100 µm nylon mesh to prevent clogging of the FlowCAM cell. Results are reported as number of colonies (trichomes) per volume of seawater. Abundance of total microzooplankton and phytoplankton (100 to 200 µm) was also determined in the same samples. Total abundance of mesozooplankton was determined by counts of aliquots of the 200 µm net preserved in 4% formalin and observed under a binocular microscope. The relative frequency of the main taxa was also recorded.

The contribution of nitrogen fixed by diazotrophs (diazotroph N) to plankton fractions was estimated using the isotope mass balance approach of Montoya et al. (2002):

$$\%\text{diazotroph} = 100 \left(\frac{\delta^{15}N_m - \delta^{15}N_{ref}}{\delta^{15}N_d - \delta^{15}N_{ref}} \right)$$

where $\delta^{15}N_m$ is the measured isotopic composition in the sample, $\delta^{15}N_{ref}$ is the isotopic reference value for plankton not influenced by diazotroph N and $\delta^{15}N_d$ is the isotopic composition for diazotrophs (-2‰, Montoya et al., 2002). Reference values $\delta^{15}N_{ref}$ were 3.7, 4.3, 5.1 and 5.8 ‰ for 200-500, 500-1000, 1000-2000 and >2000 µm size-classes, respectively, corresponding to plankton in tropical equatorial regions (Landrum et al., 2011). No estimations of diazotroph N contribution were made for the 40-200 µm class because of potential bias caused by the presence of *Trichodesmium* filaments.

RESULTS

Temperature, salinity, fluorescence and nutrients

A large range in temperature (10 to 25 °C) was found in the upper 300 m along the transect (Fig. 2). Isotherms raised in the eastern end tracing the influence of the Canary upwelling, and also at other points along the transect indicating mesoscale features favouring upwelling (e.g. near 50 and 70 °W). The highest surface temperature values were found in the western and central regions, and the lowest in the eastern region. Salinity showed a pattern similar to the described for temperature, but in this case there was a core of high salinity (>37.4) between 25 and 48 °W in the upper 150 m. Strong salinity gradients characterised the eastern region while the western region had in general low salinity values.

Low values of *in vivo* fluorescence prevailed along the transect and showed a characteristic subsurface maximum in nearly all stations. This maximum was less developed in the eastern region where fluorescence was more uniformly distributed in the upper 100 m but was sharper and deeper in the central and western regions where it reached ca. 150 m deep.

Nitrate was almost depleted (<0.05 µM) in the upper 200 m for most of the transect but in the central zone a layer of relatively high concentration was found between 75 and 100 m depth (Fig. 2). Phosphate also showed low concentrations in most of the transect but in this case the whole water column had higher concentrations (>0.05 µM) in the eastern than in the other zones. Both nutrients showed higher concentrations in deep waters at the borders of the transect.

Spatial patterns of plankton and stable isotopes

Plankton biomass decreased towards the central zone of the transect and had high values at both western and eastern zones (Fig. 3). This pattern was similar in all size classes although mean values were significantly higher in the western zone for plankton <500 µm and lower in the central zone for plankton >2000 µm (Table 1). There were significant differences in mean values of total plankton biomass, with the lowest value central zone, and the highest in the western zone.

Microzooplankton abundance was similar in all zones while phytoplankton was significantly more abundant in the western zone (Table 2). Mean abundance of mesozooplankton followed a similar pattern to total plankton biomass, with equivalent values in the eastern and western zones and minimum values in the central zone (Table 2). Copepods were generally dominant in all zones, but some genera were more frequent in the lateral zones (*Oithona*) than in the central region (*Calanus*, *Macrosetella*). Ostracoda were also more frequent in lateral zones while salps and appendicularia showed higher frequencies in central and eastern zones.

The spatial variability in nitrogen isotopes was similar to the pattern described for biomass (Fig. 4), as significant, positive correlations (P<0.05) were found between $\delta^{15}N$ and biomass in all plankton size-fractions. In this case all fractions showed mean $\delta^{15}N$ values in the central zone (<2‰) significantly lower than values in either eastern or western zones (Table 1). Isotopic enrichment was only noticeable between the smallest and largest size classes, while plankton between 200 and 2000 µm showed similar mean $\delta^{15}N$ values within zones.

In contrast, $\delta^{13}C$ displayed an opposite pattern to the one described for $\delta^{15}N$ and biomass, but with more differences between size-fractions (Fig. 5). Mean values for 40-200, 200-500 and 1000-2000 µm classes were significantly lower in the eastern zone, while no significant differences between zones were found for other classes (Table 1). Biomass was only significantly correlated with $\delta^{13}C$ for 200-500 and 500-1000 µm classes.

Relationships with surface temperature, salinity and *in vivo* fluorescence

Biomass was negatively correlated with surface temperature only for the largest size-class, and also negatively with surface salinity for <500 µm classes, while non significant correlations resulted between biomass and surface fluorescence (Fig. 6). However, fluorescence was positively correlated with $\delta^{15}N$ for all classes and with $\delta^{13}C$ for <1000 µm classes. Surface temperature was also correlated with $\delta^{15}N$ (negatively) or $\delta^{13}C$ (positively) for <1000 µm (and in case of $\delta^{13}C$ also for >1000 µm) classes.

Linearity between $\delta^{15}N$ and $\delta^{13}C$

Carbon and nitrogen isotope abundances showed a significant negative linear relationship within size-classes, except for the >2000 µm class (Fig. 7). The slopes and intercepts of the lines were equivalent for classes <1000 µm and also for classes >1000 µm, while within these groups there were no significant differences (ANCOVA, P<0.05).

***Trichodesmium* abundance and $\delta^{15}N$**

With the exception of the two easternmost stations, *Trichodesmium* was recorded at all stations of the transect (Fig. 8). Its abundance showed an abrupt increase at ca. 25ºW followed by a general decrease to the west. Mean values were significantly higher in the eastern and central zones (mean±se = 4.77±0.73 trichomes L^{-1} n=29) than in the western zone (2.04±0.57 trichomes L^{-1} n=14, ANOVA, P<0.05).

A negative linear relationship was found between $\delta^{15}N$ and log-transformed *Trichodesmium* abundance for all size-classes (Fig. 9). The slope of the line was similar for all classes (mean slope = -1.42±0.11, n=84) while there were differences in the intercept between the 40-200 µm and the other classes and between >2000 µm and dasses 200-1000 µm (ANCOVA, $P<0.05$).

Contribution of N from diazotrophs

Diazotroph N contributed to all size fractions in almost all stations (Fig. 10). Only few stations in the eastern zone without *Trichodesmium* showed zero contribution while maximum values (>70%) occurred in general in the central zone. Mean contributions were ca. 50% in the central zone but between 22 and 38% in the eastern and western zones (Table 3). The contributions of diazotroph N increased for larger classes. On average there was an increase in the contribution of diazotroph N between the 200-500 and the >2000 µm classes of 16, 10 and 4% for the eastern, central and western zones.

DISCUSSION

Plankton biomass across the subtropical Atlantic

The measured plankton biomass reflected well the oligotrophy of most of the subtropical North Atlantic. To our knowledge these results are the first obtained in this region of the deep ocean at such small spatial resolution showing a gradual decrease from zones near the continental shelves to the central basin. However, the studied transect included also productive areas. Biomass of nearly all size classes was higher in the western than in the eastern and central zones, with mean values equivalent to those previously reported for both western (Madin et al., 2001) and eastern zones (Hernández-León et al., 2007). The high productivity of lateral zones can be attributed to the influence of the Canary upwelling in the east (indicated by the upward trend in isotherms and the shallow chlorophyll maxima in Fig. 2) and to mesoscale eddy pumping (McGillicudy et al., 2001) in the west (as indicated by sharp changes in isotherms near 70°W). Zooplankton biomass has been shown to track upwelling dynamics and extend the influence of the high productive waters near north-western Africa well into the deep ocean (Hernández-León et al., 2007). Also, zooplankton biomass peaks follow winter-spring blooms in the western zone (Madin et al., 2001). The high plankton productivity is directly linked to large advective fluxes of nitrate from deep waters in both eastern (Pelegrí et al., 2005) and western zones (Lipschulz, 2001) while low production in the central zone of the basin is attributed to low nutrient advection (Marañón et al., 2000).

In contrast to lateral zones, a substantial portion of the central subtropical Atlantic was characterised by low plankton biomass in all size classes but particularly in macrozooplankon (>2000 µm). This is consistent with a larger primary production in the oceanic borders that will sustain more trophic levels than oligotrophic regions, as occurs in upwelling ecosystems (Hernández-León et al., 2007). The oligotrophic gyre in the North Atlantic, including most of the central zone in our study, is a well known nutrient-limited region because of low inputs by advection of deep waters, which explain its low levels of primary production (Behrenfeld et al., 2007). Sharp thermohaline stratification prevents major exchange between surface and deep waters and most phytoplankton biomass concentrated in a deep maximum layer (Fig. 2) where a large gradient in nutrient concentrations is expected to occur (Mouriño-Carballido et al., 2011). As the stratification is produced mainly by the warming of the surface layer, a negative correlation between plankton biomass and SST or SSS would be expected. However, in this study only macrozooplankton was correlated with SST and the smallest plankton classes (<500 µm) displayed a negative correlation with SSS, suggesting that plankton biomass was not a simple function of stratification and that other nutrient inputs would be implied.

Sources of inorganic carbon and nitrogen

Both nitrogen and carbon isotopes showed a large variation along the transect mirroring that of biomass. This variation is indicative of major changes in the source of nutrients or in the composition of plankton. Diatoms are expected to dominate in the Canary upwelling (Margalef, 1978; Bode et al., 2001) but they are less dominant in the western subtropical Atlantic (Goericke, 1998). *Trichodesmium* is a conspicuous member of phytoplankton communities in this region (Tyrrell et al., 2003; Davis and McGillicudy, 2006; Fernández et al., 2010, 2012) as found in the present study. The preferential use of different carbon sources for primary production by the different phytoplankton taxa may lead to variations in $\delta^{13}C$ that can be transmitted up the food web. Inorganic carbon uptake by cyanobacteria occurs via direct HCO_3^- transport while diatoms are able also to transport CO_2 derived from HCO_3^- dehydration (Tortell and Morel, 2002). This difference would result in a larger isotopic fractionation (and higher $\delta^{13}C$) in diatoms compared to cyanobacteria and other microalgae thus allowing to track diatom consumption along food webs (Fry and Wainright, 1991). Therefore low $\delta^{13}C$ would be expected in the central zone and high $\delta^{13}C$ in the lateral zones, particularly in the eastern region. However in our study $\delta^{13}C$ values were high in the central zone and western zones, where diatoms are not expected to dominate, and low near the

Canary upwelling. Diatom counts for some of the stations in this cruise (not shown) indicated that even in lateral zones this group never dominated the phytoplankton community. Changes in community composition may not be the primary cause of $\delta^{13}C$ variability along the transect, instead the measured $\delta^{13}C$ would reflect the main source of inorganic carbon for primary production: atmospheric in the central zone and from vertical advection in the lateral zones, as CO_2 from upwelled waters is depleted in ^{13}C (Gruber et al., 1999).

The significant linear relationships between carbon and nitrogen isotopes found along the transect also support a major role of biogeochemical processes, rather than plankton composition, in the determination of isotopic signatures of plankton. However, both the slope and the correlation were larger for <1000 µm size classes than for larger size classes, indicating that variations in the dominant plankton taxa were also important. The occasional presence of large salps and pteropods may have caused the large variability in $\delta^{13}C$ values of macrozooplankton classes, while zooplankton <1000 µm was mainly composed by copepods (Table 2) and showed comparatively less variability in $\delta^{13}C$.

Higher plankton $\delta^{15}N$ in lateral compared to central zones is consistent with a major NO_3^- input from advection of deep sea waters, as deep water NO_3^- is more enriched in ^{15}N (Montoya et al., 2002). The low $\delta^{15}N$ values found in all plankton size classes in the central zone, however, may result from a major use of regenerated nitrogen forms (mainly ammonium) or from atmospheric N_2 fixation. As heterotrophic plankton preferentially excrete isotopically light nitrogen, meso- and macrozooplankton are expected to become more enriched than subsurface nitrate in absence of significant N_2 fixation (Montoya et al., 2002) while phytoplankton (and seston) is depleted because of the uptake of light dissolved nitrogen. Alternatively, isotopic fractionation during the decomposition of dissolved organic nitrogen and subsequent assimilation by plankton in the surface layer would also lower seston $\delta^{15}N$ (Knapp et al., 2011). Both effects may confound depleted isotopic signals in seston with those caused by N_2 fixation (e.g. Mino et al., 2002). However, mean values of planktonic $\delta^{15}N$ in all zones and size classes were <3‰, and only few values in the eastern zone reached 4.5‰, a typical value for deep NO_3^- in the oligotrophic Atlantic, as summarized by Landrum et al. (2011). This result suggest that a large fraction of plankton nitrogen in the transect originates from N_2 fixation and is supported by the significant negative relationship between *Trichodesmium* abundance and planktonic $\delta^{15}N$ in all size classes (Fig. 9). Furthermore, mean $\delta^{15}N$ for the smallest size-class in the central zone was ca. 0‰, the value for atmospheric N_2, which is consistent with the low concentration of nitrate in this region. The parallel changes observed in $\delta^{15}N$ for all plankton size classes support the transfer of fixed nitrogen up the food web (Montoya et al., 2002; McClelland et al. 2003). Nevertheless, the distribution of *Trichodesmium* did not match exactly that of $\delta^{15}N$, in part because of the heterogeneous spatial distribution of this species (Davis and McGuillicuddy, 2006; Mouriño-Carballido et al., 2011) and because the possible presence of other diazotrophs (Moisander et al., 2010; Fernández et al., 2012).

Impact of diazotrophy in the subtropical North Atlantic

Our results indicate that N_2 fixation is one of the major sources of nitrogen for the pelagic ecosystem in this region. Previous studies reporting direct measurements of *Trichodesmium* abundance and nitrogen fixation have stressed the importance of this process mainly for the eastern zone, while both *Trichodesmium* abundance and diazotrophy decreased towards the central and eastern zones (Voss et al., 2004; Capone et al., 2005; Davis and McGuillicuddy, 2006; Mulholland et al., 2006; Montoya et al., 2007). Estimations from plankton $\delta^{15}N$ also highlight this effect (Montoya et al., 2002; Landrum et al., 2011) with contributions of diazotrophic nitrogen up to 38%. Biogeochemical studies based on the NO_3^-:PO_4^{3-} ratios of subsurface waters, however, suggested that the potential area for nitrogen fixation in the northern Atlantic would be much larger (Capone et al., 2005; Montoya et al., 2007; Reynolds et al., 2007), as found in our study, where contributions of diazotrophic nitrogen exceeded 50% in the central zone. These variations in the impact of the diazotrophy between the different studies may result from geographic variability in the subtropical North Atlantic, which high resolution cruises as the one in the present study, revealed more heterogeneous than expected. For instance previous cruises were further south (Montoya et al., 2002; McClelland et al., 2003; Montoya et al., 2007) or north (Landrum et al. 2011) than 24°N, and most latitudinal transects concentrated in the eastern zone (Reynolds et al., 2007; Fernández et al., 2010; 2012) while the central zone was much less studied. As diazotrophy requires phosphate and iron, besides high water temperature and the presence of diazotrophic organisms, the input of these elements to the upper layer from atmospheric (dust) or oceanic sources (upwelling) largely determines the absolute amount of fixed nitrogen (Moore et al., 2006; 2009). The influence of upwelling nutrients in subsurface layers (>100 m) was evident in the western zone of the studied transect, but while nitrate was almost depleted in the upper 100 m, phosphate concentrations were still relatively high (>0.5 µM) up to 50ºW. This suggests the input of phosphate (and likely also iron) from the Saharan dust (Sañudo-Wihelmy et al., 2001; Moore et al., 2006) that will extend the influence of diazotrophy well into the oligotrophic ocean. While iron inputs from dust cannot be neglected, recent experimental studies found a major role of diffusive fluxes of phosphate, rather than atmospheric inputs, for nitrogen fixation east of 40ºW (Fernández et al., 2012). The large gradient provided by the upwelling would favour diffusion (Mouriño-Carballido et al., 2011) but also the occasional advection of shelf waters (Pelegrí et al., 2005) that would explain the proliferation of *Trichodesmium* in the otherwise oligotrophic central zone.

Contributions of diazotrophy for >1000 µm plankton exceeded those estimated for smaller classes, as found previously by Landrum et al. (2011). This result suggests a major circulation of diazotrophic nitrogen through the water column because vertical migrations and large fecal pellet export of macrozooplankton. Experimental studies have shown the ability of some copepod species to consume *Trichodesmium* (O'Neil et al., 1996) while others are sensitive to its toxicity (Hawser et al., 1992). In our study, the dominance of *Macrosetella* in the central zone may be explained by the ability of species of this genus to graze on *Trichodesmium* and release isotopically depleted ammonium (O'Neil et al., 1996). The unbalance between carbon and N_2 fixation and the relative large releases of dissolved organic nitrogen often observed in field studies has been interpreted as an indication that most of the recently fixed nitrogen is processed by the microbial food web before reaching upper trophic levels (Mulholland, 2007). Rapid transfer of fixed N to zooplankton consumers, however, was demonstrated by analysing the stable isotope composition of essential amino acids (McClelland et al., 2003) thus showing a tight coupling of zooplankton to nitrogen fixation. While the exact mechanisms of transfer of this nitrogen from *Trichodesmium* and other diazotrophs to zooplankton are difficult to demonstrate in the field, stable isotope composition clearly show a major influence of diazotrophic nitrogen in plankton food webs through most of the subtropical North Atlantic. The participation of all the components of the food web would explain the magnitude of the impact of diazotrophic nitrogen in the oligotrophic subtropical North Atlantic, with a rapid recycling of dissolved organic nitrogen in the upper layer (Mulholland, 2007) and its transfer to particles via meso- and macrozooplankton (Montoya et al., 2002). The relatively high nitrate concentration (>4 µM) found near 100 m depth in the central zone of this study, where the estimated diazotrophic contribution reached the highest values, supports the remineralisation of recently fixed nitrogen by a coupled N_2-fixation-nitrification pathway, as suggested for the subtropical Pacific (e.g. Karl et al., 2008). This nitrogen would then be available for non-diazotrophic phytoplankton that, in turn, would be consumed by zooplankton herbivores and these by carnivores, thus amplifying the diazotrophic impact (Montoya et al., 2002).

In conclusion, this study confirms the major dependence of the planktonic food web from atmospheric carbon and nitrogen fixation in the subtropical North Atlantic. The high spatial resolution data revealed that the influence of diazotrophy is high in the central region of the subtropical gyres (>50%), but it is still important on the edges of the gyres (20-38%), while previous studies stressed the major impact of diazotrophy in the eastern zone. Future studies will elucidate the importance of diazotrophic nitrogen through the food web, for instance by calculating precise estimates of trophic levels using compound-specific $\delta^{15}N$ determinations.

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REFERENCES

Barcelos e Ramos, J., Biswas, H., Schulz, K. G., Laroche, J. and Riebesel, U. (2007) Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*. Global Biogeochem. Cycles, 21, doi:10.1029/2006GB002898.

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., Mcclain, C. L., Sarmiento, J. L., Feldman, G. C., Milligan, A. J., Falkowski, P. G., Letelier, R. M. and Boss, E. S. (2006) Climate-driven trends in contemporary ocean productivity. Nature, 444, 752-755.

Benavides, M., Agawin, N. S. R., Arístegui, J., Ferriol, P. and Stal, L. J. (2011) Nitrogen fixation by *Trichodesmium* and small diazotrophs in the subtropical northeast Atlantic. Aquat. Microb. Ecol., 65, 43-53.

Bode, A., Carrera, P. and Lens, S. (2003) The pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) during spring: Natural abundance of stable carbon and nitrogen isotopes. ICES J. Mar. Sci., 60, 11-22.

Bode, A., Barquero, S., Varela, M., Braun, J. G. and Armas, D. D. (2001) Pelagic bacteria and phytoplankton in oceanic waters near the Canary Islands in summer. Mar. Ecol. Prog. Ser., 209, 1-17.

Breitbarth, E., Oschlies, A. and Laroche, J. (2007) Physiological constraints on the global distribution of *Trichodesmium* – effect of temperature on diazotrophy. Biogeosci., 4, 53-61.

Bunn, S. E., Loneragan, N. R. and Kempster, M. A. (1995) Effects of acid washing on stable isotope ratios of C and N in Penaeid shrimp and seagrass: Implications for food-web studies using multiple stable isotopes. Limnol. Oceanogr., 40, 622-625.

Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F. and Carpenter, E. J. (2005) Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic ocean. Global Biogeochem. Cycles, 19, doi:10.1029/2004GB002331.

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. and Carpenter, E. J. (1997) *Trichodesmium*, a globally significant marine cyanobacterium. Science, 276, 1221-1229.

Davis, C. S. and McGillicuddy, D. J. (2006) Transatlantic abundance of the N_2-fixing colonial cyanobacterium *Trichodesmium*. Science, 312, 1517-1520.

Duce, R. A., Laroche, J., Altieri, K., Arrigo, K. R., Baker, A. R., Capone, D. G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R. S., Geider, R. J., Jickells, T., Kuypers, M. M., Langlois, R., Liss, P. S., Liu, S. M., Middelburg, J. J., Moore, C. M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L. L., Uematsu, M., Ulloa, O., Voss, M., Ward, B. and Zamora, L. (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science, 320, 893-897.

Emerson, S., Quay, P., Karl, D., Winn, C., Tupas, L. and Landry, M. (1997) Experimental determination of the organic carbon flux from open-ocean surface waters. Nature, 389, 951-954.

Fernández, A., Mouriño-Carballido, B., Bode, A., Varela, M. and Marañón, E. (2010) Latitudinal distribution of *Trichodesmium* spp. and N_2 fixation in the Atlantic Ocean. Biogeosci., 7, 3167-3176.

Fernández, A., Graña, R., Mouriño-Carballido, B., Bode, A., Varela, M., Domínguez, J. F., Escánez, J., De Armas, D. and Marañón, E. (2012) Community N_2 fixation and *Trichodesmium* spp. abundance along longitudinal gradients in the eastern subtropical North Atlantic. ICES J. Mar. Sci., in press.

Fry, B. and Wainright, S. C. (1991) Diatom sources of ^{13}C-rich carbon in marine food webs. Mar. Ecol. Prog. Ser., 76, 149-157.

Glibert, P. M. and Bronk, D. A. (1994) Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp. Appl. Environ. Microbiol., 60, 3996-4000.

Goericke, R. (1998) Response of phytoplankton community structure and taxon-specific growth rates to seasonally varying physical forcing in the Sargasso Sea off Bermuda. Limnol. Oceanogr., 43, 921-935.

Grasshoff, K., Ehrhardt, M. and Kremling, K. (1983) Methods of seawater analysis. Verlag Chemie, Weinheim.

Gruber, N. and Sarmiento, J. (1997) Global patterns of marine nitrogen fixation and denitrification. Global Biogeochem. Cycles, 11, 235-266.

Gruber, N., Keeling, C. D., Bacastow, R. B., Guenther, P. R., Lueker, T. J., Wahlen, M., Meijer, H. A. J., Mook, W. G. and Stocker, T. F. (1999) Spatiotemporal patterns of carbon-13 in the global surface oceans and the oceanic Suess effect. Global Biogeochem. Cycles, 13, 307-335.

Hawser, S.P., O'Neil, J.M., Roman, M.R. and Codd, G.A. (1992) Toxicity of blooms of the cyanobacterium *Trichodesmium* to zooplankton. J. Appl. Phycol.. 4, 79-86.

Hernández-León, S., Gómez, M. and Arístegui, J. (2007) Mesozooplankton in the Canary Current system: The coastal–ocean transition zone. Prog. Oceanogr., 74, 397-421.

Karl, D. M., Bidigare, R. R., Church, M. J., Dore, J. E., Letelier, R. M., Mahaffey, C. and Zehr, J. P. (2008) The nitrogen cycle in the North Pacific Trades biome: An evolving paradigm. In Capone, D. G., Bronk, D. A., Mulholland. M. R. and Carpenter, E. J. (eds) Nitrogen in the marine environment. Elsevier, pp. 705-769.

Knapp, A. N., Sigman, D. M., Lipschultz, F., Kustka, A. B. and Capone, D. G. (2011) Interbasin isotopic correspondence between upper-ocean bulk don and subsurface nitrate and its implications for marine nitrogen cycling. Global Biogeochem. Cycles, 25, doi:10.1029/2010GB003878.

Landrum, J. P., Altabet, M. A. and Montoya, J. P. (2011) Basin-scale distributions of stable nitrogen isotopes in the subtropical North Atlantic Ocean: Contribution of diazotroph nitrogen to particulate organic matter and mesozooplankton. Deep Sea Res., 58, 615-625.

Lipschultz, F. (2001) A time-series assessment of the nitrogen cycle at BATS. Deep Sea Res. II, 48, 1897-1924.

Luo, Y.-W., Doney, S. C., Anderson, L. A., Benavides, M., Bode, A., Bonnet, S., Boström, K. H., Böttjer, D., Capone, D. G., Carpenter, E. J., Chen, Y. L., Church, M. J., Dore, J. E., Falcón, L. I., Fernández, A., Foster, R. A., Furuya, K., Gómez, F., Gundersen, K., Hynes, A. M., Karl, D. M., Kitajima, S., Langlois, R. J., Laroche, J., Letelier, R. M., Marañón, E., McGillicuddy Jr., D. J., Moisander, P. H., Moore, C. M., Mouriño-Carballido, B., Mulholland, M. R., Needoba, J. A., Orcutt, K. M., Poulton, A. J., Raimbault, P., Rees, A. P., Riemann, L., Shiozaki, T., Subramaniam, A., Tyrrell, T., Turk-Kubo, K. A., Varela, M., Villareal, T. A., Webb, E. A., White, A. E., Wu, J. and Zehr, J. P. (2012) Database of diazotrophs in global ocean: Abundances, biomass and nitrogen fixation rates. Earth Syst. Sci. Data Discuss., 5, 47-106.

Madin, L. P., Horgan, E. F. and Steinberg, D. K. (2001) Zooplankton at the Bermuda Atlantic Time-Series study (BATS) station: Diel, seasonal and interannual variation in biomass, 1994-1998. Deep Sea Res. II, 48, 2063-2082.

Marañón, E., Holligan, P. M., Varela, M., Mouriño, B. and Bale, A. J. (2000) Basin-scale variability of phytoplankton biomass, production and growth in the Atlantic Ocean. Deep Sea Res., 47, 825-857.

Margalef, R. (1978) Phytoplankton communities in upwelling areas. The example of NW Africa. Oecologia Aquatica, 3, 97-132.

McClelland, J. W., Holl, C. M. and Montoya, J.-P. (2003) Relating low $\delta^{15}N$ values of zooplankton to N_2-fixation in the Tropical North Atlantic: Insights provided by stable isotope ratios of amino acids. Deep Sea Res., 50, 849-861.

McGillicuddy Jr., D. J., Kosnyrev, V. K., Ryan, J. P. and Yoder, J. A. (2001) Covariation of mesoscale ocean color and sea-surface temperature patterns in the Sargasso Sea. Deep Sea Res. II, 48, 1823-1836.

Mino, Y., Saino, T., Suzuki, K. and Marañón, E. (2002) Isotopic composition of suspended particulate nitrogen ($\delta^{15}N_{sus}$) in surface waters of the Atlantic Ocean from 50ºN to 50ºS. Global Biogeochemical Cycles, 16, doi:10.1029/2001GB001635.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P. and Zehr, J. P. (2010) Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. Science, 327, 1512-1514.

Montoya, J. P., Carpenter, E. J. and Capone, D. G. (2002) Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. Limnol. Oceanogr., 47, 1617-1628.

Montoya, J. P., Voss, M. and Capone, D. G. (2007) Spatial variation in N_2-fixation rate and diazotroph activity in the Tropical Atlantic. Biogeosci., 4, 369-376.

Moore, K., Doney, S. C., Lindsay, K., Mahowald, N. and Michaels, A. F. (2006) Nitrogen fixation amplifies the ocean biogeochemical response to decadal timescale variations in mineral dust deposition. Tellus, 58B, 560-572.

Moore, C. M., Mills, M. M., Achterberg, E. P., Geider, R. J., Laroche, J., Lucas, M. I., Mcdonagh, E. L., Pan, X., Poulton, A. J., Rijkenberg, M. J. A., Suggett, D. J., Ussher, S. J. and Woodward, E. M. S. (2009) Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nature Geoscience, 2, 867-871.

Mouriño-Carballido, B., Graña, R., Fernández, A., Bode, A., Varela, M., Domínguez, J. F., Escánez, J., De Armas, D. and Marañón, E. (2011) Importance of N_2 fixation vs. Nitrate eddy diffusion along a latitudinal transect in the atlantic ocean. Limnol. Oceanogr., 56, 999-1007.

Mulholland, M. R. (2007) The fate of nitrogen fixed by diazotrophs in the ocean. Biogeosci., 4, 37-51.

Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A. and O'neil, J. M. (2006) Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. Limnol. Oceanogr., 51, 1762-1776.

O'Neil, J.M., Metzler, P.M. and Glibert, P.M. (1996) Ingestion of $^{15}N_2$-labelled *Trichodesmium* spp. and ammonium regeneration by the harpacticoid copepod *Macrosetella gracilis*. Mar. Biol. , 125, 89-96.

Oschlies, A. and Garçon, V. (1998) Eddy-induced enhancement of primary production in a model of the North Atlantic Ocean. Nature, 394, 266-269.

Owens, N. J. P. (1987) Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol., 24, 389-451.

Pelegrí, J. L., Arístegui, J., Cana, L., González-Dávila, M., Hernández-Guerra, A., Hernández-León, S., Marrero-Díaz, A., Montero, M. F., Sangrá, P. and Santana-Casiano, M. (2005) Coupling between the open ocean and the coastal upwelling region off Northwest Africa: Water recirculation and offshore pumping of organic matter. J. Mar. Systems, 54, 3-37.

Reynolds, S. E., Mather, R. L., Wolff, G. A., Williams, R. G., Landolfi, A., Sanders, R. and Woodward, E. M. S. (2007) How widespread and important is N_2 fixation in the North Atlantic Ocean? Global Biogeochem Cycles, 21, doi:10.1029/2006GB002886.

Sañudo-Wilhelmy, S. A., Kustka, A. B., Gobler, C. J., Hutchins, D. A., Yang, M., Lwiza, K., Burns, J., Capone, D. G., Raven, J. A. and Carpenter, E. J. (2001) Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature, 411, 66-69.

Sohm, J. A., Webb, E. A. and Capone, D. G. (2011) Emerging patterns of marine nitrogen fixation. Nat. Rev. Microbiol., 9, 499-508.

Torres-Valdes, S., Roussenov, V. M., Sanders, R., Reynolds, S., Pan, X., Mather, R., Landolfi, A., Wolff, G. A., Achterberg, E. P. and Williams, R. G. (2009) Distribution of dissolved organic nutrients and their effect on export production over the Atlantic Ocean. Glob. Biogeochem. Cycles, 23, doi:10.1029/2008GB003389

Tortell, P. D. and Morel, F. M. M. (2002) Sources of inorganic carbon for phytoplankton in the eastern subtropical and equatorial Pacific Ocean. Limnol. Oceanogr., 47, 1012-1022.

Tyrrell, T., Marañón, E., Poulton, A. J., Bowie, A. R., Harbour, D. S. and Woodward, E. M. S. (2003) Large-scale latitudinal distribution of *Trichodesmium* sp. in the Atlantic Ocean. J. Plankton Res., 25, 405-416.

Voss, M., Croot, P., Lochte, K., Mills, M. and Peeken, I. (2004) Patterns of nitrogen fixation along 10º N in the Tropical Atlantic. Geophys. Res. Lett., 31, doi:10.1029/2004GL020127.

Wannicke, N., Liskow, I. and Voss, M. (2010) Impact of diazotrophy on N stable isotope signatures of nitrate and particulate organic nitrogen: Case studies in the north-eastern tropical Atlantic Ocean. Isotopes Environ. Health Stud., 46, 337-354.

Waser, N. A. D., Harrison, W. G., Head, E. J. H., Nielsen, B., Lutz, V. A. and Calvert, S. E. (2000) Geographic variations in the nitrogen isotope composition of surface particulate nitrogen and new production across the North Atlantic Ocean. Deep-Sea Res., 47, 1207-1226.

Williams, R. G. and Follows, M. J. (1998) The Ekman transfer of nutrients and maintenance of new production over the North Atlantic. Deep-Sea Res., 45, 461-489.

Figure legends

Figure 1. CTD and plankton sampling stations during cruise Leg 8 of Malaspina-2010 expedition along 24 ^{0}N (dashed line). The vertical lines indicate the limits of the eastern (E), central (C) and western (W) regions described in the text.

Figure 2. Temperature, salinity and in vivo fluorescence in the upper 300 m along 24 ^{0}N. CTD stations are indicated in the upper panel. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

Figure 3. Accumulated biomass (mg dry weight m^{-3}) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

Figure 4. Natural abundance of stable nitrogen isotopes (δ^{15}N, ‰) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

Figure 5. Natural abundance of stable carbon isotopes (δ^{13}C, ‰) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

Figure 6. Correlation coefficients (Pearson r) between size fractionated plankton: a) biomass (mg DW m-3), b) δ15N or c) δ13C of and sea surface temperature (SST, ºC), salinity (SSS) or *in vivo* fluorescence (Sfluor) along 24 ºN. The dashed lines indicate the significance value (P<0.05).

Figure 7. Relationships between δ^{15}N and δ^{13}C for size fractionated plankton (µm). All regression lines are significant with P<0.01 except for the >2000 µm fraction (dashed line, P<0.05).

Figure 8. Abundance of *Trichodesmium* (trichomes L^{-1}) along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

Figure 9. Relationships between $\delta^{15}N$ and *Trichodesmium* abundance (log_{10}(trichomes L^{-1})). All regression lines are significant with $P<0.001$.The dashed line indicates the regression line for the >2000 µm fraction.

Figure 10. Diazotrophic N contribution (%) to plankton size-classes >200 µm estimated from $\delta^{15}N$ along 24^{0} N.

Table 1. Mean (±se) biomass (mg DW m^{-3}), $\delta^{15}N$ and $\delta^{13}C$ by size-fractions in the western (W), central (C) and eastern (E) zones along 24°N. Total biomass (Total) is the sum of biomass for all size-fractions. n: number of data. Shaded values and letters indicate significant differences between means (ANOVA and C-Dunnett *a posteriori* test, P<0.05).

	DW			$\delta^{15}N$			$\delta^{13}C$		
Size fraction (µm)	W	C	E	W	C	E	W	C	E
40-200	4.65±0.24[b]	3.38±0.11[a]	3.46±0.18[a]	1.7±0.2[b]	0.6±0.1[a]	2.1±0.2[b]	-19.6±0.2[b]	-19.7±0.1[b]	-20.3±0.1[a]
200-500	3.00±0.24[b]	1.89±0.08[a]	2.04±0.12[a]	2.0±0.2[b]	1.2±0.1[a]	2.5±0.3[b]	-19.7±0.1[b]	-19.6±0.3[b]	-20.4±0.2[a]
500-1000	2.69±0.24[a]	1.95±0.09[a]	2.48±0.22[a]	2.3±0.2[b]	1.2±0.1[a]	2.5±0.3[b]	-20.0±0.2[a]	-20.1±0.3[a]	-20.6±0.2[a]
1000-2000	2.31±0.16[a]	1.74±0.20[a]	2.08±0.11[a]	2.4±0.2[b]	1.5±0.2[a]	2.6±0.3[b]	-19.4±0.4[b]	-18.7±0.4[b]	-20.9±0.7[a]
>2000	2.39±0.17[b]	1.65±0.11[a]	2.71±0.23[b]	3.2±0.3[b]	1.6±0.3[a]	2.9±0.3[b]	-19.5±0.5[a]	-19.3±0.5[a]	-20.9±0.6[a]
Total	15.04±0.93[c]	10.60±0.38[a]	12.78±0.66[b]						
n	14	12	17	14	12	17	14	12	17

Table 2. Mean (±se) abundance of microplankton (n L^{-1}), and dominant taxa (% frequency) and mean (±sd) total abundance (n m^{-3}) of mesozooplankton in the western (W), central (C) and eastern (E) zones along 24°N. Shaded values and letters indicate significant differences between means (ANOVA and C-Dunnett *a posteriori* test, P<0.05).

Group		**Taxa**	**zone** W	**zone** C	**zone** E
			Total abundance (n L^{-1})		
Microplankton (40-200 µm)					
	Phytoplankton	Mean ± se	8.1±1.0[b]	4.5±0.5[a]	3.7±0.4[a]
	Zooplankton	Mean ± se	11.0±1.0[a]	7.3±0.7[a]	7.5±1.4[a]
			Frequency (%)		
Mesozooplankton (>200 µm)		*Calanus*	4.7	9.9	10.0
		Corycaeus	8.1	8.3	5.6
		Macrosetella	4.0	7.4	4.4
		Oithona	8.7	7.4	10.0
		Appendicularia	2.0	2.5	6.1
		Chaetognatha	6.7	7.4	7.8
		Ostracoda	8.7	5.0	7.2
		Polychaeta	7.4	3.3	5.6
		Salps	2.7	5.8	5.0
			Total abundance (n m^{-3})		
		Mean ± se	186.9±29.1[a]	124.3±16.6[a]	178.0±32.4[a]
		n	14	12	17

Table 3. Mean (±se) contribution of N from diazotrophs (%) to plankton size-fractions in the western (W), central (C) and eastern (E) zones along 24°N. N: number of data. Shaded values and letters indicate significant differences between means (ANOVA and C-Dunnett *a posteriori* test, P<0.05).

Size fraction (µm)	W	C	E
200-500	29.4±3.9[a]	43.1±1.4[b]	22.5±3.9[a]
500-1000	31.9±2.7[a]	49.2±1.9[b]	29.0±4.4[a]
1000-2000	38.3±3.2[a]	50.4±3.2[b]	35.5±4.1[a]
>2000	33.2±4.3[a]	53.5±4.1[b]	38.7±4.7[a]
n	14	12	17

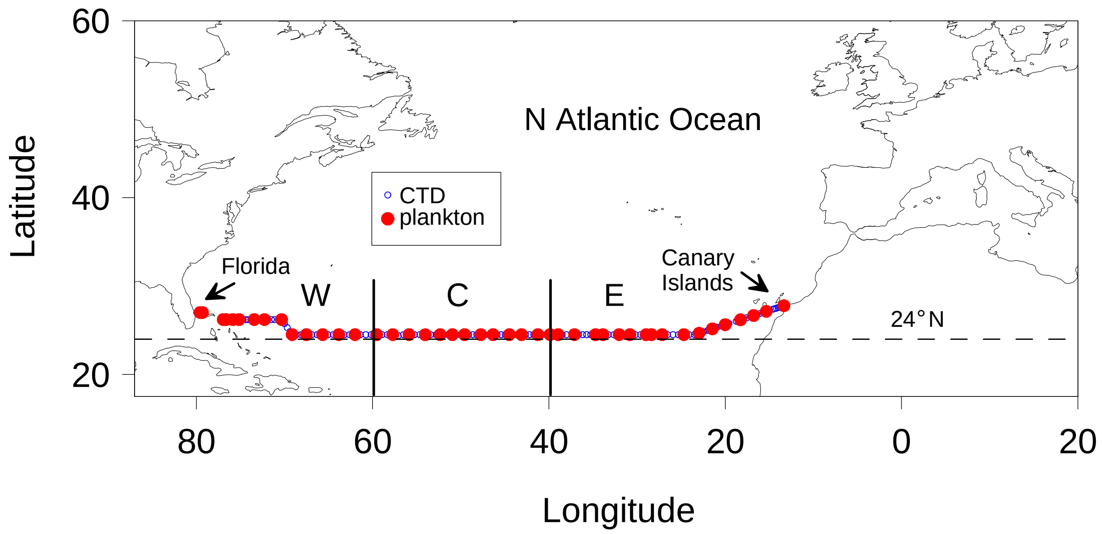


Figure 1. CTD and plankton sampling stations during cruise Leg 8 of Malaspina-2010 expedition along 24 ^{0}N (dashed line). The vertical lines indicate the limits of the eastern (E), central (C) and western (W) regions described in the text.

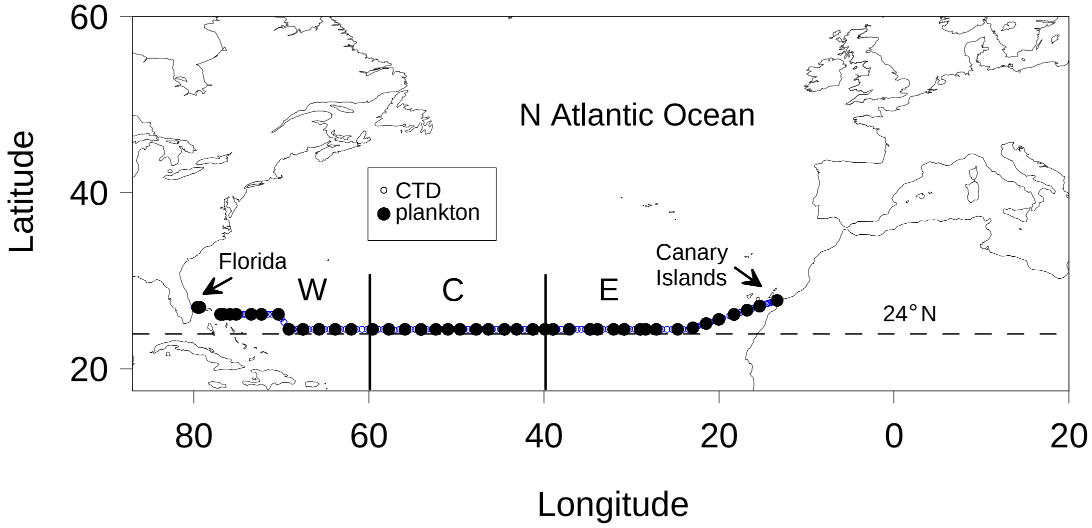


Figure 1. CTD and plankton sampling stations during cruise Leg 8 of Malaspina-2010 expedition along 24 ^{0}N (dashed line). The vertical lines indicate the limits of the eastern (E), central (C) and western (W) regions described in the text.

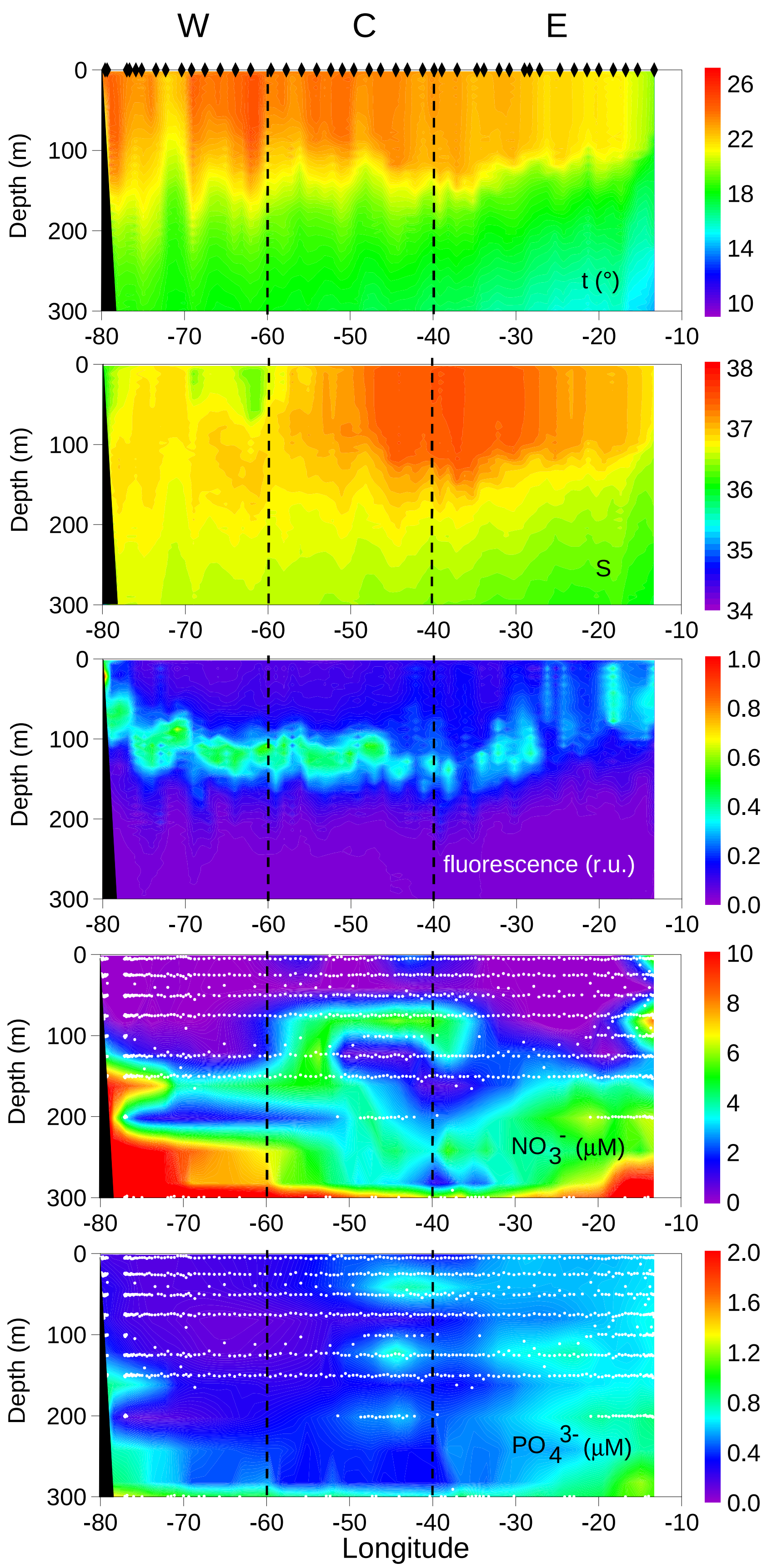
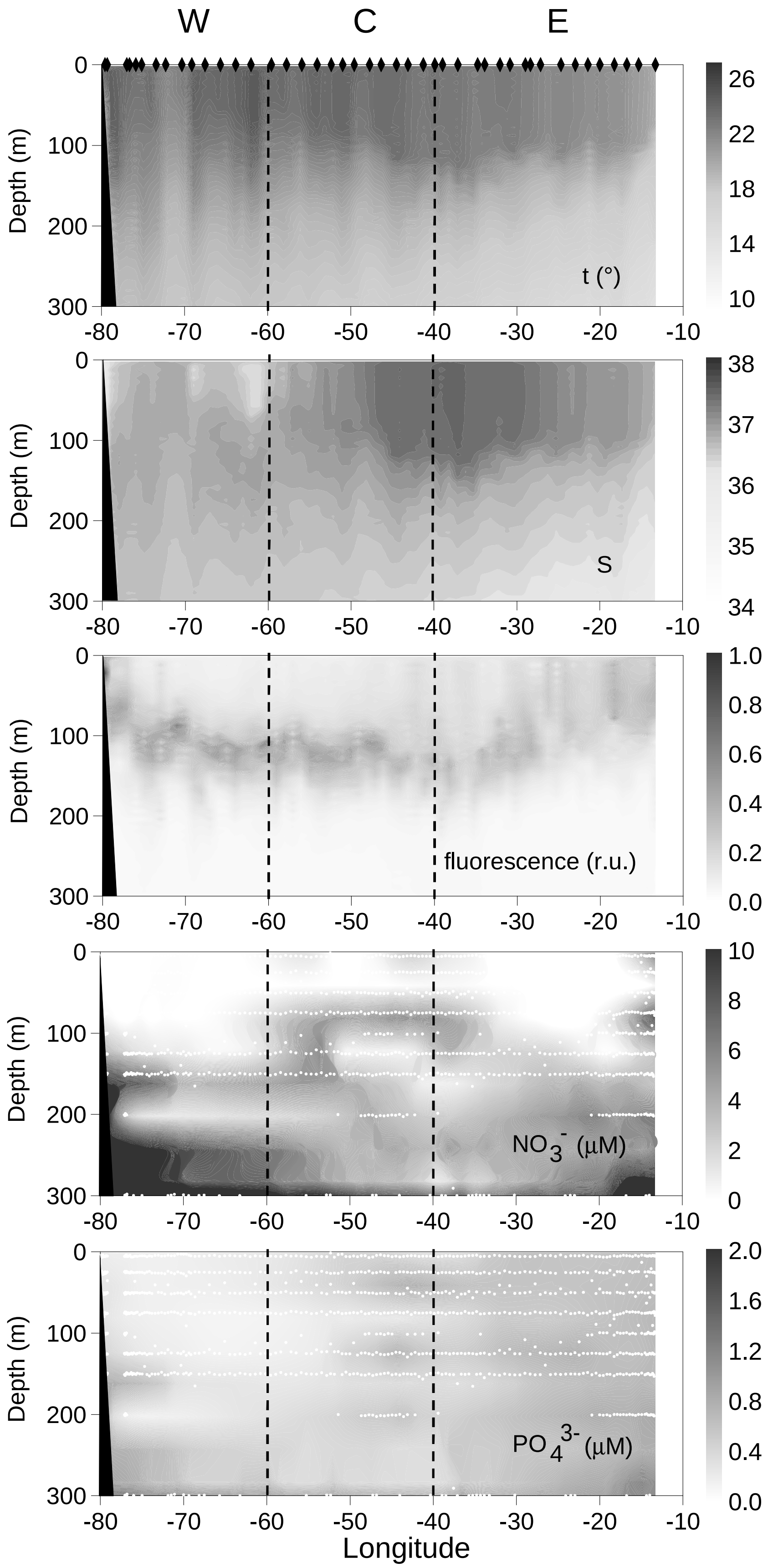


Figure 2. Temperature, salinity, in vivo fluorescence, nitrate and phosphate in the upper 300 m along 24 ^{0}N. CTD stations are indicated in the upper panel. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.



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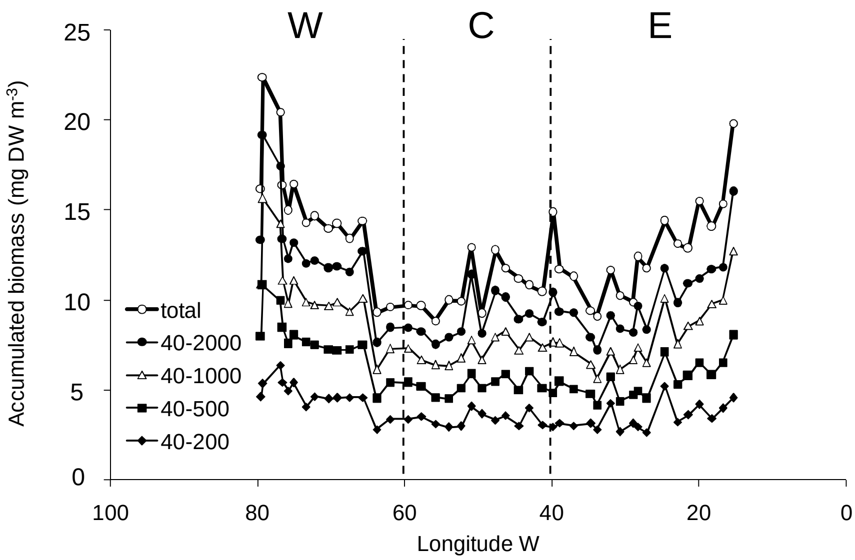

Figure 3. Accumulated biomass (mg dry weight m^{-3}) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

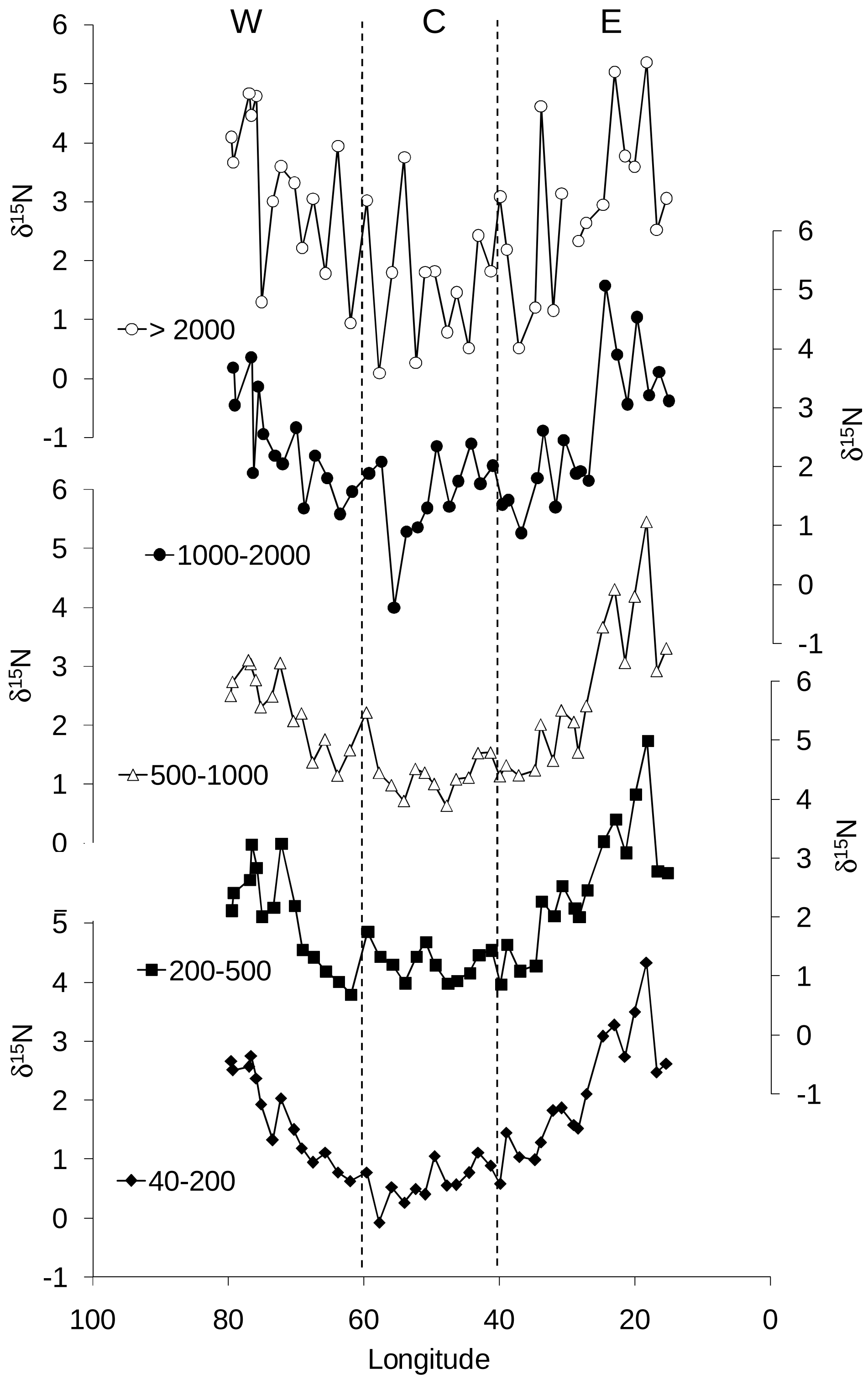

Figure 4. Natural abundance of stable nitrogen isotopes ($\delta^{15}N$, ‰) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

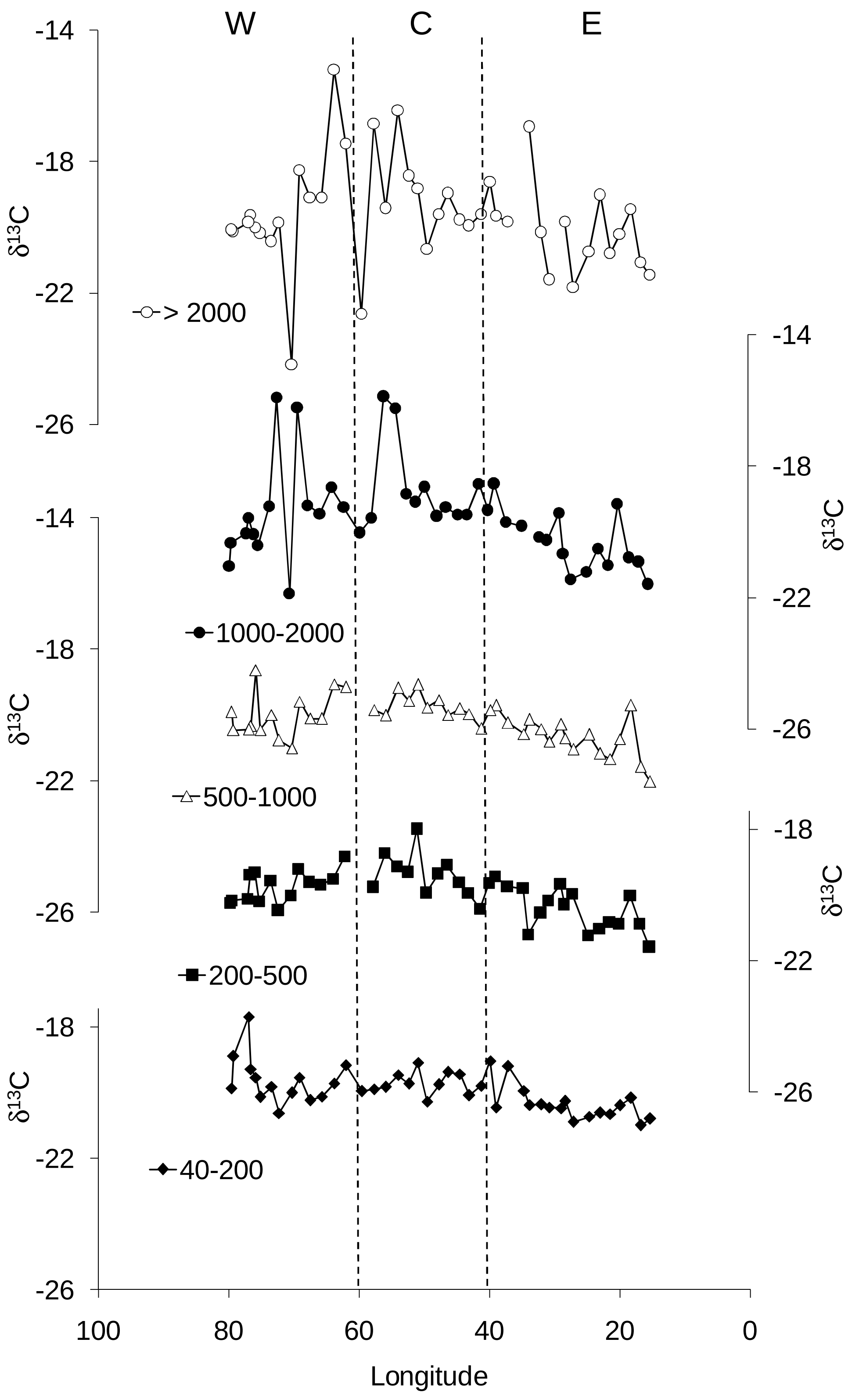

Figure 5. Natural abundance of stable carbon isotopes ($\delta^{13}C$, ‰) of size fractionated plankton along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

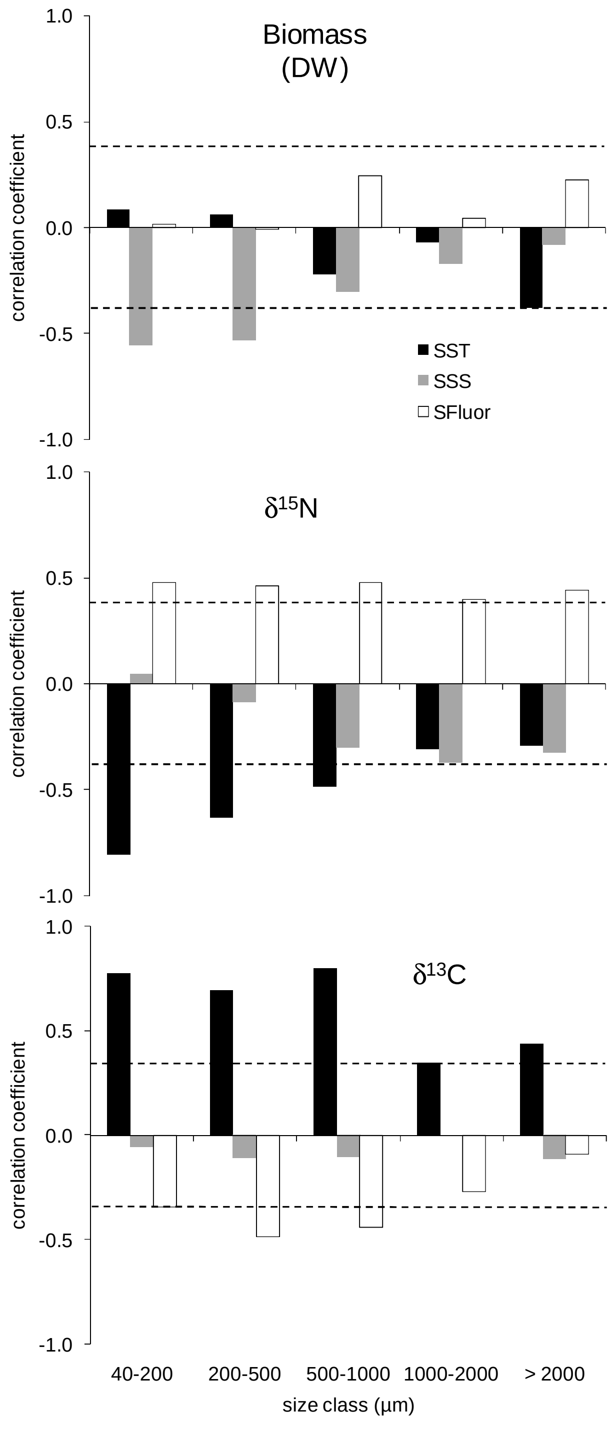

Figure 6. Correlation coefficients (Pearson r) between size fractionated plankton biomass (mg DW m^{-3}) $\delta^{15}N$ or $\delta^{13}C$ of and sea surface temperature (SST, ºC), salinity (SSS) or *in vivo* fluorescence (Sfluor) along 24 ºN. The dashed lines indicate the significance value (P<0.05).

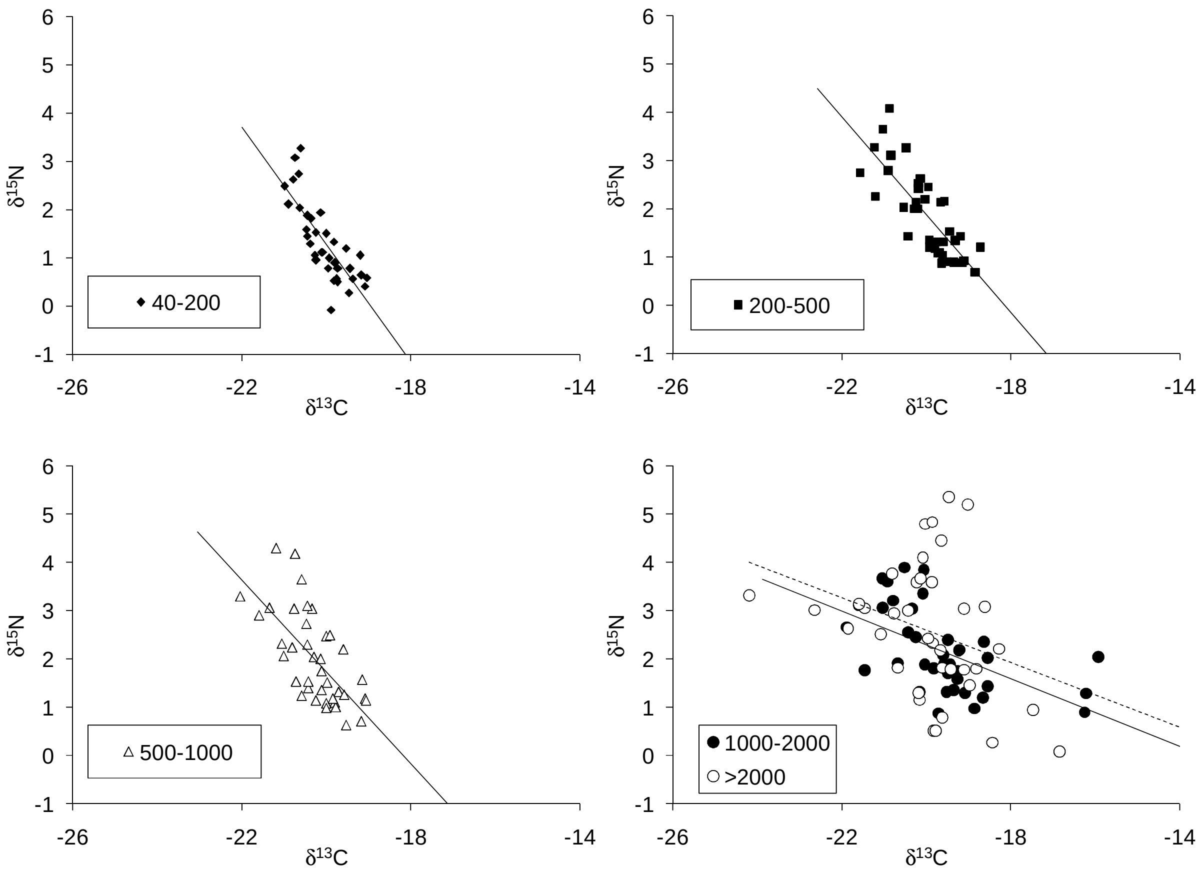

Figure 7. Relationships between $\delta^{15}N$ and $\delta^{13}C$ for size fractionated plankton (µm). All regression lines are significant with $P<0.01$ except for the >2000 µm fraction (dashed line, $P<0.05$).

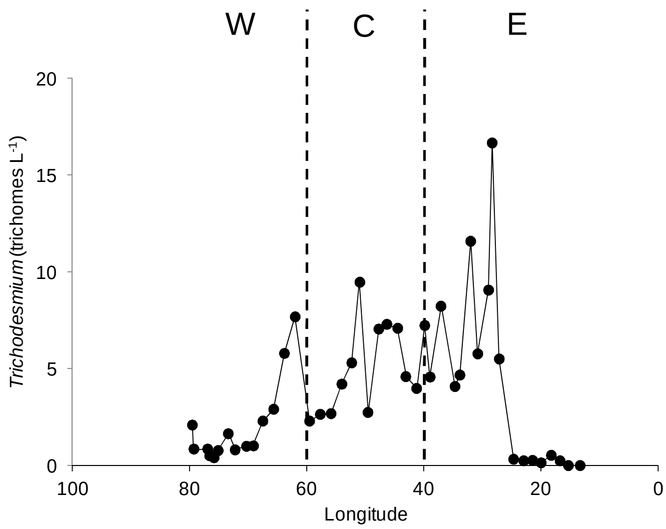

Figure 8. Abundance of *Trichodesmium* (trichomes L^{-1}) along 24 ^{0}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

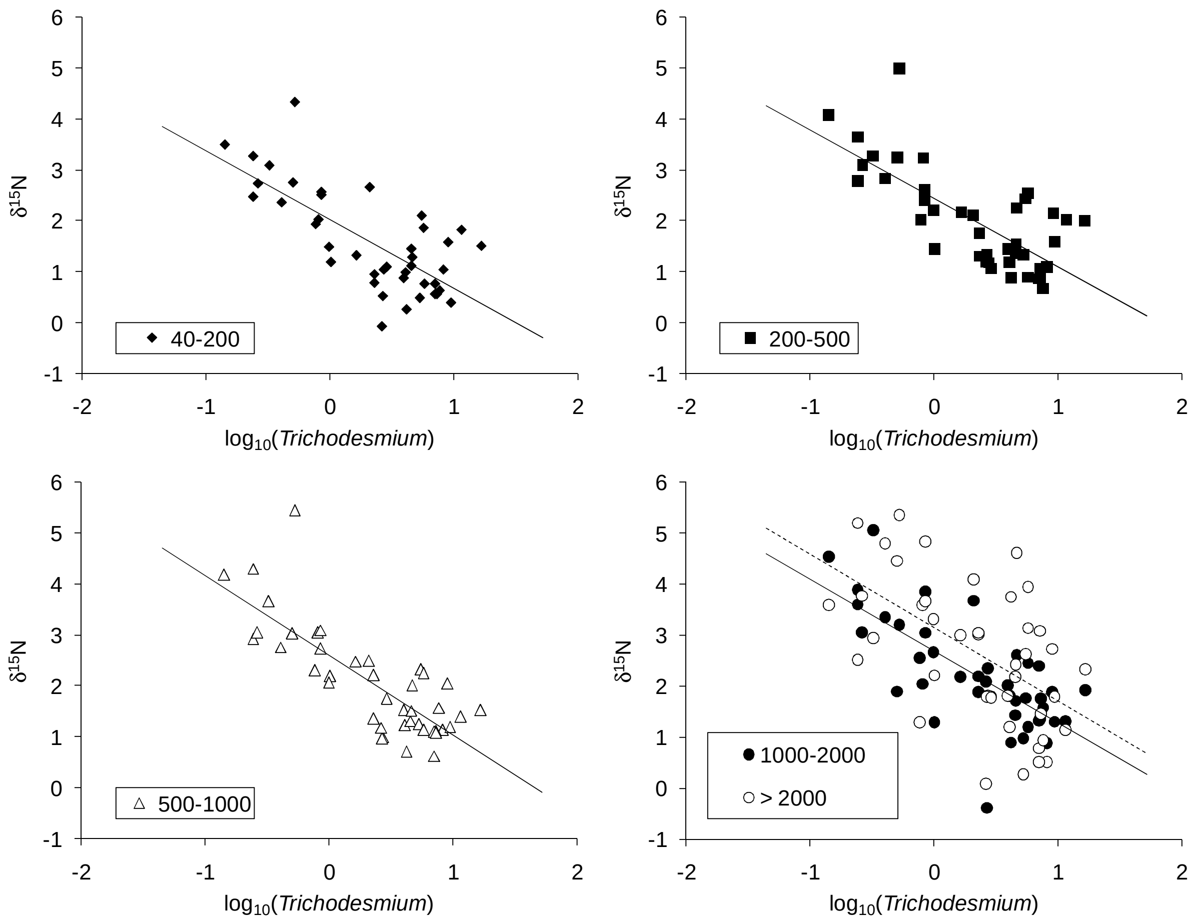

Figure 9. Relationships between $\delta^{15}N$ and *Trichodesmium* abundance (log_{10}(trichomes L^{-1})). All regression lines are significant with P<0.001.The dashed line indicates the regression line for the >2000 µm fraction.

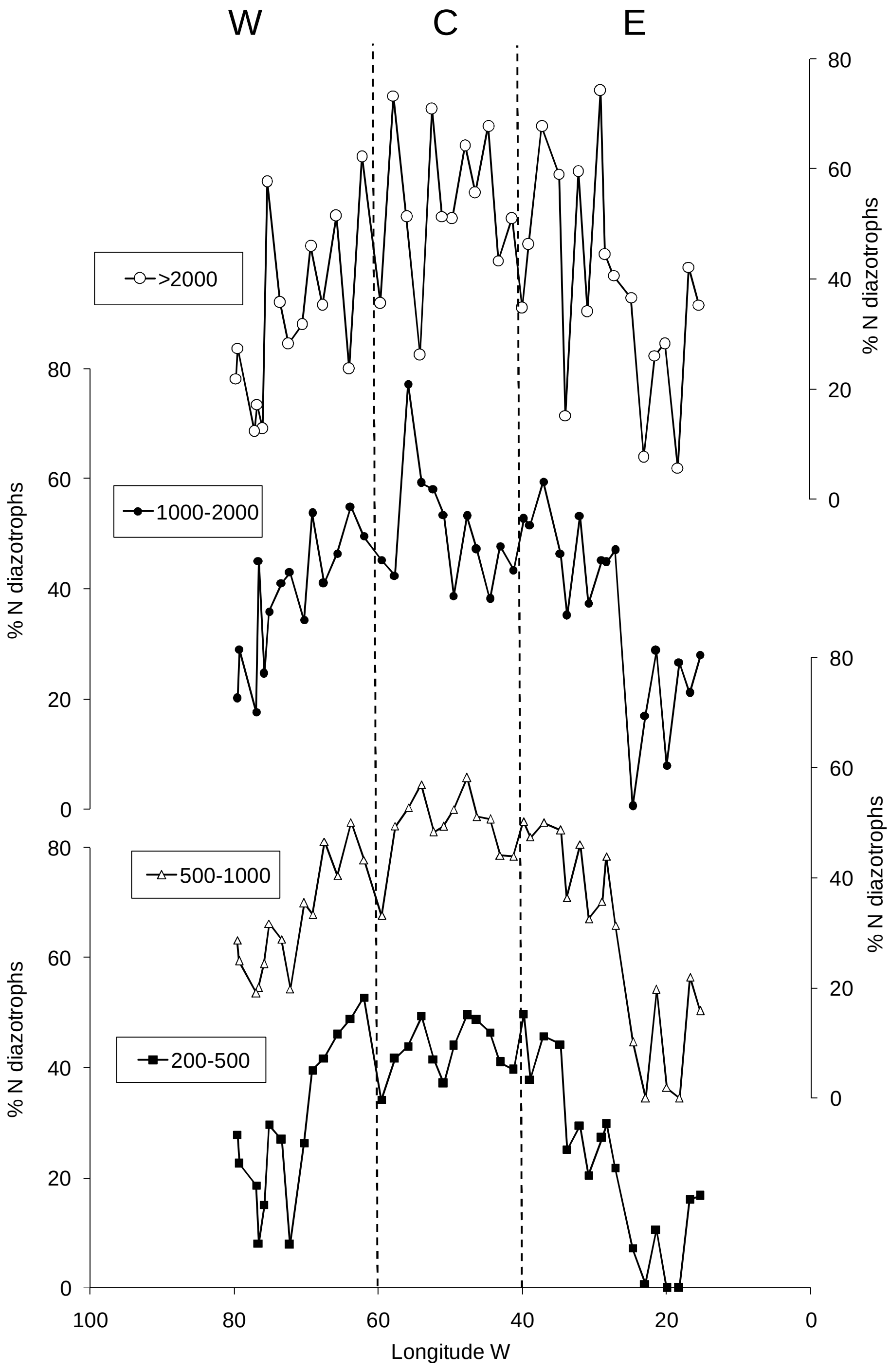

Figure 10. Diazotrophic N contribution (%) to plankton size-classes >200 µm estimated from δ[15]N along 24º N.