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Macroecological Patterns of Trophic Variability in the Oceanic Plankton

Patrones Macroecológicos de Variabilidad Trófica en el Plancton Oceánico

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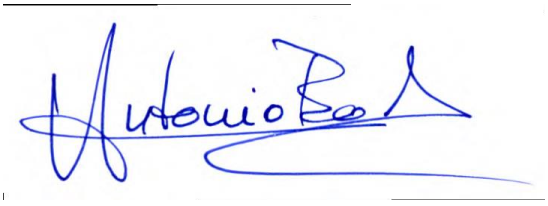
D. Antonio Bode Riestra, Científico Titular del Centro Oceanográfico de A Coruña del Instituto Español de Oceanografía,

HACE CONSTAR que la presente memoria, titulada "*Patrones macroecológicos de variabilidad trófica en el plancton oceánico*", presentada por la licenciada en Biología Dña. Carmen Mompeán de la Rosa para optar al grado de doctora, ha sido realizada bajo mi dirección, cumpliendo con las condiciones exigidas para su presentación, la cual autorizo.

Para que así conste y surta los efectos oportunos, firmo la presente en A Coruña, a 28 de setiembre de 2015.



Fdo. Antonio Bode Riestra

Los trabajos que se incluyen en esta tesis se desarrollan en el marco del proyecto EXPEDICIÓN DE CIRCUNNAVEGACIÓN MALASPINA 2010: CAMBIO GLOBAL Y EXPLORACIÓN DE LA BIODIVERSIDAD DEL OCÉANO GLOBAL (CONSOLIDER INGENIO CSD 2008-00077). El capítulo 3 de esta memoria ya ha sido publicado como Mompeán et *al.* 2013, los capítulos 2 y 4 se encuentran en fase de revisión.

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Del sur al norte y del norte al sur,
a quienes creyeron en mí.

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Introducción

El estudio de los isótopos estables, especialmente de los isótopos del nitrógeno, nos va a llevar a lo largo de este trabajo a ahondar en el conocimiento de la estructura trófica del plancton, conocer su distribución global gracias a una expedición a nivel mundial a lo largo de los tres grandes océanos del planeta (Atlántico, Pacífico e Indico). Al mismo tiempo profundizar en una zona donde el estudio del nitrógeno es especialmente relevante como es el Atlántico Norte subtropical, a través no sólo del estudio de la biomasa y los isótopos estables de carbono y nitrógeno, sino también por medio del análisis del isótopo estable de nitrógeno en aminoácidos que es una de las técnicas más precisas para conocer el comportamiento de este isótopo a lo largo de la cadena trófica.

1.1 EL PLANCTON OCEÁNICO

La distribución de la biomasa planctónica y la producción primaria neta en los océanos está definida por la disponibilidad de luz y nutrientes (nitrógeno, fosfato, hierro). Estos factores limitantes del crecimiento están regulados por los procesos físicos de circulación del océano, dinámicas de capas de mezcla, afloramientos o afloramientos (upwelling), deposición de polvo atmosférico y el ciclo solar (Behrenfeld et al. 2006). En su estudio Behrenfeld et al. recopiló datos de satélite durante una década (1997 a 2006) en todos los océanos. Estas medidas de satélite proporcionan una media de la productividad oceánica cuantificada a nivel global y asociada a la variabilidad de factores ambientales. La media anual de la producción primaria neta mostró altos valores en las zonas donde los nutrientes superficiales fueron elevados, y bajos donde la temperatura superficial fue alta. Así la variabilidad en la temperatura y estratificación está unida a la disponibilidad de nutrientes y por tanto a la producción primaria en los océanos (Behrenfeld et al. 2006).

El fitoplancton crece en las zonas bien iluminadas de la superficie del océano, formando la base de la red trófica marina ya que no sólo proporciona el alimento de zooplancton y éstos a su vez de mamíferos y peces de importancia comercial, sino que juega un papel fundamental en el funcionamiento de los ecosistemas marinos, proporcionando la mitad de la producción primaria global y contribuyendo de manera importante a los ciclos biogeoquímicos del carbono y muchos otros elementos en el mar. La biomasa fitoplanctónica puede variar con un factor de ~100 en las aguas superficiales, ya que su distribución geográfica está determinada por la circulación de los océanos y zonas de afloramientos, con los niveles mayores encontrados en el Ecuador, en latitudes templadas y polares y a lo largo de los bordes oeste de los continentes (Doney 2006).

La mayor conexión entre el plancton y los factores ambientales se encuentra en trópicos y latitudes medias donde está limitada la mezcla vertical ya que la columna de agua está estabilizada por una estratificación térmica (que es cuando aguas cálidas recubren aguas densas y frías). En estas áreas las bajas concentraciones de nutrientes en superficie limitan el crecimiento de fitoplancton, el calentamiento de las aguas inhibe la mezcla, reduciendo el ascenso de aguas profundas con nutrientes y por tanto la productividad en la capa superficial (Figura 1.1a). Sin embargo a mayores latitudes el fitoplancton está limitado por la luz, se producen mezclas verticales que hacen que los nutrientes penetren cientos de metros donde la luz del sol no penetra. En estas regiones el posible calentamiento futuro y las mayores entradas de agua dulce, principalmente por aumento de las precipitaciones y de los deshielos, contribuirá a reducir la mezcla lo que debería así incrementar la productividad en la superficie (Figura 1.1b).

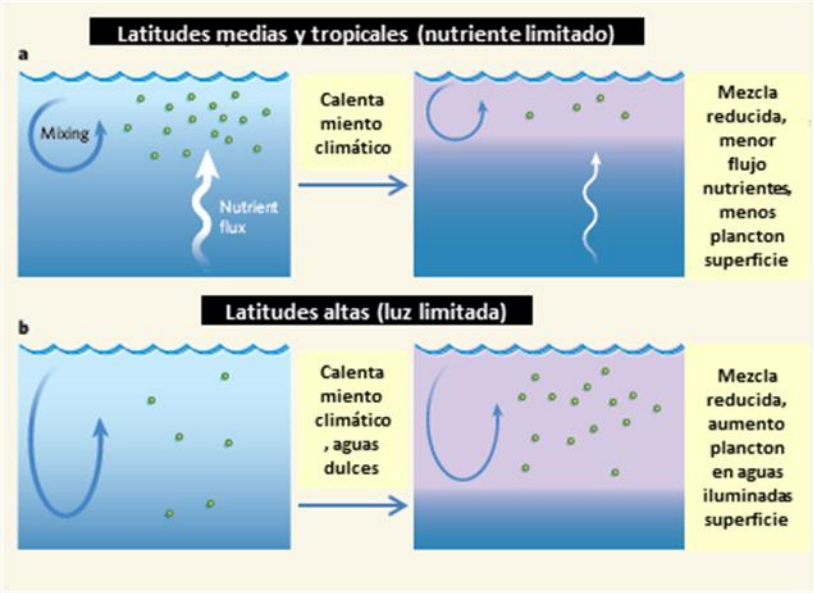

Figura 1.1 Respuesta del fitoplancton al incremento de la temperatura en las aguas superficiales de los océanos. a, en los trópicos y latitudes medias, el fitoplancton está limitado por nutrientes y los datos de satélite muestran baja productividad biológica y reducción del flujo nutrientes hacia la capa superficial tras su calentamiento. b, a latitudes más altas, la respuesta biológica debería ser opuesta al calentamiento futuro, y entradas extra de aguas dulces. En estas regiones, el fitoplancton está limitado por la luz; una mezcla reducida conservaría al plancton cerca de la superficie donde los niveles de luz son mayores. Adaptada de la figura 1 de Doney (2006)

1.2 ESTRUCTURA DE LOS ECOSISTEMAS PLANCTÓNICOS

Para conocer mejor la distribución del plancton a través de los océanos, previos estudios han definido la estructura de los ecosistemas planctónicos a partir del tamaño de los organismos. Antes se creía a los herbívoros como red trófica dominante en los ecosistemas marinos, hasta que se vio que la red trófica microbiana era el componente más importante de los sistemas acuáticos. El microzooplancton es la unión más importante entre el bucle microbiano y los niveles superiores en la cadena trófica, son los consumidores principales de pico y nanoplancton (Calbet and Landry 2004) y representa un gran valor alimenticio para el mesozooplancton (Sanders and Wickham 1993; Broglio et al. 2004), sobretodo en los sistemas poco productivos (Calbet 2001). En los ecosistemas productivos, donde abundan los nitratos, el mesozooplancton resulta el principal consumidor de carbono fitoplanctónico, sin embargo en zonas oligotróficas como las del Pacífico ecuatorial el picoplancton es el principal consumidor de este carbono (Richardson et al. 2004).

El tamaño del fitoplancton marino cubre al menos cuatro órdenes de magnitud (Legendre and Rassoulzadegan, 1996) desde especies de diatomeas de ~2000µm (*Ethmodiscus rex*) en aguas oceánicas cálidas, hasta pequeños proclorófitos de menos 0.6-0.7 µm. Para simplificar en su estudio Legendre and Rassoulzadegan dividieron a los productores primarios en 3 clases: fitoplancton grande (>5µm), fitoplancton pequeño (0.2-5µm) y el carbono orgánico disuelto, DOC (<0.2µm). En esta división se estableció el límite de 5µm para separar el fitoplancton pequeño del grande porque estudiaron las interacciones tróficas y el mesozooplancton no se alimenta eficientemente de partículas inferiores a 5µm (Fortier et al. 1994). Aunque lo más usual es dividir en picoplancton (<2 µm) y nanoplancton (2 a 20 µm). Estos diferentes criterios de división del fitoplancton se producen debido a que el espectro de los productos fotosintéticos es continuo, desde el fitoplancton más grande a las más pequeñas moléculas orgánicas exudadas. Por tanto los límites entre fitoplancton grande, pequeño y DOC, son, en general, bastante arbitrarios.

Se puede conocer la forma de las pirámides de biomasa en los océanos conociendo la relación de biomasa entre autótrofos y heterótrofos (Morán et al. 2004). Gasol et al. (1997) a partir de una extensa recopilación bibliográfica, mostraron que la relación entre la biomasa total de heterótrofos (bacterias, protozoos y mesozooplancton) y de autótrofos (fitoplancton), la relación H:A, desciende cuando aumenta la biomasa fitoplanctónica y la producción primaria. Estos autores sugieren que en las aguas oceánicas el control de la biomasa del fitoplancton se realiza por los consumidores de arriba abajo (top-down), y por el contrario en las zonas de afloramientos y áreas costeras el control es por los nutrientes, de abajo a arriba (bottom-up).

Sin embargo estos patrones no siempre se encuentran en los océanos; una excepción clara son las zonas oligotróficas, como el giro del Pacífico Norte (Cortés et al. 2001) donde la limitación de nitratos y luz es la que controla el crecimiento de los cocolitóforos y no el herbívorismo de los niveles tróficos superiores.

Estos ejemplos nos muestran la complejidad de las redes tróficas y de los ciclos de carbono y nutrientes en los ecosistemas planctónicos sobre todo en las regiones oligotróficas del océano.

1.3 CARACTERÍSTICAS DEL PLANCTON EN LOS BIOMAS OCEÁNICOS

Longhurst (Longhurst 2007) propone una división de los océanos en cuatro biomas: Polar, Westerlies, Trades y Coastal. Esta división propone que los diferentes mecanismos de producción pelágica en los océanos se pueden agrupar en seis modelos simples.

Bioma Polar: donde la profundidad de la capa de mezcla está forzada por una capa salobre superficial que se forma cada primavera en la zona de los márgenes del hielo. Este bioma está caracterizado por una baja diversidad taxonómica a todos los niveles tróficos. Durante el breve periodo en el que el sol incide en un ángulo alto, la biomasa fitoplanctónica está dominada por células grandes (>90% diatomeas y cocolitofóridos) aunque las fracciones menores de pico- y nanofitoplancton son sin embargo responsables del 10-25% de la producción. Este caso sería el primer modelo de producción pelágica: caso 1: Pico de producción dependiente de la irradiancia solar.

Bioma Westerlies (vientos del oeste): donde la profundidad de la capa de mezcla está forzada por los vientos y la irradiancia locales. Se dan dos modelos de producción: caso 2: Nutrientes limitantes en primavera, con pulso de producción y caso 3: Producción de invierno-primavera con limitación de nutrientes. Aquí ambos casos describen el ciclo de producción estacional, dependiendo de la latitud y de la profundidad relativa de la capa de mezcla producida por los vientos del oeste.

En el caso 2 se producen unas condiciones para los nutrientes al final del invierno que están relacionados con la profundidad de la capa de mezcla de invierno y los nitratos subsuperficiales. Estas condiciones pueden causar una proliferación de fitoplancton (bloom primaveral) en la capa superficial si las condiciones de luz son favorables. En verano se produce una situación oligotrófica formándose un máximo de clorofila profundo al agotarse los nutrientes en la capa superficial.

A bajas latitudes donde los vientos del oeste son relativamente ligeros se daría el modelo de producción denominado caso 3 donde la producción primaria es siempre baja y estacional dependiendo de la profundidad de la capa de mezcla en invierno. A estas bajas latitudes las pequeñas células fotosintéticas dominan la biomasa fitoplanctónica durante aquellos largos periodos donde la estratificación es marcada, el

flujo de nutrientes es reducido y el bloom de algas no se produce. La fracción de picoplancton, dominado por cianobacterias y proclorófitos, comprende el 70-90% de la clorofila y contribuye al 80-90% de la producción primaria a 20-30º de latitud. Igualmente la fracción hervíbora del zooplancton es más diversa que en el caso 2 y comprende muchos géneros de pequeños copépodos y tunicados.

Bioma Trades (vientos alisios): donde la profundidad de la capa de mezcla está forzada por ajuste geostrófico desde una escala oceánica local o por vientos lejanos forzados, en este bioma se producen también dos modelos diferentes de producción: caso 4: Respuestas de pequeña amplitud por vientos alisios estacionales y caso 5: Respuestas de gran amplitud por giro monzónico de los vientos alisios.

La productividad en este bioma raramente está limitada por la luz y responde rápidamente a la entrada de nutrientes dentro de la zona fótica por afloramientos costeros, picos de Ekman o ajustes de la picnoclina asociados con la inversión de los vientos monzónicos. Los cambios que se dan en la biomasa fitoplanctónica coinciden con cambios estacionales en la producción. Las proliferaciones estacionales de algas están dominadas por diatomeas, con picocianobaterias y otros proclorófitos contribuyendo sólo al 30-40% del carbono del fitoplancton, una fracción pequeña comparada con la observada en las regiones oligotróficas.

Aunque la fijación de nitrógeno se produce principalmente en los organismos pelágicos del bioma Westerlies, especialmente por células epífitas en el mar de los Sargazos en el Atlántico Norte y como endosimbiontes dentro de diatomeas, los blooms de cianofitas fijadoras de nitrógeno en este bioma ha llegado a ser el paradigma de la fijación de N_2 entre las células fitoplanctónicas.

En este bioma el ecosistema pelágico es el más diverso taxonómicamente y representa la comunidad pelágica climax. Todos los otros sistemas pelágicos pueden ser vistos como derivados de éste. El zooplancton de los océanos tropicales está dominado numéricamente por los copépodos, aunque sólo representan el 33% de la biomasa total. En contraste los copépodos representan el 68% de la biomasa en los océanos polares, lo

que resalta la mayor importancia relativa que tiene otros grupos del zooplancton en los mares tropicales.

Bioma Coastal (costero): donde muy diversos procesos costeros modifican la profundidad de la capa de mezcla y las entradas de nutrientes, se produce el sexto caso de mecanismo de producción: caso6: Producción intermitente por divergencias costeras y afloramientos (upwelling).

El plancton, especialmente el zooplancton, del bioma costero difiere en todas las latitudes respecto al plancton de mar abierto debido a que está formado en gran proporción de meroplancton (larvas planctónicas de invertebrados bentónicos y litorales). Pero como los invertebrados bentónicos en latitudes mayores tienden a un desarrollo directo, la relativa contribución del meroplancton al plancton costero es otra variable que se modifica con la latitud. El resto de especies que forman el holoplancton costero se pueden considerar en su mayor parte especies congenéricas de las oceánicas, sólo algún grupo taxonómico (como los cladóceros) tienen géneros y especies exclusivamente costeros.

En zonas de afloramiento es típica la presencia de zooplancton hervíboro especializado como los copépodos calanoides que migran estacionalmente y son frecuentes en bajas latitudes (p. ej. *Calanoides carinatus*).

Situadas en latitudes subtropicales hay grandes regiones del océano que están determinadas por giros de corrientes que rotan en el sentido de las agujas del reloj en el Hemisferio Norte y viceversa en el sur. Las regiones centrales de estos giros se caracterizan por una baja producción biológica debido a una escasa entrada de nutrientes, mientras que en los bordes la producción aumenta (Behrenfeld et al. 2006). Así en el centro de los giros oceánicos, donde la termoclina es profunda y los gradientes de nutrientes suaves, las tasas de suministro de nutrientes por difusión o advección son bajas (Mouriño-Carballido et al. 2011). A pesar de su baja producción estos giros, debido a su tamaño, contribuyen a una gran parte del carbono exportado hacia el océano profundo (Emerson et al. 1997, Karl et al. 2008).

En las zonas con giros oligotróficos se conocen una gran variedad de mecanismos físicos que contribuyen a la entrada de nutrientes a la zona fótica, como por ejemplo la turbulencia (Oschlies & Garçon 1998), el transporte lateral desde otras regiones (Williams & Follows 1998, Torres-Valdés et al. 2009b) y la deposición atmosférica (Duce, LaRoche, et al. 2008). Sin embargo la fijación biológica de N_2 atmosférico (diazotrofía) puede ser la entrada más importante de nitrógeno en el océano oligotrófico (Gruber & Sarmiento 1997, Capone et al. 2005, Moore et al. 2009).

1.4 FIJACIÓN DE NITRÓGENO: *Trichodesmium*

Aunque varios elementos pueden limitar la producción primaria (ej. P, Fe, etc.), el nitrógeno es típicamente el principal factor limitante en grandes áreas de los océanos (Ryther & Dunstan 1971, Graziano et al. 1996, Moore et al. 2008). La mayor parte de la producción primaria se sostiene con formas de nitrógeno inorgánico regenerado mediante procesos biológicos en la superficie del océano (ej. NH_4^+ que proviene de la descomposición de la materia orgánica) , pero el balance biogeoquímico requiere la entrada de nitrógeno externo (nuevo) que mantenga la producción primaria en la superficie a pesar de las pérdidas por sedimentación (Capone et al., 2005; Ward et al., 2007) . Este nitrógeno alóctono puede entrar en grandes cantidades por mezcla vertical (ej. en invierno) o advección en zonas de afloramiento (upwelling o, en menores cantidades por difusión (McGillicuddy et al. 1998), deposición atmosférica (Duce, La Roche, et al. 2008) o por fijación biológica del N_2 atmosférico (Mahaffey et al. 2005, Capone et al. 2005, Karl & Letelier 2008).

Para entender un poco más el ciclo del nitrógeno en los océanos vamos a desarrollar la siguiente figura (Figura 1.2 según (Arrigo 2005)) donde se representa el balance global del océano entre fijación de N_2 y la pérdida de N, así como la relación N:P. En zonas de afloramiento generalmente el N está empobrecido respecto al P. Donde los afloramientos son importantes (regiones eutróficas), la producción primaria es alta, produciéndose el hundimiento de mayores cantidades de materia orgánica. Esta materia orgánica se descompone solubilizando el N y P y consumiendo la mayor parte del

oxígeno. En estas aguas con limitación de oxígeno los procesos de oxidación anaerobia de amonio (anammox) y desnitrificación convierten el NH_4 y NO_3 a N_2, resultando en una pérdida de N disponible biológicamente y por tanto un marcado descenso en las aguas profundas de la relación N:P. Por el contrario, donde los aportes de N y P son bajos (regiones oligotróficas), la producción primaria es reducida y suele estar dominada por cianobacterias fijadoras de N_2, que ven favorecido su crecimiento en aguas con baja concentración de nutrientes, especialmente N. La fijación de N_2 aumenta la relación N:P de la materia orgánica a valores superiores a la relación Redfield (N:P = 16), que es finalmente remineralizado para producir aguas enriquecidas en N. Sin embargo la fijación de N_2 no es suficiente para equilibrar las pérdidas de N fijado por anammox y desnitrificación. Por tanto, los océanos contemporáneos tienen una relación media de N:P ligeramente menor que la relación de Redfield (Arrigo 2005).

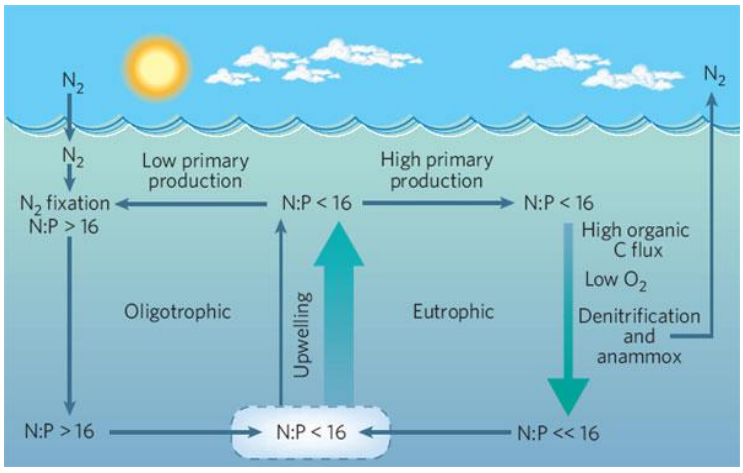

Figura 1.2. Balance global del océano entre fijación de N_2 y la pérdida de N. Relación de N:P. (Arrigo 2005)

Los organismos fijadores de N_2 proporcionan una fuente de N nuevo a los océanos del mundo, especialmente en giros oligotróficos oceánicos (Capone et al. 2005, Montoya et al. 2007), como señalábamos en el apartado anterior. Así en el Atlántico Norte la diazotrofía (fijación biológica de N_2) contribuye a una gran parte de la nueva producción primaria excediendo incluso las contribuciones por difusión de nitrato a través de la picnoclina (Capone et al. 2005, Fernández et al. 2010, Mouriño-Carballido et al. 2011). La

fijación de nitrógeno está controlada por la temperatura (Breitbarth et al. 2007), CO_2 (Barcelos e Ramos et al. 2007) y la disponibilidad de otros nutrientes, como fósforo y hierro, este último proporcionado por entradas de polvo atmosférico (Moore et al. 2009, Sohm et al. 2011). El nitrógeno de origen diazotrófico está disponible para la red trófica pelágica fundamentalmente a través de la excreción y mortalidad de las cianobacterias (Glibert & Bronk 1994) cuya materia orgánica es procesada posteriormente por microorganismos y metazoos planctónicos (Montoya et al. 2002).

El género *Trichodesmium* es una cianobacteria colonial que se encuentra ampliamente distribuida por las regiones tropicales y subtropicales de los océanos (Capone et al. 1997, Luo et al. 2012). Son los diazotrofos mejor conocidos y habitan estas regiones donde la superficie del agua supera los 20ºC (Breitbarth et al. 2007). Los filamentos reciben el nombre de tricomas y en el medio marino aparecen tanto tricomas libres como colonias que a su vez pueden presentar dos morfologías diferentes: en forma esférica o en forma de haz (Figura 1.3)

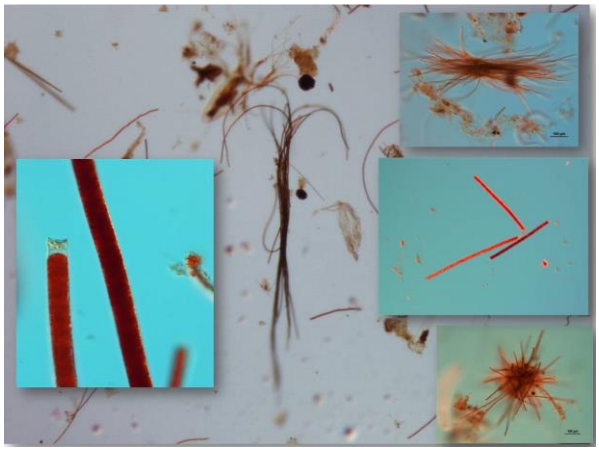

Figura 1.3. Diferentes formas de presentarse *Trichodesmium* en el medio marino: Tricomas libres y en forma de colonias.

La mayoría de grupos de zooplancton pelágico no consume a los representantes de *Trichodesmium* debido a su toxicidad (Hawser et al. 1992). Por lo tanto la exudación directa de nitrógeno orgánico disuelto, que puede representar hasta un 50% del total de

N_2 recién fijado (Glibert & Bronk 1994), sería la vía principal de transmisión de nitrógeno recién fijado por *Trichodesmium* a la red trófica.

En el Atlántico Norte *Trichodesmium* es más abundante entre 20ºN y 20ºS (Tyrrell et al. 2003, Davis & McGillicuddy 2006, Fernández et al. 2010, 2012) aunque la mayoría de los estudios sobre fijación de nitrógeno se han realizado en las zonas tropicales y subtropicales donde son más frecuentes las proliferaciones de esta especie (Voss et al. 2004, Capone et al. 2005, Mulholland et al. 2006, Montoya et al. 2007). Así las medidas de la abundancia natural de isótopos de nitrógeno estable en seston y plancton (Waser et al. 2000, Montoya et al. 2002, Mino et al. 2002, Reynolds et al. 2007, Landrum et al. 2011) proporcionaron evidencias del impacto de la diazotrofía a grandes escalas geográficas.

1.5 EL ANÁLISIS DE ISÓTOPOS ESTABLES EN ZOOPLANCTON

Cuando decimos que dos organismos o especies de un ecosistema tienen el mismo nivel trófico es que coinciden por el lugar de su hábitat que ocupan, en el flujo de energía y nutrientes, es decir, que ocupan un lugar equivalente en la cadena alimenticia. Así por ejemplo los productores primarios serían nivel trófico= 1, los consumidores primarios nivel trófico= 2, etc. éstos serían los valores teóricos. Sin embargo dentro de estos grandes niveles tróficos los organismos ocupan diferentes posiciones tróficas, que son valores fraccionales, reales y observables.

El análisis de isótopos estables, en particular la composición del nitrógeno isotópico ha sido utilizado para investigar la estructura de las redes tróficas, identificar las posiciones tróficas de los organismos y cuantificar el flujo de nitrógeno en dichas redes (Hobson & Welch 1992, Yoshii et al. 1999, Ogawa et al. 2001, Fry 2006). Muchos estudios determinan las posiciones tróficas de los organismos porque son simples de definir, caracterizan a los organismos y facilitan las estimaciones de los flujos de energía y masas a través de los ecosistemas (Hairston & Hairston 1993).

En el análisis de isótopos estables se define δ como:

$$\delta = (R_{muestra}/R_{estándar} - 1)*1000$$

Donde R es la relación entre el isótopo pesado y el ligero en la muestra o en el valor estándar, así por ejemplo para el isótopo del nitrógeno, la relación quedaría:

$$\delta = \left[\frac{(^{15}N_{muestra}/^{14}N_{muestra})}{(^{15}N_{estándar}/^{14}N_{estándar})} - 1 \right] *1000$$

La relación de isótopos estables de nitrógeno ($\delta^{15}N$) se puede usar como estimador de la posición trófica porque el $\delta^{15}N$ de un consumidor está enriquecido con respecto a su dieta (Deniro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 2012). Por el contrario la relación de isótopos de carbono ($\delta^{13}C$) cambia poco a través de las redes tróficas (Rounick & Winterbourn 1986, Peterson & Fry 1987, France & Peters 1997) y así se usa para evaluar las fuentes de carbono para un organismo cuando la señal isotópica de las fuentes es diferente.

La posición trófica (TP) se estimó por la ecuación:

$$TP_{consumidor} = \frac{\delta^{15}N_{consumidor} - \delta^{15}N_{referencia}}{FE} + TL_{referencia}$$

Siendo $\delta^{15}N_{referencia}$ el valor medido en un nivel trófico (productor o consumidor primario) de referencia ($TL_{referencia}$= 1 o 2, respectivamente) y FE el factor de enriquecimiento entre niveles tróficos.

Se dedujo tras observaciones empíricas que ~3.4‰ era el incremento que tenía cada nivel trófico (Deniro & Epstein 1981, Minagawa & Wada 1984, Post 2002).

Sin embargo se ha encontrado que el factor de enriquecimiento varía entre diferentes especies o según su fisiología o ecología trófica (Vander Zanden & Rasmussen 2001, McCutchan Jr et al. 2003, Martinez del Rio et al. 2009). Por ejemplo McCutchan et al. (2003) encontraron una variación del factor de enriquecimiento de ^{15}N desde -2.1‰ a 5.4‰ para insectos y peces. Recientemente se ha encontrado que la variación en FE afecta especialmente a los niveles tróficos superiores (Hussey et al., 2014).

Otro problema surgía al estimar el valor de $\delta^{15}N$ en productores primarios ya que por ejemplo en ambientes acuáticos (por ejemplo cianobaterias y algas) muestran gran variabilidad en sus valores de $\delta^{15}N$ (más del 10‰ en algunos casos que es 3 veces el factor de enriquecimiento de ^{15}N (Hannides et al. 2009)). Esta variación se debía a que estos organismos incorporan el nitrógeno de varias fuentes (N_2, NO_3^-, NH_4^+) y al mismo tiempo son organismos con periodo de vida corto (Bronk & Glibert 1993, Rolff 2000, Dore et al. 2002). En un estudio realizado en el Mar Báltico, Rolff (2000) encontró un rango anual con valores de $\delta^{15}N$ para fitoplancton de 0‰ a 7‰ y un rango para zooplancton de 3‰ a 10‰.

Así este método fue mejorado con el análisis del isótopo de nitrógeno en aminoácidos (McClelland & Montoya 2002, McClelland et al. 2003, Chikaraishi et al. 2007) que desarrollaremos en el siguiente apartado, ver cuadro comparativo de los dos métodos (Figura 1.4).

1.6 EL ANÁLISIS DEL ISÓTOPO ESTABLE DE NITRÓGENO ($\delta^{15}N$) EN AMINOÁCIDOS

Los primeros estudios publicados sobre el análisis de isótopos estables en aminoácidos (McClelland & Montoya 2002, McClelland et al. 2003) mostraron como los valores de $\delta^{15}N$ de aminoácidos individuales ($\delta^{15}N$-AA) cambian con la transferencia trófica. Pero estos cambios son diferentes dependiendo del aminoácido que observemos, por ejemplo el ácido aspártico (Asp) o el ácido glutámico (Glu) incrementan su $\delta^{15}N$ con cada nivel trófico, mientras que fenilalanina (Phe) o serina (Ser) tienen valores de $\delta^{15}N$ que permanecen constantes. McClelland y Montoya (2002) realizaron experimentos con cultivos de una especie de alga verde y su consumidor zooplanctónico, obteniendo un enriquecimiento de ~7‰ en aminoácidos como Glu y pocos cambios en otros como Phe con cada nivel trófico. Así se sugirió que la comparación de estos dos valores de $\delta^{15}N$, indicarían la posición trófica de un organismo.

En estudios posteriores (Chikaraishi et al. 2007) se observó esta misma relación entre macroalgas y gasterópodos en el medio natural y de los valores obtenidos de $\delta^{15}N$ se dedujo que ciertos aminoácidos (alanina, valina, isoleucina, ácido glutámico) se

enriquecen en cada transferencia trófica (hasta 10‰) por lo que se denominan aminoácidos "tróficos", mientras otros (metionina o fenilalanina) apenas cambia su valor de δ^{15}N y se denominan aminoácidos "fuente", ya que mantienen los valores de δ^{15}N de las fuentes de N inorgánico empleadas por los productores primarios.

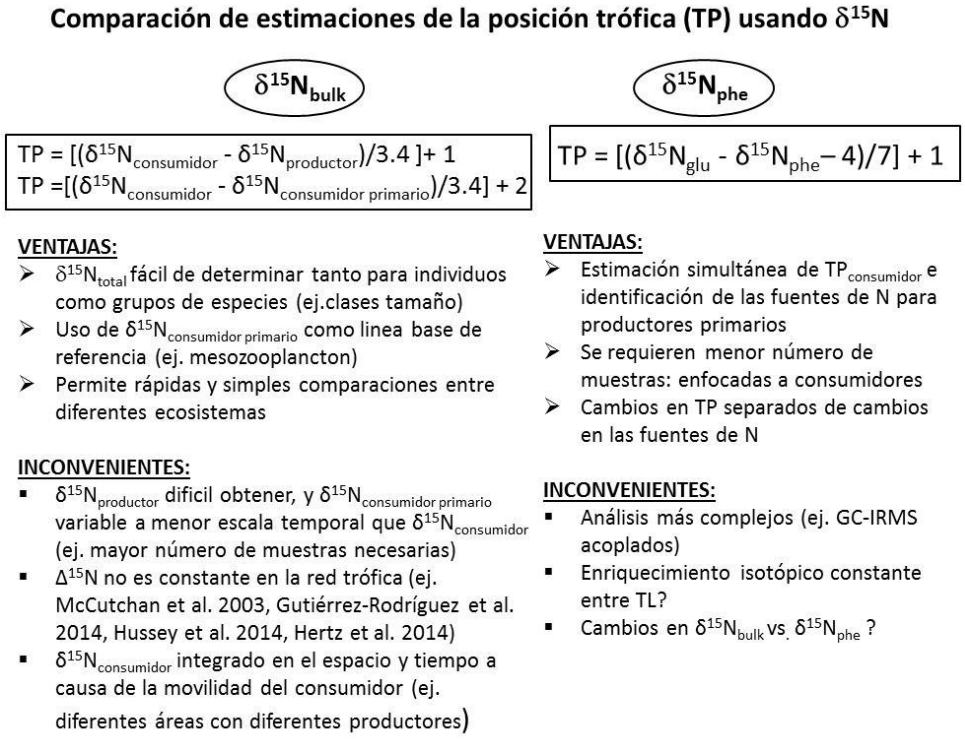

Figura 1.4 Cuadro comparativo de estimaciones de la posición trófica (TP) usando δ^{15}N. Muestra las ventajas e inconvenientes de los dos métodos utilizados en este estudio, para la estimación de la TP de organismos planctónicos.

Una de las mayores ventajas del método de análisis de isótopos en aminoácidos es que la posición trófica se estima a partir de dos aminoácidos de un sólo organismo, por tanto no se requiere la caracterización de los valores de δ^{15}N de los productores primarios o de otros organismos usados como referencia. Además se necesita una muestra bastante pequeña (contenido de nitrógeno nanomolar) para el análisis de nitrógeno estable en aminoácidos usando un espectrómetro de masas de relaciones isotópicas acoplado por combustión a un cromatógrafo de gases (gas chromatography /combustion/isotope ratio mass spectrometry GC/C/IRMS). Así el método de los aminoácidos facilita la estimación de los niveles tróficos incluso en muestras pequeñas, lo que permite avanzar

en la comprensión de las estructura de las redes tróficas y el flujo de nitrógeno en ambientes naturales (Chikaraishi et al. 2009). En varios estudios ecológicos se empezó a utilizar el método de los aminoácidos para clarificar los niveles tróficos de organismos como por ejemplo: gambas en el archipiélago sub-Antártico (Pakhomov et al. 2004), krill en el Antártico (Schmidt et al. 2004, 2006), plancton en el Pacífico Central (McCarthy et al. 2007), zooplancton cerca de Hawaii (Hannides et al. 2009), corales en el Pacífico Norte (Sherwood et al., 2014), sedimentos oceánicos (Batista et al., 2014), mejillones en la costa de California (Vohkshoori and McCarthy, 2014) y predadores superiores de la corriente californiana (Ruiz-Cooley et al., 2014).

En la mayoría de estos estudios los aminoácidos más utilizados para el cálculo del nivel trófico es Phe como mejor indicador del valor base de $\delta^{15}N$ y Glu como indicador de la transferencia trófica. La ecuación para el cálculo de la posición trófica es:

$$TP = \left(\frac{(\delta^{15}N_{glu} - \delta^{15}N_{phe}) - 3.4‰}{7.6‰} \right) + 1$$

Donde se asume que $\delta^{15}N_{glu}$ está enriquecido un 7.6‰ con cada transferencia trófica por encima del fitoplancton cuyo $\Delta\delta^{15}N_{glu\text{-}phe}$ es 3.4‰ (McClelland & Montoya 2002, Hannides et al. 2009, Chikaraishi, 2009).

1.7 HIPÓTESIS Y OBJETIVOS

En esta tesis se aborda el estudio de la variabilidad de la composición de isótopos estables con el tamaño del plancton. La hipótesis de trabajo es que estas variables reflejan propiedades fundamentales de la red trófica que pueden formularse para analizar y predecir a distintas escalas espaciales.

Así, el objetivo principal que se plantea es analizar los patrones de variabilidad en las fuentes de nutrientes y la complejidad de la red trófica planctónica a distintas escalas espaciales. Para ello se emplearán medidas de la abundancia natural de isótopos

estables en distintas clases de tamaño individual de los organismos del plancton en muestras recogidas en la capa superficial de los principales océanos.

Objetivos parciales:

- Determinar la variabilidad geográfica en la composición isotópica del plancton de distintos tamaños a escalas espaciales de cientos a miles de kilómetros.
- Determinar la existencia de patrones macroecológicos en la composición isotópica del plancton en función del tamaño y variables ambientales (principalmente temperatura y nutrientes).
- Estimar propiedades de la red trófica (ej. relación de tamaños, diversidad trófica, estado metabólico) a partir de la composición isotópica de los aminoácidos del plancton.

1.8 ESTRUCTURA DE LA TESIS

Los datos de esta tesis se recogieron durante la campaña de circunnavegación expedición MALASPINA-2010, que recorrió tres grandes océanos a bordo de dos buques oceanográficos; BO Sarmiento de Gamboa (Enero-Marzo 2011) que realizó un transecto a lo largo del paralelo 24^{o}N entre las Islas Canarias y Florida (leg 8), y BIO Hespérides (Diciembre 2010-Julio 2011) que realizó un muestreo desde Cádiz hasta Cartagena (Murcia) recorriendo a lo largo de 7 tramos (legs) los océanos Atlántico, Indico y Pacífico.

El muestreo de plancton se realizó en los dos buques oceanográficos cada 24 horas en horario diurno. Se tomaron muestras en 145 estaciones oceánicas a lo largo de tres cuencas oceánicas y 16 provincias biogeográficas, situadas en tres de los cuatro grandes biomas descritos anteriormente Trades, Westerlies y Coastal, únicamente quedaría fuera de este muestreo el Bioma Polar.

La campaña MALASPINA-2010 es un estudio multidisciplinar en el que se tomaron muestras físicas, químicas y biológicas del océano profundo y superficial, tanto para su estudio inmediato como para servir de material científico a generaciones futuras. En

nuestro caso utilizamos las muestras de plancton recolectadas en los primeros 200m y las propiedades estimadas del agua a través de lances de CTD en los primeros 300m desde la superficie. Las variables medidas en cada estación diaria incluyeron: temperatura, salinidad, fluorescencia, clorofila *a*, la profundidad del máximo de clorofila, la estratificación por la frecuencia de Brunt- Väisälä, nitrato y fosfato (utilizado en este estudio sólo del Leg 8), abundancia de *Trichodesmium*, y abundancia natural de isótopos en pescas de plancton de 5 fracciones de tamaño (40-200, 200-500, 500-1000, 1000-2000 y >2000µm). También se han empleado bases de datos de satélite disponibles *on-line* para obtener información sobre la producción primaria anual media y la deposición de polvo atmosférico en la cercanía de las estaciones muestreadas

El análisis de los datos se divide en tres capítulos:

En el primer capítulo se estudian los patrones espaciales en la estructura de la red trófica a gran escala aplicando modelos continuos de distribución por tamaños de la biomasa y el $\delta^{15}N$ para el plancton de las capas superficiales del océano. Este capítulo se ha enviado a publicar y se titula **"Capítulo 2**: **Atmospheric and subthermocline nitrogen inputs influence on biomass and trophic structure of ocean plankton studied using biomass and stable isotope size-spectra".**

En el siguiente capítulo se caracterizan los patrones espaciales del plancton en la zona subtropical y oligotrófica del Atlántico Norte por medio del análisis de la biomasa del plancton fraccionado por tamaños y de la abundancia natural de los isótopos de carbono y nitrógeno. Estos patrones están relacionados con la abundancia de *Trichodesmium* e indican una gran influencia de la diazotrofía en esta zona. Este capítulo fue publicado en Journal of Plankton Research (2013) y se titula: **"Capítulo 3: Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer Trichodesmium along the subtropical North Atlantic".**

A continuación se profundiza en esta zona del Atlántico Norte a través del análisis del isótopo $\delta^{15}N$ en aminoácidos, revisando así las estimaciones previas de las posiciones tróficas y el estado metabólico del zooplancton fraccionado por tamaño en una zona subtropical y la importancia de la fijación de nitrógeno a través de la red trófica. Este

capítulo se ha enviado a publicar y se titula: **“Capítulo 4: Bulk vs. aminoacid stable N isotope estimations of metabolic status and contributions of nitrogen fixation to size-fractionated zooplankton biomass in the subtropical N Atlantic”.**

Finalmente se hace una síntesis y discusión conjunta de los principales resultados encontrados, para terminar con las conclusiones de la tesis.

CAPÍTULO 2:

Atmospheric and subthermocline nitrogen inputs influence on biomass and trophic structure of ocean plankton studied using biomass and stable isotope size-spectra

ABSTRACT

Nitrogen sources and their transfer up marine food webs are studied through the natural abundance of stable nitrogen isotopes ($\delta^{15}N$). To date these studies are generally limited to a few species or assemblages within a particular food web. Here we investigate large scale patterns in food web structure by applying continuous size-scaled models of biomass and $\delta^{15}N$ to plankton from the surface layer of the open ocean. Plankton samples were collected at 145 oceanic stations during the Malaspina-2010 Expedition across three ocean basins and 16 biogeographic provinces. Carbon biomass and $\delta^{15}N$ was determined in size-fractionated samples (40 to 5000 µm) collected by vertical hauls (0-200 m). Biomass-normalized size-spectra were constructed to summarize planktonic food web structure and spatial patterns in spectral parameters were analyzed using geographically-weighted regression analysis. Except in the northwestern Atlantic, parameters of biomass and $\delta^{15}N$ size-spectra showed low variability in most regions, reflecting a large homogeneity in nitrogen sources and food web structure for the central oceans, despite local variability in biomass. The largest changes in nitrogen sources were related to inputs of atmospheric nitrogen, either from the diazotrophic cyanobacterium *Trichodesmium* (e.g. in the Westerlies) or other atmospheric nitrogen inputs (e.g. in the Trades). The comparative analysis of $\delta^{15}N$ and biomass size-spectra revealed as a practical tool to estimate the sources of inorganic nitrogen and their net transfer up the food web as illustrated at large spatial scales with ocean plankton. Differences between $\delta^{15}N$ and biomass size-spectra suggest geographic homogeneity in the net transfer of nitrogen up the food web.

2.1 INTRODUCTION

Ocean plankton is an essential component of the earth climate system. Phytoplankton drawdown of CO_2 is made at the expenses of nutrients as nitrogen, and the fate of the organic matter produced depends on the characteristics of the food web, that in turn are largely determined by the size of planktonic organisms (Legendre & Lefevre 1995). The effects of warming and increasing stratification imply reductions in the input of nutrients from deep waters to the surface ocean and subsequent changes in primary production in large ocean areas (Behrenfeld *et al.* 2006). Depending on the structure of the food web the climate effects can be amplified or modified as shown by both *in situ* (Richardson & Schoeman 2004) and modeling studies (Chust *et al.* 2014). Thus, determining the structure of the food web is capital for understanding the behavior of the ocean ecosystems under a changing climate.

Based on the dependency of most physiological processes on organism size, models relating organism abundance (or biomass) and individual size have been developed with the purpose of describing the continuous change of biomass across the whole food web (Platt and Denman 1978, Blanco *et al.* 1994). The so-called size-spectrum models have been used to synthesize the food web structure in comparative analysis of oceanic ecosystems (Rodriguez & Mullin 1986, Piontkovski *et al.* 2003, Quiñones *et al.* 2003, San Martin *et al.* 2006). Furthermore, they have been used to infer the maximum number of trophic levels that a particular ecosystem can support (Zhou 2006, Basedow *et al.* 2010). However, their applicability has been questioned as their predictions require steady state conditions and uniformity in the size-dependent physiological rates (Poulin & Franks 2010).

Stable isotope analysis provides an important tool for elucidating trophic structure. For instance, enrichment in ^{15}N has been observed with increasing trophic position, thus

allowing to infer trophic structure (e.g. Post 2002). This enrichment can also be expected across plankton size classes, as pelagic food webs were strongly size-structured, the smallest organisms being generally primary producers and large organisms consumers of smaller prey (Platt & Denman 1978). However, only a few field studies explored the implications of the variability in ^{15}N with plankton size (Fry and Quiñones 1994, Rolff 2000, Bode *et al.* 2003, Mompeán *et al.* 2013). One of the main limitations was the difficulty in obtaining measurements over a large number of size classes representative of the different trophic levels. The adjustment of simple continuous functions was not always possible because of the frequent exceptions to the general increase of ^{15}N with the average size of the organisms sampled size. For instance, plankton of total length smaller than 200 µm tend to show lower variability in average ^{15}N content than plankton in larger size classes (Rolff 2000, Bode et al. 2003). In addition, the presence of large or colonial plankton that feed on phytoplankton caused a decrease in ^{15}N at large sizes, while there is a lineal increase with plankton size when these large herbivores were not present (Fry & Quiñones 1994). Besides, because most studies used net sieves with a logartihmic increase in mesh size, the direct comparison of ^{15}N content with size did not allowed for the computation of significant linear functions in some the samples (e.g. Rolff 2000). As for the biomass distributions, the use of logarithmic transformations and normalization of the variable of interest (^{15}N in this case) may lead for the construction of size-spectra that allow inferences on the trophic structure of plankton independently of the actual range of sizes measured. To our knowledge, this study represents the first application of biomass-normalized size spectra using stable isotopes.

Large regions of the open ocean are poorly sampled. Here, food webs are generally characterized by low nutrient concentrations, low biomass and dominance of microbial recycling of nutrients with some exceptions due to external nutrient supply (Longhurst 2007). Studies analyzing the planktonic size structure at large oceanic scales are scarce (Piontkovstki *et al.* 2003, Quiñones *et al.* 2003, San Martín *et al.* 2006) while there are no studies addressing the changes of stable nitrogen isotopes with plankton size at such scales. In this study we use biomass and ^{15}N size spectra to assess whether the type of dominant source of inorganic nutrients affects the trophic structure of plankton in the open ocean. The underlying hypothesis is that the trophic structure of plankton in the

open ocean will be determined by the balance between nutrient inputs from deep or continental waters or from the atmosphere. Advective inputs can be expected to be of importance at relatively small spatial or temporal scales, as those related to mesoscale dynamics (Oschlies & Garçon 1998), while most of the nutrients for the oligotrophic ocean are provided by transport across the pycnocline (Mouriño-Carballido et al. 2011, Torres-Valdés *et al.* 2009). Atmospheric inputs include biological N_2 fixation (Capone et al. 2005, Mulholland 2007) or deposition of inorganic and organic nutrients, the latter noticeably enhanced by anthropogenic emissions (Duce et al. 2008). Advection of deep nutrients is expected to lead to blooms of phytoplankton of relatively large size, high primary production and metazoan food webs, typical of most temperate and polar regions, with a strong seasonal variability (Longhurst 2007). Most of the open ocean is expected to depend on non-seasonal, relatively small inputs of nutrients by diffusion, leading to phytoplankton of small size, low primary production and rapid remineralization of organic matter in microbial food webs. Atmospheric N fixation can be due to either large phytoplankton (as the colony-forming cyanobacteria *Trichodesmium*) or to microbial forms, but in all cases low primary production and high microbial remineralization is expected before effective transfer of the fixed N up the food web (Mulholland 2007). Because of the dependence of N fixation on micronutrients, as Fe provided by dust deposition (Moore et al. 2009), atmospheric inputs of nutrients may have also a clear seasonal component. In all cases, the trophic structure of the food web will depend on the net amount of nutrients transferred from the primary producers to the various types of consumers. This implies, by assuming a constant transfer efficiency between trophic levels (Zhou 2006), that most of the oligotrophic open ocean would have shorter food webs than productive seasonal regions, as the biomass of pelagic predators depends on the amount of primary production (Chassot et al. 2007).

The objective of this study is to investigate the relationships between plankton trophic structure, primary production and the source of N at large-scale spatial scales in the ocean. For this purpose we analyzed carbon biomass and the natural abundance of stable nitrogen isotopes in a large set of size-fractionated plankton samples from the research expedition Malaspina-2010 across three ocean basins.

2.2 METHODS

2.2.1 Plankton sampling and analysis

Plankton samples were collected during Malaspina-2010 expedition between December 2010 and July 2011 (Fig. 2.1).

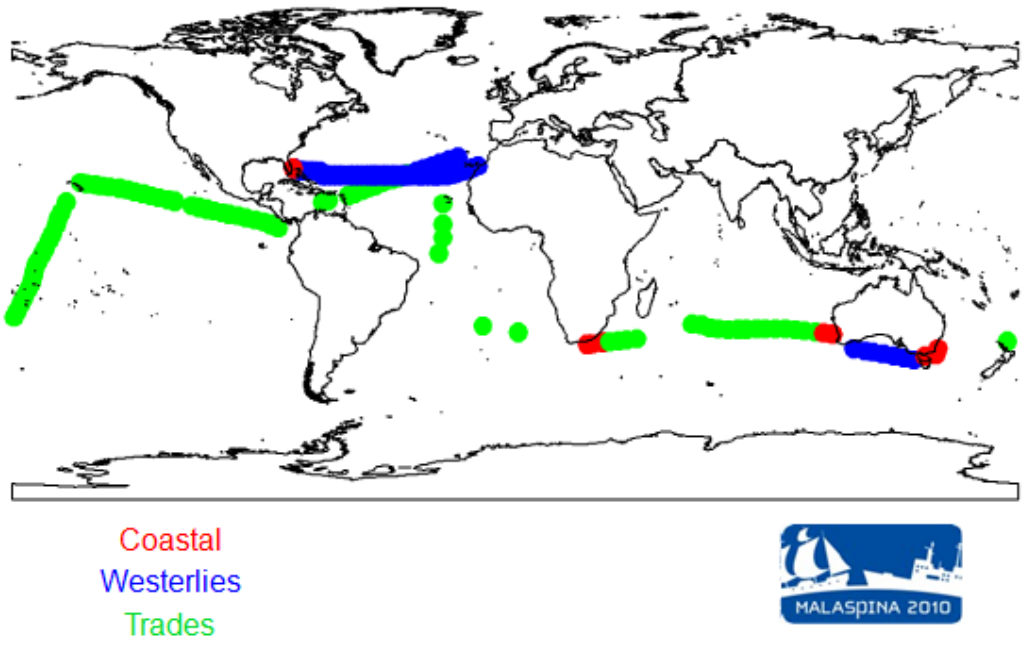

Figure 2.1 Position of stations sampled during the Malaspina-2010 expedition for determination of size-fractionated plankton biomass and natural abundance of stable nitrogen isotopes. The colours indicate the biomes (red: Coastal, blue: Westerlies, green: Trades) according to Longhurst (2007).

The expedition employed two oceanographic ships to make observations and collect water, seston and plankton samples across three major ocean basins (http://metamalaspina.imedea.uib-csic.es/geonetwork/srv/en/main.home). In this study plankton samples from 145 stations were considered. Samples were collected by vertical hauls of bongo-type nets (30 cm diameter, 40 µm mesh size and 50 cm diameter, and 200 µm mesh size) between 200 m depth and the surface during early morning hours. Plankton was size-fractionated using sieves of 200, 500, 1000, 2000 and 5000 µm,

collected on pre-weighted glass-fiber filters and oven dried (60°C, 24 h) on board. In addition, sample aliquots were preserved in formalin (4% final concentration) for later determination of the abundance of trichomes of the N-fixing cyanobacterium *Trichodesmium*. Counts were made using a semiautomatic image analysis flow-through system (FlowCam).

Biomass was later determined in the laboratory for each size fraction as carbon content (C) using an elemental analyzer (Carlo Erba CHNSO 1108). Natural abundance of stable nitrogen isotopes ($\delta^{15}N$) was determined using a mass spectrometer (Finnigan Mat Delta Plus) coupled to the elemental analyzer. Details on the sampling and analysis can be found in Mompeán *et al.* (2013) and Moreno-Ostos et al. (2012).

2.2.2 Oceanographic conditions *in situ*

Hydrographic information was obtained from CTD-rosette casts at the same stations and chlorophyll-a (Chla) was determined from acetonic extracts of phytoplankton collected at up to 8 discrete depths in the photic layer (>0.1% of surface photosynthetically active irradiance). Here Chla values were integrated in the photic layer as a proxy for phytoplankton biomass. In addition, the vertical extent of phytoplankton was estimated by the depth of the chlorophyll maximum (DCM). The stratification of the upper water column at each station, a proxy for nutrient supply from deep waters, was estimated primarily by the Brunt-Väisälä frequency (N^2) values computed at 1 m intervals for the upper 200 m. Various estimates were employed: the mean (N^2m) and standard deviation (N^2sd) of Brunt-Väisälä values, and the mixing layer depth (MLD), the latter estimated using a density difference criterion ($\Delta\delta$ = 0.25 kg m^{-3}). Additional details on the sampling and on the analytical methods employed can be found in Moreno-Ostos (2012).

2.2.3 Satellite observations

As the observed plankton properties (i.e. biomass, stable isotopes) at each station would be the consequence not only of local conditions but also of general oceanographic conditions prevailing over a certain amount of space and time, additional variables, estimated from satellite observations, were also considered. Annual averages of primary production for 2010 (PP, mg C m^{-2} d^{-1}) were generated by averaging monthly data for

primary production downloaded from the Ocean Productivity website (http://www.science.oregonstate.edu/ocean.productivity/index.php) from the grid (0.17° x 0.17°) closest to each station position.

Dust deposition, as a proxy for atmospheric inputs of key nutrients for primary production (e.g. Fe or P), was also estimated from Aqua-MODIS Aerosol Optical Depth at 550 nm and Aerosol Small Mode Fraction data provided by the Giovanni online data system (NASA Goddard Earth Sciences). These data were retrieved from a grid of 1º resolution and centered at the closest location to each station and combined with wind speed derived from AVISO (http://las.aviso.oceanobs.com/las/getUI.do) to estimate the monthly average atmospheric dust column concentration (MDU, g m^{-2}) at each station (Kaufman *et al.* 2005).

2.2.4 Creation of size spectra

Biomass and $\delta^{15}N$ values by size fractions of plankton were combined in the form of linear regressions with the median size of individuals in the corresponding size fraction (Rodriguez and Mullin 1986). Logarithmic transformations (log_2) and normalization by the width of the interval of individual biomass were applied to obtain functions independent on size-class width (Blanco et al. 1994, Zhou 2006). The resulting lines (obtained by least squares regression) characterized the size distribution of biomass or $\delta^{15}N$ by two parameters: the intercept (C_a, $\delta^{15}N_a$), a proxy for the values in the first size class, and the slope (C_b, $\delta^{15}N_b$), indicative of the rate of change in the values with increasing individual size (Fig. 2). In addition the slope of the biomass spectra allowed for an estimation of the maximum number of trophic levels (TLC) at each station using the model of Zhou (2006). It must be noted that the $\delta^{15}N$ spectrum is equivalent to a biomass spectrum where the distribution of biomass is replaced by $\delta^{15}N$ values. These spectra cannot be considered a mere artifact of the normalization procedure as both biomass and $\delta^{15}N$ show a natural asymmetric tendency with organism size (Blanco et al. 1994).

2.2.5 Spatial analysis

Stations were first grouped by biomes (Longhurst 2007) (Fig. 2.2) to investigate large scale differences in biomass and spectral parameters using ANOVA and Dunnett-C post-hoc tests. Visualization and further analysis of trends at various spatial scales were made using the tools provided by the package Spatial Analysis in Macroecology (SAM V 4.0) which allowed mapping and computation of various spatial statistics from metrics descriptive of spatial autocorrelation to advanced spatial regression (Rangel *et al.* 2010).

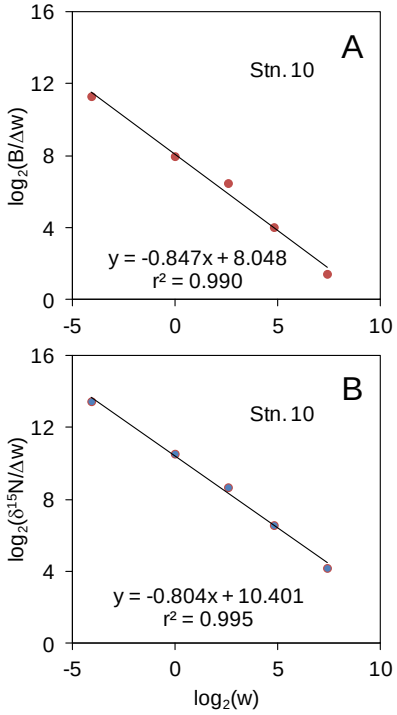

Figure 2.2 Examples of size spectra of carbon biomass (A) and $\delta^{15}N$ for plankton samples collected at Stn. 10 of the Malaspina-2010 expedition. The least squares regression parameters for each spectrum are shown.

Spatial autocorrelation of variables was analyzed by means of correlograms of the Moran's I coefficient computed for groups of samples (stations) of increasing spatial distance. The relationships between spectral parameters and environmental variables were investigated using principal components analysis (PCA) to determine the main correlations. Then, we selected the most representative environmental variables to construct regression models explanatory of each of the spectral parameters at each station using Geographically Weighted Regression (GWR). This method computed a

series of local regressions, one for each station location, between the independent variable (i.e. spectral parameters) and the explanatory variables (i.e. selected environmental variables) taking into account the information from the surrounding stations weighted by a spatial function. In this case we used a moving spatial window with an adaptive Kernel of 15% of neighboring stations optimized using the Akaike's Information Criterion. The explanatory power of GWR (r^2) for each spectral parameter was in general higher than those of ordinary least squares, as measured by ANOVA tests (Rangel *et al.* 2011).

2.3 RESULTS

2.3.1 Spatial distributions

Biomass and $\delta^{15}N$ showed an uneven distribution across the ocean as illustrated by the maps for the 40-200 µm size-class (Fig. 2.3).

In contrast, the values for the parameters characterizing biomass and $\delta^{15}N$ spectra showed in general low variability, resulting in homogeneous distribution at large spatial scales (Fig. 2.4). An exception was the Subtropical North Atlantic region, mostly in the Westerlies biome. Here, the intercepts for both biomass and $\delta^{15}N$ spectra (C_a and $\delta^{15}N_a$) were lower, and the slope for the biomass spectra (C_b) steeper, than in other regions, while the values of the $\delta^{15}N$ spectra ($\delta^{15}N_b$) were flatter in this region. This pattern is a response to the low values measured in the 40-200 µm size-class (Fig. 2.3).

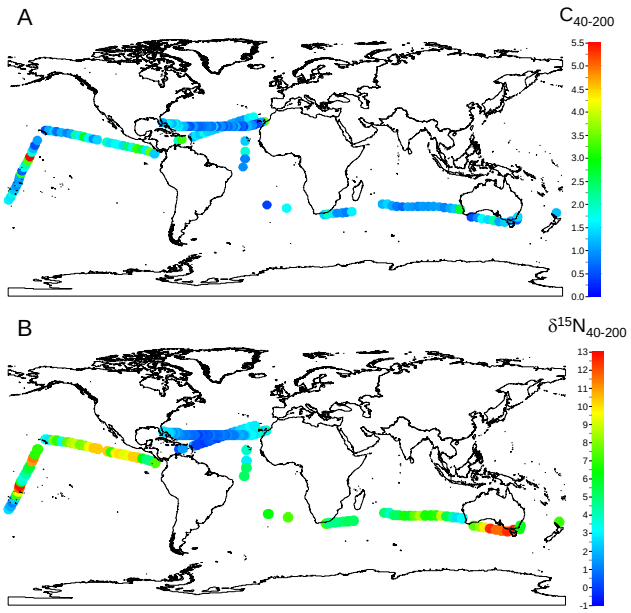

Figure 2.3 Distribution of plankton biomass (A, $C_{40\text{-}200}$, mg C m^{-3}) and $\delta^{15}N$ (B, $\delta^{15}N_{40\text{-}200}$, ‰) in the 40-200 µm size fraction for stations sampled during the Malaspina-2010 expedition.

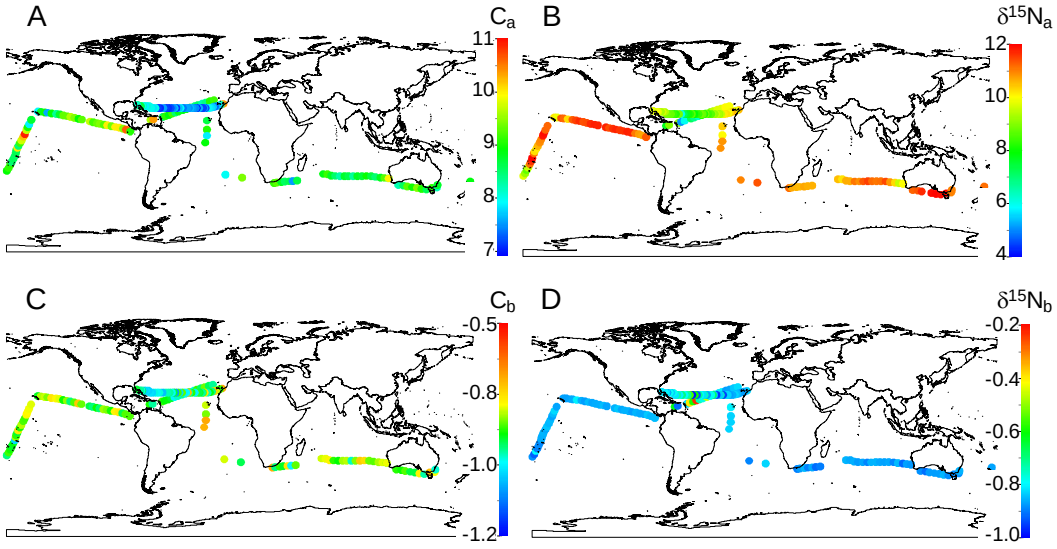

Figure 2.4 Distribution of parameters of biomass (A, C) and $\delta^{15}N$ (B, D) plankton size spectra computed for stations sampled during the Malaspina-2010 expedition. A, B: intercept of biomass (C_a) and $\delta^{15}N$ ($\delta^{15}N_a$) spectra, respectively. C, D: slope of biomass (C_b) and $\delta^{15}N$ ($\delta^{15}N_b$) spectra, respectively.

When grouped by biomes, the differences were significant in the Westerlies (including the southern Australia region) for all parameters and variables, except for $\delta^{15}N_b$ (Table 2.1). Thus, the lower biomass and $\delta^{15}N$ values at the base of the planktonic food web in the Westerlies biome ($C_{40\text{-}200}$ and $\delta^{15}N_{40\text{-}200}$) affected the food web structure represented by size spectra.

Table 2.1 Mean±se values of the parameters of the size spectra for biomass (C_a: intercept; C_b: slope) and $\delta^{15}N$ ($\delta^{15}N_a$: intercept; $\delta^{15}N_b$: slope) and of carbon biomass ($C_{40\text{-}200}$, mg C m^{-3}) and $\delta^{15}N$ ($\delta^{15}N_{40\text{-}200}$, ‰) for the 40-200 µm size class in the three biomass sampled. Means not significantly different are marked in the same group (ANOVA and Dunnett-C test, P<0.05). n: number of data.

variable	biome	mean	se	n	group
C_a	Coastal	8.961	0.071	79	a
	Westerlies	7.959	0.093	56	b
	Trades	8.902	0.171	10	a
C_b	Coastal	-0.884	0.006	79	a
	Westerlies	-0.940	0.009	56	b
	Trades	-0.868	0.023	10	a
$\delta^{15}N_a$	Coastal	10.444	0.183	79	a
	Westerlies	9.444	0.163	56	b
	Trades	10.589	0.230	10	a
$\delta^{15}N_b$	Coastal	-0.832	0.012	79	a
	Westerlies	-0.824	0.010	56	a
	Trades	-0.867	0.008	10	a
$C_{40\text{-}200}$	Coastal	1.491	0.090	79	a
	Westerlies	1.073	0.068	56	b
	Trades	1.395	0.193	10	a
$\delta^{15}N_{40\text{-}200}$	Coastal	5.810	0.365	79	a
	Westerlies	2.808	0.423	56	b
	Trades	5.324	0.949	10	a

The distribution of primary production was also quite homogeneous across the ocean sampled, while variables indicative of different nutrient inputs, as the abundance of *Trichodesmium*, the atmospheric dust deposition or the depth of the chlorophyll maximum were more heterogeneously distributed (Fig. 2.5). *Trichodesmium* abundance reached maximum values in the North Atlantic but it was almost undetected in the rest of regions, with the exception of a few stations close to New Zealand and to South Africa. Relatively high dust deposition was estimated in the North Pacific and in the

North Atlantic, but in the latter there were large differences between the values corresponding to different cruises in this region. For instance, the largest dust deposition corresponded to the spring-summer cruise and the lowest to the winter cruise. In turn, the chlorophyll maximum was deepest in the central regions of all ocean basins. The distribution of other variables as the depth of the mixing layer or the Brunt-Väisälä frequency had also variability at intermediate and large scales (Fig. 2.6) but otherwise they were significantly correlated with the depth of the chlorophyll maximum (Fig. 2.5).

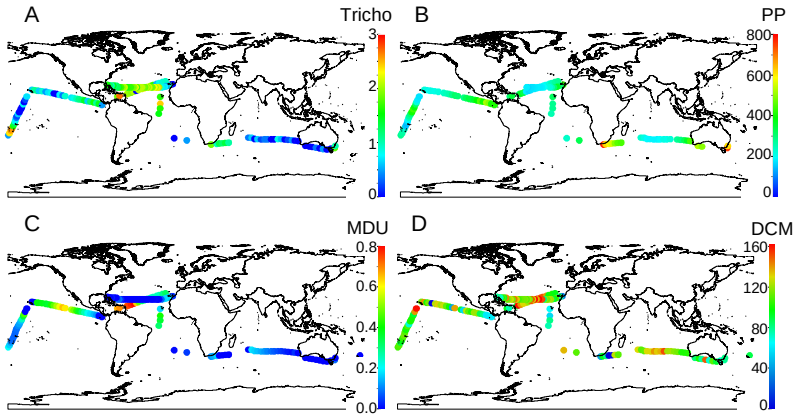

Figure 2.5 Distribution of *Trichodesmium* abundance (A, Tricho, log trichomes m^{-3}), primary production (B, PP, mg C m^{-2} d^{-1}), mean dust deposition (C, MDU, g m^{-2}) and depth of the chlorophyll maximum (D, DCM, m) measured or estimated for stations sampled during the Malaspina-2010 expedition.

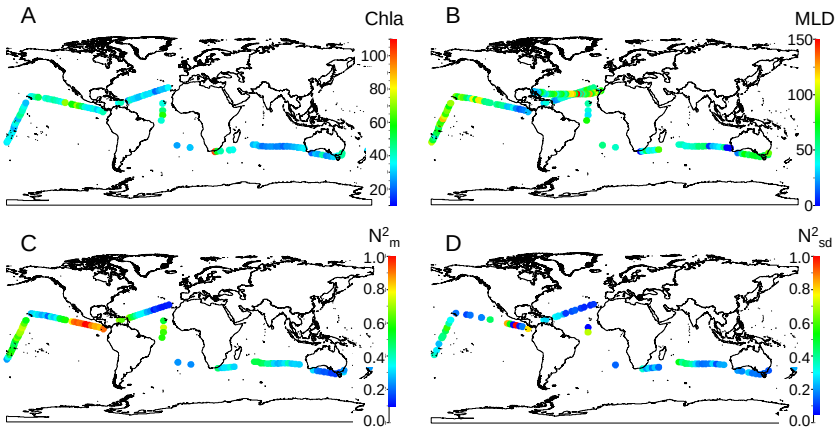

Figure 2.6 Distribution of integrated chlorophyll-a concentration (A, Chla, mg m^{-2}), mixed layer depth (B, MLD, m), mean Brunt-Väisälä frequency (C, N^2_m, s^{-2},*10^-4), and standard deviation of the Brunt-Väisälä frequency (D, N^2_{sd}, s^{-2},*10^-4) measured for stations sampled during the Malaspina-2010 expedition.

2.3.2 Correlations of spectral and other variables

We found correlation between intercept and slope within each spectrum type (biomass or $\delta^{15}N$), and between the intercepts and the values measured at the smallest size class (40-200 µm), as expected. There was also a negative correlation between the slopes of biomass and $\delta^{15}N$ spectra (Table 2.S1).

Table 2.S1 Correlations between spectral parameters and environmental variables. C_a and C_b: intercept and slope of the biomass spectra, respectively; $\delta^{15}N_a$ and $\delta^{15}N_b$: intercept and slope of the $\delta^{15}N$ spectra, respectively; $C_{40\text{-}200}$ and $\delta^{15}N_{40\text{-}200}$: biomass and $\delta^{15}N$ of the 40-200 µm size-class, respectively; Tricho: *Trichodesmium* abundance; Chla: integrated chlorophyll concentration; DCM: depth of the chlorophyll maximum; MLD: mixing layer depth; N^2_m and N^2_{sd} mean and standard deviation of Brunt-Väisälä frequency, respectively; MDU: mean dust deposition; PP: primary production. *: P<0.05; **: P<0.01; ***: P<0.001.

	C_a	C_b	$\delta^{15}N_a$	$\delta^{15}N_b$
C_a	1.000	---	---	---
C_b	0.324 *	1.000	---	---
$\delta^{15}N_a$	0.393 *	0.411 *	1.000	---
$\delta^{15}N_b$	-0.091	-0.212 ***	-0.699 *	1.000
$C_{40\text{-}200}$	0.813 *	0.008	0.161	-0.016
$\delta^{15}N_{40\text{-}200}$	0.469 *	0.395 *	0.866 *	-0.424 *
Tricho	-0.024	-0.157	-0.290 *	0.079
Chla	0.203 ***	0.110	0.145	-0.046
DCM	-0.357	-0.240 **	-0.329 *	0.234 **
MLD	-0.218 ***	0.010	0.062	-0.155
N^2_m	0.470 *	0.127	0.111	0.042
N^2_{sd}	0.123	0.197	0.094	-0.048
MDU	0.487 *	0.153	-0.049	0.301 **
PP	0.301 **	0.174	0.258 **	-0.169

All these correlations can be characterized by the angle between vectors in the space of the two main components of the PCA (Fig. 2.7). It should be highlighted the negative correlations between $\delta^{15}N_a$ and the depth of the chlorophyll maximum (DCM) and the abundance of *Trichodesmium*, and the positive correlations between C_a and chlorophyll and primary production. In turn, $\delta^{15}N_b$ showed positive correlation with mean dust deposition and DCM depth while C_b was negatively correlated with DCM depth.

Interestingly, DCM depth can be considered an index of nutrient inputs of nutrients across the thermocline, as it was negatively correlated with chlorophyll-a, primary production, biomass and $\delta^{15}N$ of the 40-200 µm size-class and positively correlated with the depth of the mixing layer.

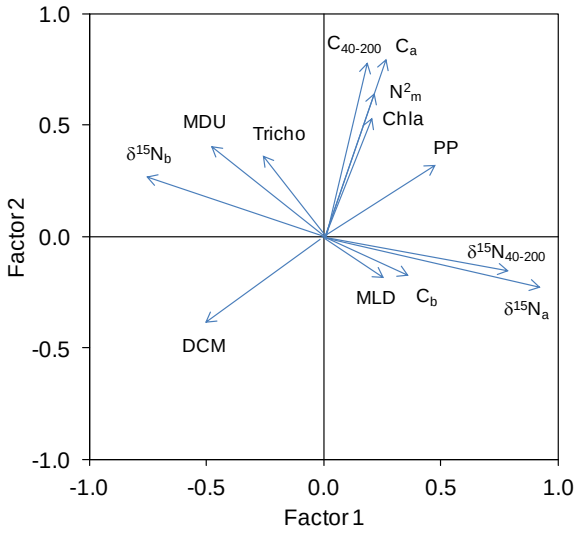

Figure 2.7 Vectors of variables projected in the space of the two main components of the PCA. The angle between vectors is proportional to their correlation (minimum correlation for 90º). C_a and C_b: intercept and slope of the biomass spectra, respectively; $\delta^{15}N_a$ and $\delta^{15}N_b$: intercept and slope of the $\delta^{15}N$ spectra, respectively; PP: primary production; Tricho: *Trichodesmium* abundance; MDU: mean dust deposition; MLD: mixed layer depth; DCM: depth of the chlorophyll maximum; $C_{40\text{-}200}$ and $\delta^{15}N_{40\text{-}200}$: biomass and $\delta^{15}N$ of the 40-200 µm size-class, respectively; Chla: chlorophyll concentration; N^2_m: mean Brunt-Väisälä frequency

2.3.3 Geographically weighted regression models

From the global correlation and PCA results we selected the independent variables DCM (as an index of the vertical transport of nutrients across the thermocline), *Trichodesmium* abundance (as a proxy for atmospheric nitrogen fixation) and MDU (as a proxy for other atmospheric nitrogen inputs) to construct regression models explanatory of the spectral parameters (C_a, C_b, $\delta^{15}N_a$, $\delta^{15}N_b$) at local scale using GWR models (Table 2.2).

Table 2.2. Median, minimum (min) and maximum (max) values of GWR coefficients for spectral parameters (C_a, $\delta^{15}N_a$ and $\delta^{15}N_b$) estimated from logarithmic *Trichodesmium* abundance (Tricho, log trichomes m^{-3}), depth of chlorophyll maximum (DCM, m) and mean monthly dust deposition (g m^{-2} $month^{-1}$). All models were fit with P<0.05 and include a constant term. Values from models for C_b were not shown as GWR was not significant for this variable.

	constant			Tricho			DCM			MDU		
Dependent	median	min	max	median	min	max	median	min	max	median	min	max
C_a	9.162	5.842	12.391	0.001	-0.036	0.058	-0.002	-0.032	0.004	0.327	-5.685	7.134
$\delta^{15}N_a$	11.194	7.444	15.799	-0.004	-0.076	0.063	-0.002	-0.045	0.029	-2.416	-20.418	3.535
$\delta^{15}N_b$	-0.879	-1.473	-0.722	0.000	-0.003	0.004	0.000	-0.001	0.004	0.089	-0.088	0.682

Most GWR models were significant (P<0.001) and only the GWR model for C_b resulted non significant (P=0.070) and with less explanatory power than ordinary least squares (the latter with a relative decrease of 33.035 in the Akaike Information Criterion). The spatial autocorrelation of spectral variables and residuals indicated by Moran's I values (Fig. 2.8) showed that the spatial structure was better represented at all scales in the case of $\delta^{15}N$ models, with lower residuals than models for biomass.

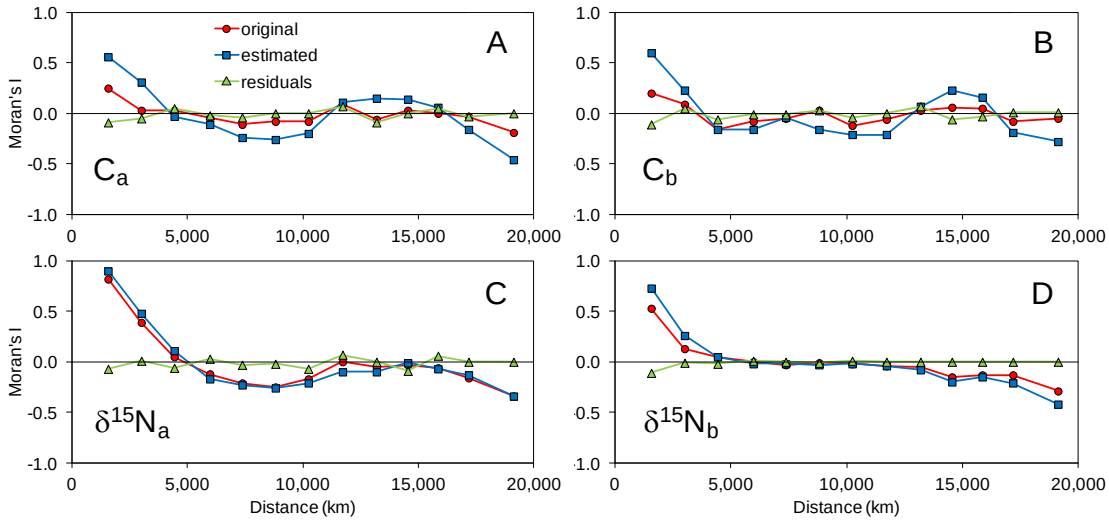

Figure 2.8 Spatial autocorrelation (Moran's I) for spectral parameters estimated by GWR at different spatial scales. C_a and C_b: intercept and slope of the biomass spectra, respectively; $\delta^{15}N_a$ and $\delta^{15}N_b$: intercept and slope of the $\delta^{15}N$ spectra, respectively.

The median values of the model parameters indicated that the distribution of the dependent variables was determined mainly by a constant value modulated by the

coefficients of the independent variables (Table 2.2). *Trichodesmium* abundance and DCM had marginal influence in general (median values close to 0) but their range of values indicated some local influence. In contrast, median values for MDU coefficients ranked second after the constant values, suggesting that this variable was important for determining the shape of $\delta^{15}N$ spectra and the biomass at the base of the food web (C_a).

2.3.4 Trophic structure

The maximum number of trophic levels (TLC) estimated from C_b ranged from 2.26 to 3.24 (mean = 2.70) over the whole transect, with a distribution (not shown) matching that of C_b (Fig. 2.3C). However, there was no clear correspondence between TLC (or C_b) and the slope of the $\delta^{15}N$ spectra (Fig. 2.3C and D), despite the significant correlations between C_b and the parameters of the $\delta^{15}N$ spectra in the whole data set (Table 2.S1). When corrected for spatial autocorrelation C_b and $\delta^{15}N_b$ were not significantly correlated ($r = -0.203$, $P = 0.213$, $n = 145$).

2.4 DISCUSSION

The results of this study showed that the size spectra of stable nitrogen isotopes added a new dimension to characterize the structure of planktonic food webs across the ocean. The parameters defining the size spectrum at each particular region were correlated with the main sources of nutrients for primary production, as the vertical transport across the thermocline and atmospheric inputs. Thus, the use of stable isotope spectra complement the information provided by biomass spectra, which were already established as an useful tool for describing and modeling the structure and function of pelagic ecosystems (Rodriguez & Mullin 1986, Quiñones *et al.* 2003, San Martin *et al.* 2006, Zhou 2006). The large number of observations collected across three ocean basins in our study suggests that the resulting relationships are of general application to all pelagic environments.

2.4.1 Methodological considerations

Our samples do not cover all possible oceanic conditions as most of the observations were made in central areas of the ocean. Also, temporal variability (e.g. seasonality) was not taken into account for most regions. However, three of the four ocean biomes were covered (only the polar biome was not included in the study), and the range of primary production values was representative of most of the open ocean, even including some data from the most productive upwelling regions (e.g. Benguela). In addition, the observations across the subtropical N Atlantic were made in two different seasons (winter-early spring and late spring-summer) thus providing some hints for temporal variability, at least in this region. In agreement with the low relative importance of seasonality for plankton in the tropical and subtropical ocean (Longhurst 2007), we found small differences in size-spectra in this region, even when there were large differences in dust inputs between the transects made in different seasons (Fig. 2.4C). Short term variability (e.g. those caused by nycthemeral migrations of plankton between deep and surface layers) is likely to have little effect on our results as the sampling was conducted approximately at the same period of the day through all cruises.

The range of individual sizes considered could also influence the estimation of the parameters of the spectra (Blanco et al. 1994). Although we employed only 5 discrete size-classes to construct the spectra, they included organisms ranging 10 ng C and 0.5 mg C individual weight (i.e. four orders of magnitude). An inspection of some of the samples collected indicated a good representation of phytoplankton (including diatoms, dinoflagellates, phytoflagellates and cyanobacteria), and zooplankton filter-feeders and predators (Mompeán *et al.* 2013). This wide range of organisms, along with the robust character of log-normalized size-spectra that allows for estimations of properties of the plankton community outside the actual range of fitted sizes (Blanco *et al.* 1994), supports the validity of our estimations for the plankton food web. In the case of δ^{15}N, previous studies showed that the spectra are continuous across the whole pelagic food web (Jennings *et al.* 2001, Bode *et al.* 2003).

2.4.2 Size-spectra and nutrient sources

When considering biome scales, our results show large homogeneity in the parameters defining the size spectra. Only the Westerlies biomes resulted with lower values of intercept and slope in the biomass spectra, and also lower intercept values of the δ^{15}N spectra, than those computed for the Trades and Coastal biomes. Such homogeneity in the size structure of the open ocean plankton can be interpreted in terms of the main controls of the productivity. For most ocean regions, primary production is controlled by the availability of nutrients in the photic layer, and the main inputs depend on eddy diffusion through the pycnocline or advection of subsurface waters (Longhurst 2007). This is likely the main fertilization mechanism for the Trades and Coastal biomes, including productive upwelling in equatorial and coastal waters, while most of the subtropical oceans are occupied by oligotrophic gyres with reduced nutrient inputs and productivity (Behrenfeld *et al.* 2006). Nevertheless, various mechanisms can provide local nutrient inputs, as mesoscale turbulence (Oschlies & Garçon 1998), lateral transport from productive regions (Torres-Valdés *et al.* 2009), atmospheric deposition (Duce *et al.* 2008) and atmospheric nitrogen fixation (Capone *et al.*, 2005). Notably, the subtropical N Atlantic is a region within the Westerlies biome where the fixation of nitrogen by diazotrophic plankton is favored by the deposition of Fe and phosphorus in dust particles (Capone *et al.* 2005, Moore *et al.* 2009, Fernández *et al.* 2010). Notwithstanding the relative small fixation rates recorded in tropical and subtropical waters of the S Atlantic, they also contributed to a large fraction of total nitrogen inputs when compared to those from eddy diffusion (Mouriño-Carballido *et al.* 2011).

Our results in this study will show the importance of these fertilization mechanisms translates in a significant correlation between indices of the different nutrient sources and plankton biomass structure, the latter represented in this study by the parameters of the size spectra. Nutrient inputs from subsurface waters are indicated by the DCM, the layer where phytoplankton biomass accumulates because the growth is maximized by a compromise between light levels (from above) and nutrient inputs (from below). The DCM, generally, is deeper when advection is low (e.g. at the centre of oligotrophic gyres) and shallower when is high (e.g. near upwelling areas), also could be affected for

season of the year. Studies of DCM distribution across the ocean have revealed that DCM is a better proxy for productivity than instantaneous nutrient concentrations in the surface layer (Mouriño *et al.* 2004). In the case of the atmospheric inputs, nitrogen fixation was generally associated to the abundance of the colony-forming *Trichodesmium*, although unicellular diazotrophs may be also important (Luo *et al.* 2012). Dust deposition favors nitrogen fixation, as it provides the necessary additional micronutrients (Moore *et al.* 2009, Fernández *et al.* 2010), but also it introduces significant nitrogen and phosphorus amounts (Morin *et al.* 2009). Thus, the main nutrient inputs can be used to model the biomass at the base of the food web, represented in our model by C_a. However, these mechanisms were not enough to predict the slope of the biomass spectrum, a classical proxy for food web structure (Zhou 2006).

To our knowledge, this study represents the first application of biomass-normalized size spectra using stable isotopes. Previous studies of $\delta^{15}N$ in different size classes of plankton failed to demonstrate a regular increase in $\delta^{15}N$ with size, as there were many exceptions both at large and small organism sizes (Fry & Quiñones 1994, Rolff 2000, Bode *et al.* 2003, Landrum *et al.* 2011, Mompeán *et al.* 2013). However, the increase in $\delta^{15}N$ with organism size is a general rule in pelagic food webs (Jennings *et al.* 2001, Bode *et al.* 2003, Jennings *et al.* 2008). The planktonic exception can be attributed to the presence of large herbivores (as the colony-forming salps) but also the different turnover times of biomass in organisms of different size (Jennings *et al.*, 2008). Besides, there are evidences of a small enrichment in $\delta^{15}N$ in the microbial food web (Rau *et al.* 1990, Fawcett *et al.* 2011). The normalization procedure applied in this study overcomes the lack of a regular increase in each local spectrum. As for biomass spectra, the normalized spectrum allows for a synthetic representation of the continuous variability of the variable of interest across organism sizes (Blanco *et al.* 1994, Zhou, 2006). In this case, changes in the intercept of the spectrum are related to the dominant source of nitrogen for primary producers while changes in the slope are indicative of the overall isotopic fractionation along the food web. Lower intercepts are thus expected in areas with significant atmospheric inputs, either by diazotrophy or by inputs of anthropogenic nitrogen, as these sources have $\delta^{15}N$ values near zero (Morin *et al.* 2009). This was the

case of our observations in the Westerlies biome that had lower $\delta^{15}N_a$ than the Trades and Coastal biomes (Table 2.1), in agreement with the large importance of atmospheric nitrogen fixation in the former. In turn, variations in the $\delta^{15}N$ spectrum slope would indicate changes in the rate of transfer of nitrogen across trophic level. However, we did not find significant differences in $\delta^{15}N_b$ between biomes and the detailed distribution of this parameter was very homogeneous across the ocean (Fig. 2.2D). This suggests that the transfer of nitrogen up the plankton food web proceeds at similar rates in all ocean regions. Both the intercept and the slope of the $\delta^{15}N$ spectrum can be estimated from the major nitrogen sources, thus providing a tool for testing the dominant sources at local or regional scales.

2.4.3 Size-spectra and trophic structure

In this study, the two approaches employed to characterize the structure of the planktonic food web (size-spectra of planktonic C biomass or $\delta^{15}N$) produced different results. The biomass size-spectra varied across large ocean regions (biomes) and also at small spatial scales. The biomass of primary producers, indicated by the intercept of the biomass spectrum, influenced the structure of the entire food web, indicated by the slope of the biomass spectrum. In the Westerlies biome, the low biomass at the base of the food web was associated to spectra with flatter slopes than those obtained for Coastal and Trades biomes, the latter with higher biomass values. These results agree with previous studies of planktonic size-spectra in various regions of the ocean (Rodriguez & Mullin 1986, Piontkovski *et al.* 2003, Quiñones *et al.* 2003, San Martin *et al.* 2006). On the other hand, while the intercept of the $\delta^{15}N$ and the biomass spectra showed similar variability, the $\delta^{15}N$ slope was more homogeneous than the biomass slope. Notably, all the biomes showed a similar $\delta^{15}N$ slope, suggesting that the trophic structure of plankton described by nitrogen isotopes was constant across the ocean. These results imply that either the biomass structure is not solely due to trophic processes or that $\delta^{15}N$ variability does not reflect the trophic structure because of variations in the isotopic fractionation between trophic levels.

The interpretation of the biomass spectra is conditioned by trophic assimilation efficiency and the dynamic state of the community. When in steady state, the slope of

the biomass size spectrum of a planktonic community is determined by the biomass fluxes leading to individual growth, losses by mortality, and transfers to upper trophic levels (Platt & Denman 1978). Thus it can be related to the assimilation efficiency and to the number of trophic levels (Zhou 2006). By considering a fixed mean efficiency of 70%, typical of copepod grazers, other studies have estimated the number of trophic levels of various planktonic communities (Zhou *et al.* 2009; Basedow *et al.* 2010), as in our study. Using this approach boreal plankton result with up to 5.6 trophic levels (Basedow *et al.* 2010) while in our study in temperate and tropical biomes only 3.2 trophic levels were reached. This result implies that boreal communities can support more trophic levels because they have more biomass of primary producers and more efficient transmission of energy through the size spectrum (i.e. flatter slopes) than temperate and tropical communities. However, assimilation efficiencies are dependent, among other factors, on the type of food consumed, and may be higher for predators than for grazers (Mauchline 1998). Modeling studies have pointed out that not all size-dependent physiological processes (e.g. respiration, grazing) affected in the same way the slope on the biomass spectrum, as the interaction of the various biological processes can lead to complex changes in the planktonic size spectra (Poulin & Franks 2010). In addition, it is possible that not all the sampled plankton communities were in steady state. We have found variability in the spectra and other variables in the North Atlantic between different transects, even when most samples were obtained in tropical and subtropical areas of the ocean with low seasonality (Longhurst 2007). Therefore, both the uncertainty in assimilation efficiency and the presence of communities out from steady state limit the application of the use of biomass spectra to estimate trophic structure.

The low variability observed in $\delta^{15}N$ slopes suggests that the overall trophic structure is similar across different planktonic communities, even when there are large variations in the nutrient sources and in the transmission of the energy through the food web. Organisms included in our spectra covered four orders of magnitude in individual size, implying a large difference in growth rates and biomass turnover times, and thus larger variability in $\delta^{15}N$ composition of the small plankton versus large plankton (Jennings *et al.* 2008). Such differences explain the reported delays in the transmission of the $\delta^{15}N$ signal from the N source (e.g. atmospheric N) to zooplankton predators (Rolff 2000,

Mompeán *et al.* 2013). The normalization procedure applied in our study overcomes the time-lags and local variability of $\delta^{15}N$ and, as normalized biomass spectra, provides a better tool to compare different communities than unnormalized spectra. Similarity in $\delta^{15}N$ spectrum slopes and trophic structure across communities can be expected if the underlying physiological processes are similar. Even when the nutrient (nitrogen) sources are different the transmission up the food web is made by similar biochemical reactions for all planktonic communities. In the case of tropical and subtropical communities living in oligotrophic waters, recycling of dissolved organic matter through microbial communities is an important mechanism for transmission of nitrogen to upper trophic levels (Mulholland 2007). In the case of boreal communities, the transmission is mainly through grazing and predation by large organisms (Basedow *et al.* 2010). By taking into account that the processes leading to nutrient acquisition (uptake by phytoplankton, grazing by herbivores and predation by carnivores) and loss (excretion) are size-dependent (Poulin & Franks 2010), there is no reason to expect that boreal ecosystems have more trophic levels than subtropical ones, as the latter have most of the energy and nutrient exchanges in microbial components at different trophic levels. The homogeneity in trophic structure across pelagic ecosystems was already suggested by the first studies of biomass and abundance size spectra (Platt and Denman 1978). Now, the application of normalized $\delta^{15}N$ spectra to whole food webs, including fish and upper consumers, can be used to analyze their variability by considering nitrogen exchanges.

CAPÍTULO 3:

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

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 (δ^{15}N<1‰) in the central zone, and were inversely correlated to carbon stable isotopes (δ^{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.

3.1 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 (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 & Letelier 2008).

A variety of physical mechanisms are known to contribute to nitrogen inputs in oligotrophic gyres, including mesoscale and submesoscale turbulence (Oschlies & Garçon 1998), lateral transport from other regions (Williams & Follows 1998, Torres-Valdés 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 & 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. 2011). 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 & 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 & McGillicuddy 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, Montoya et al. 2002, Mino 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 (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 (Montoya et al. 2002, Mino 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 & McGillicuddy 2006, Fernández et al. 2010). Direct measurements revealed significant N_2 fixation also in the eastern subtropical Atlantic (Wannicke et al. 2010, Fernández et al. 2010, 2012, Benavides et al. 2011), 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.

3.2 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. 3.1.). The transect was arbitrarily divided in eastern, central, and western zones to summarize its oceanographic and plankton characteristics.

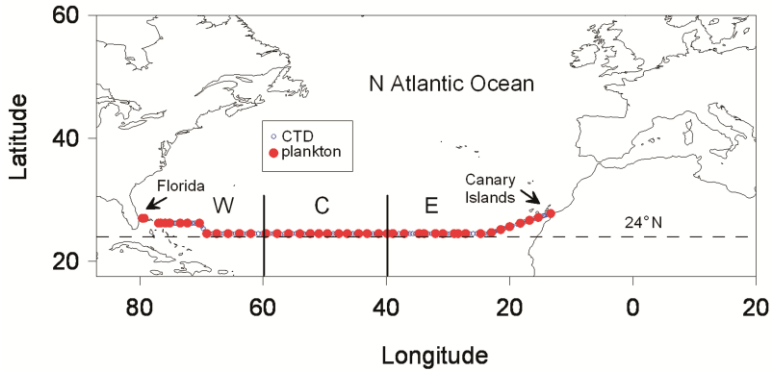

Figure 3.1 CTD and plankton sampling stations during cruise Leg 8 of Malaspina-2010 expedition along 24 °N (dashed line). The vertical lines indicate the limits of the eastern (E), central (C) and western (W) regions described in the text.

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 et 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.

3.3 RESULTS

3.3.1 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. 3.2). 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.

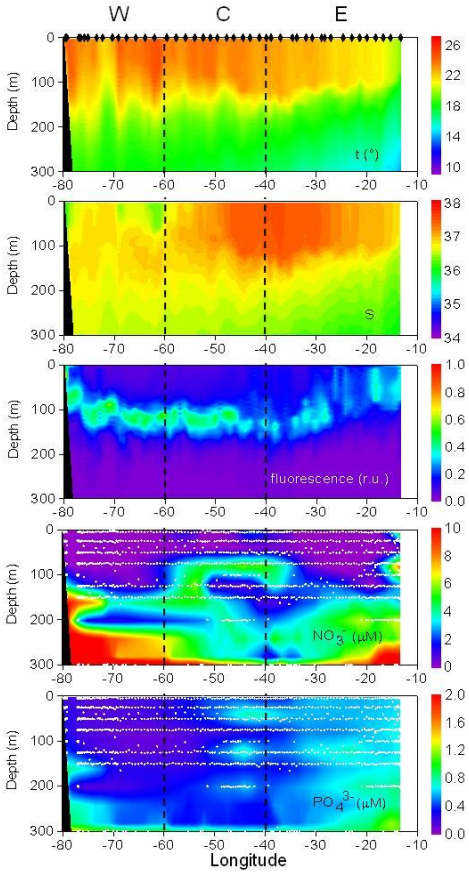

Figure 3.2 Temperature, salinity, in vivo fluorescence, NO_3^-, and PO_4^{3-} in the upper 300 m along 24 ºN. CTD stations are indicated in the upper panel. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

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. 3.2). 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.

3.3.2 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. 3.3). 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 3.1). There were significant differences in mean values of total plankton biomass, with the lowest value central zone, and the highest in the western zone.

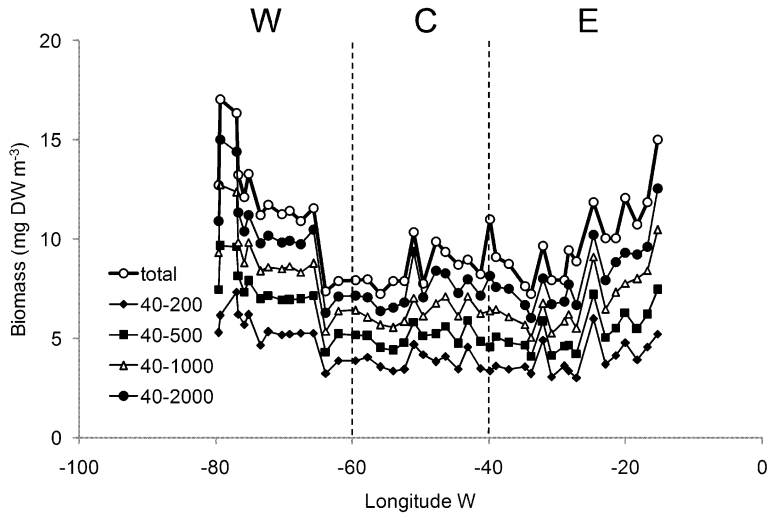

Figure 3.3 Accumulated biomass (mg dry weight m^{-3}) of size fractionated plankton along 24 ^{o}N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text

Table 3.1 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

Microzooplankton abundance was similar in all zones while phytoplankton was significantly more abundant in the western zone (Table 3.2). 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 3.2). 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.

Table 3.2 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).

		zone		
Group	**Taxa**	**W**	**C**	**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

The spatial variability in nitrogen isotopes was similar to the pattern described for biomass (Fig. 3.4), 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 3.1). 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.

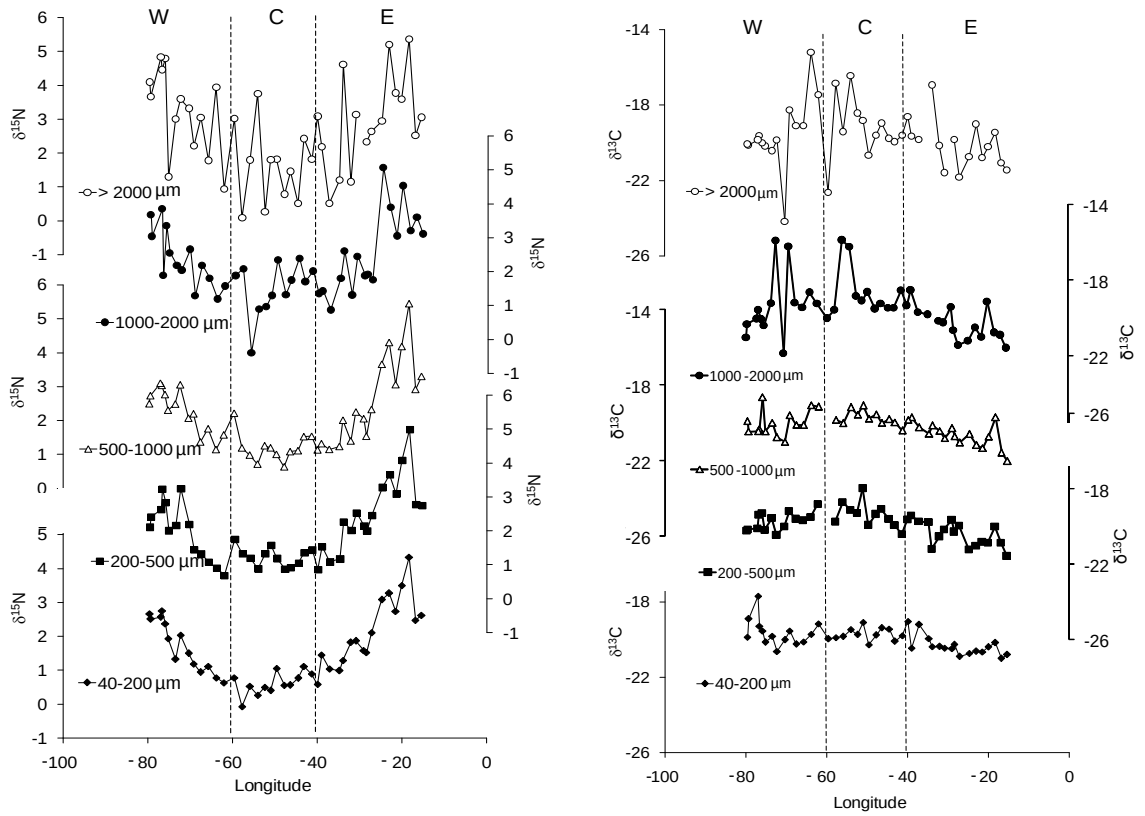

Figure 3.4 and 3.5 Natural abundance of stable nitrogen and carbon isotopes ($\delta^{15}N$, $\delta^{13}C$, ‰) of size fractionated plankton along 24 °N. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

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. 3.5). 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 3.1). Biomass was only significantly correlated with $\delta^{13}C$ for 200-500 and 500-1000 µm classes.

3.3.3 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. 3.6). 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.

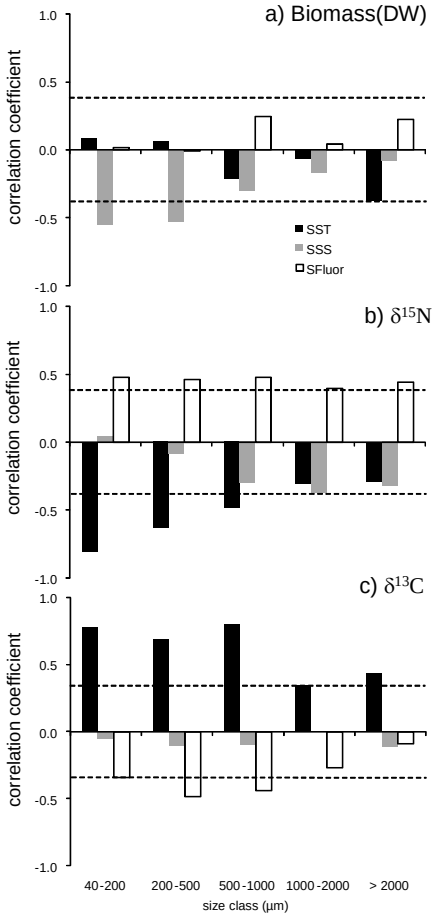

Figure 3.6. Correlation coefficients (Pearson r) between size fractionated plankton: a) biomass (mg DW m-3), b) $\delta^{15}N$ or c) $\delta^{13}C$ of and sea surface temperature (SST, ^{o}C), salinity (SSS) or *in vivo* fluorescence (Sfluor) along 24 ^{o}N. The dashed lines indicate the significance value (P<0.05).

3.3.4 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. 3.7). 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).

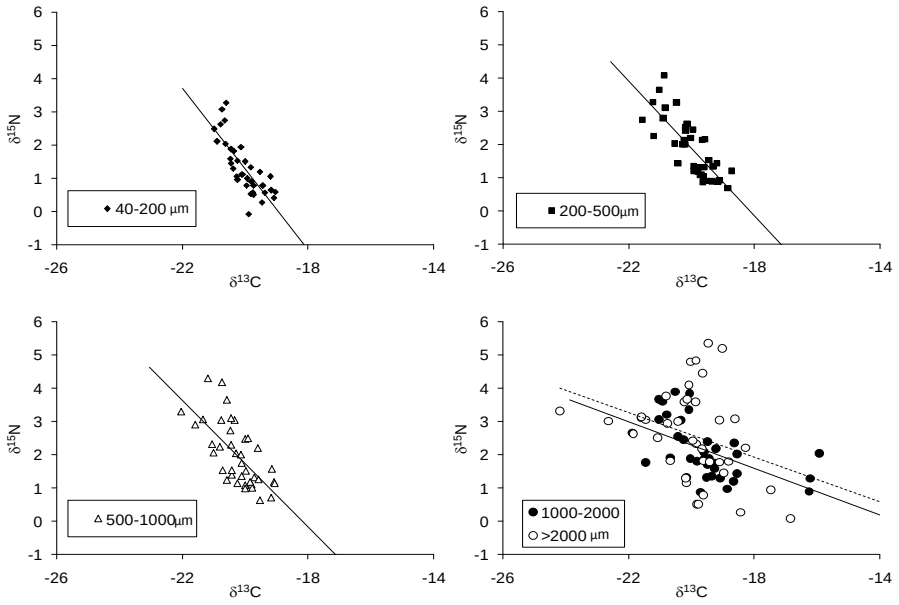

Figure 3.7 Relationships between $\delta^{15}N$ and $\delta^{13}C$ for size fractionated plankton (µm). All regression lines are significant with P<0.01 except for the >2000 µm fraction (dashed line, P<0.05).

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

With the exception of the two easternmost stations, *Trichodesmium* was recorded at all stations of the transect (Fig. 3.8). 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).

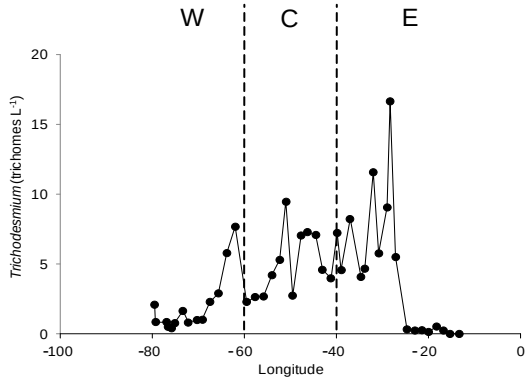

Figure 3.8 Abundance of *Trichodesmium* (trichomes L^{-1}) along 24 ºN. The dashed lines indicate the limits between eastern (E), central (C) and western (W) zones described in the text.

A negative linear relationship was found between $\delta^{15}N$ and log-transformed *Trichodesmium* abundance for all size-classes (Fig. 3.9). 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 classes 200-1000 µm (ANCOVA, P<0.05).

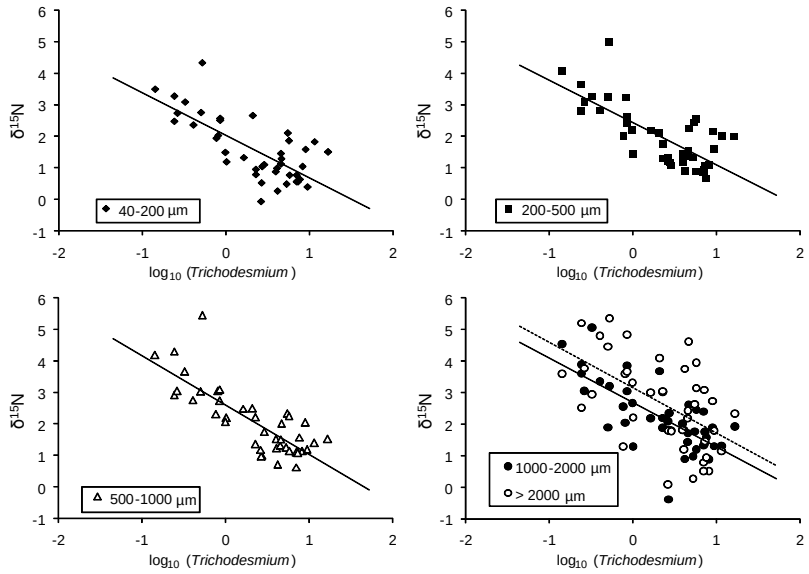

Figure 3.9 Relationships between $\delta^{15}N$ and *Trichodesmium* abundance (log_{10}(trichomes L^{-1})). All regression lines are significant with P<0.001.The dashed line indicates the regression line for the >2000 µm fraction.

3.3.6 Contribution of N from diazotrophs

Diazotroph N contributed to all size fractions in almost all stations (Fig. 3.10). 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 3.3). 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.

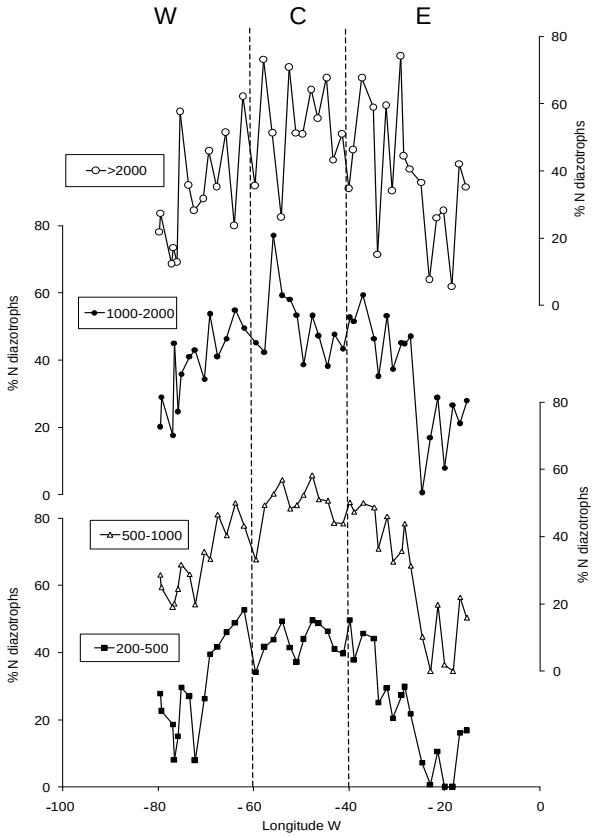

Figure 3.10 Diazotrophic N contribution (%) to plankton size-classes >200 µm estimated from $\delta^{15}N$ along 24° N.

Table 3.3 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

3.4 DISCUSSION

3.4.1 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 (McGillicuddy 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 (Lipschultz 2001) while low production in the central zone of the basin is attributed to low nutrient advection (Marañon 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. 2006). Sharp thermohaline stratification prevents major exchange between surface and deep waters and most phytoplankton biomass concentrated in a deep maximum layer (Fig. 3.2) 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.

3.4.2 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 & McGillicuddy 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 & 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 & 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 3.2) 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. (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. 3.9). 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 & McGillicuddy 2006, Mouriño-Carballido et al. 2011) and because the possible presence of other diazotrophs (Moisander et al. 2010, Fernández et al. 2012).

3.4.3 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, Mulholland et al. 2006, Davis & McGillicuddy 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, 2007, McClelland et al. 2003) 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-Wilhelmy 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. (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.

CAPÍTULO 4:

Bulk vs. amino acid stable N isotope estimations of metabolic status and contributions of nitrogen fixation to size-fractionated zooplankton biomass in the subtropical N Atlantic

ABSTRACT

A comparative analysis of natural abundance of stable N isotopes ($\delta^{15}N$) in individual amino acids and bulk organic matter of size-fractionated plankton revealed the differential impact of nitrogen fixation through the food web in a transect across the subtropical North Atlantic. All $\delta^{15}N$ measurements showed low values in the central region, followed by the western zone, while maximum $\delta^{15}N$ values were found in the eastern zone. These results were consistent with the prevalence of nitrogen fixation in the central and western zones, and the influence of the west Africa upwelling in the eastern zone. Use of compound-specific amino acid isotope data (CSI-AA) revealed relatively low variability in the impact of diazotrophic nitrogen within the different plankton size fractions, while $\delta^{15}N$ of bulk organic matter showed high variability with size. Moreover these results indicated a greater importance of diazotrophy than suggested by bulk $\delta^{15}N$, even in zones having less abundant nitrogen fixing organisms and lower concurrent measurements of nitrogen fixation previously reported. Explicit CSI-AA trophic position estimates for different plankton size classes varied in a relatively narrow range (1.8 to 2.5), with the lowest values in the central zone, and showed the expected general increase with mean plankton size class. Investigations of individual amino acids (in particular Phe and Thr), as well as reconstructed total protein $\delta^{15}N$ values, revealed strong correlations with bulk plankton $\delta^{15}N$, suggesting a set of new relationships that may be important in using CSI-AA data to tracing direct plankton contributions to many detrital organic nitrogen pools in the ocean. Overall, these new results represent the most detailed investigation of CSI-AA data in zooplankton size classes to date, and point to a key role of large zooplankton in the transmission of the diazotrophic nitrogen up oceanic food webs.

4.1 INTRODUCTION

Large regions of the surface Atlantic Ocean are characterized by nutrient deficient waters, where stratification of the surface reduce the input of nutrients from deep waters and the winds induce downwelling over subtropical gyres, to the north and south of the equator (Reynolds et al., 2007). In these conditions, inputs of nitrogen from the atmosphere may represent one of the main drivers of primary production (Montoya et al., 2007; Mouriño-Carballido et al., 2011). Stable isotopes can trace N_2 inputs because atmospheric nitrogen is relatively depleted in heavy (^{15}N) isotopes compared with marine nitrate (Montoya et al., 2002; Wannicke et al., 2010). Assimilation of the light N_2 by diazotrophs produces organic matter with a characteristic isotopic signature ($\delta^{15}N$, the excess in ^{15}N relative to atmospheric N) that can be traced along the food web. Therefore nitrogen isotope values in seston reflect the relative contributions of nitrate coupled with degree of uptake of atmospheric N_2 by cyanobacteria (Montoya et al., 2002, 2004; Fernández et al., 2014), while those in zooplankton reflect both trophic transfer and degree assimilation of organic matter initially produced by diazotrophs (McClelland et al., 2003).

Previous studies in the oligotrophic North Atlantic (Montoya et al., 2004; Landrum et al., 2011; Mompeán et al., 2013; Fernández et al., 2014) showed in general higher plankton $\delta^{15}N$ in eastern compared with western and central zones, consistent with the variable influence of deep water advection vs. atmospheric nitrogen fixation (Montoya et al., 2007; Benavides et al., 2013; Fernández et al., 2013). When analyzed by size fractions (Landrum et al., 2011; Mompeán et al., 2013; Fernández et al., 2014), different plankton size classes showed in general similar geographic change in $\delta^{15}N$, consistent with the propagation of the source N up the food web. However, the low $\delta^{15}N$ values measured in these regions may result either from atmospheric N_2 fixation or from a major use of regenerated nitrogen forms (mainly ammonium). As heterotrophic plankton

preferentially excrete isotopically light nitrogen, meso- and macrozooplankton are expected to become more enriched than subsurface nitrate in the absence of significant N_2 fixation (Montoya et al., 2002), while phytoplankton (and seston) will be have lower $\delta^{15}N$ values because of the uptake of light dissolved nitrogen. Further processing of organic matter up the food web would affect the $\delta^{15}N$ of consumers depending on the fraction of their diet including N directly derived from diazotrophy vs. other sources.

Taking into account that pelagic food webs are strongly size-structured, as consumers are generally much larger than their prey, and that life span and mobility also depend on organism size, $\delta^{15}N$ signals of producers and consumers may become uncoupled (Jennings et al., 2008). This uncoupling has been observed several times in the subtropical North Atlantic in the form of lower $\delta^{15}N$ for large sized compared to small plankton (Landrum et al., 2011; Mompeán et al., 2013; Fernández et al., 2014), and implies a differential impact of N fixation across the food web. For instance, Mompeán et al. (2013) estimated a mean contribution of N from biological fixation of 43% for the 200-500 µm plankton and 54% for plankton >2000 µm in the central region of the subtropical N Atlantic, while Landrum et al. (2011) estimated contributions of >60% of N fixation for >4000 µm plankton in the western basin. These contributions were estimated by comparing bulk $\delta^{15}N$ of plankton samples between areas of presumably different nitrogen sources for primary producers. However, both the transient nature of blooms of the most conspicuous diazotrophic organisms (filament-forming *Trichodesmium*), and the persistence of low rates of fixation by small non-colonial cyanobacteria (Montoya et al., 2004) make the characterization of nitrogen sources actually contributing to local primary production difficult. Therefore more precise estimations of the contribution of different nitrogen sources, in particular N fixation, and impacts for the different organisms of the food web are required to understand main factors limiting the productivity of oceanic ecosystems.

Empirical observations have demonstrated that the $\delta^{15}N$ values in consumers increase with each trophic transfer (trophic enrichment factor, TEF), with a commonly applied average change of approximately ~3.4‰ (Post, 2002). The trophic position (TP) of consumer organisms has therefore been commonly estimated as the difference

between the values of $\delta^{15}N$ of the consumer and that of primary producers scaled by the average increase between trophic transfers. Generally, whole individuals or tissues have been employed for $\delta^{15}N$ determinations ($\delta^{15}N_{bulk}$) because this analysis requires relatively small sample sizes and simple analytical preparation (e.g., Mompeán et al., 2013).

However, there are several important drawbacks to TP estimation based only $\delta^{15}N_{bulk}$. First, accumulating evidence in recent years has clearly demonstrated that the commonly used ^{15}N-enrichment factor of ~3.4‰ is far from universal, but instead can vary substantially with different species, physiology and trophic ecology. For example McCutchan et al. (2003) reported that the ^{15}N-enrichment factor varied over a range of almost 8 ‰ (from -2.1‰ to 5.4‰) for insects and fish. Second, the classical TP estimation requires an integrated $\delta^{15}N$ value for primary producers in a given system (often referred to as baseline $\delta^{15}N$ value). One single representative baseline $\delta^{15}N$ value typically cannot be directly measured, and is rather assumed from literature values for most common primary producers in a given system. However, primarily producers in marine ecosystems (mainly cyanobacteria and algae) can themselves show very high spatial and temporal variability in $\delta^{15}N$, due to the assimilation of various nitrogen sources (i.e., N_2, NO_3^-, NH_4^+, urea) and to their short life span (Varela et al., 2005; Fernández et al., 2013). Consequently, variability in $\delta^{15}N_{bulk}$ of marine particles (as a proxy for marine phytoplankton) has been shown to be over a factor of three greater than assumed ^{15}N-trophic enrichment factor (Hannides et al., 2009). Finally, higher TP consumers themselves are often highly mobile. Therefore, they may feed at multiple depths or across wide geographic locations, potentially having substantially different baseline $\delta^{15}N$ values that are very difficult to determine.

Amino acids (AA) are key components of organic matter participating in most metabolic and growth process. Compound specific isotope analysis of individual amino acids (CSI-AA) is a rapidly growing technique that can address many limitations of bulk $\delta^{15}N$ analysis (McClelland and Montoya, 2002; McClelland et al., 2003; McCarthy et al., 2007; Chikaraishi et al., 2009). The first published CSI-AA studies in marine plankton (McClelland and Montoya, 2002) showed that $\delta^{15}N$ values of individual amino acids

($\delta^{15}N_{AA}$) have strongly variable isotopic enrichment with trophic transfer. Trophic enrichment factors (TEF) measured for individual AA reveal that at the molecular level, they fall into two broadly different groupings. One group (now commonly termed the "trophic" AA, Popp et al., 2007) include those AA which are rapidly transaminated, and so isotopically closely linked to an organisms central N pool (e.g., McCarthy et al, 2013). Trophic AA therefore have high $\delta^{15}N_{AA}$ TEF values, typically much greater than the canonical 3.4 ‰ value for $\delta^{15}N_{bulk}$. In contrast, a second group (commonly termed "source" AA) have far lower $\delta^{15}N_{AA}$ TEF values. For several AA, in particular Phenlyalanine (Phe) and Methionine (Met), $\delta^{15}N$ values have been shown to remain essentially unchanged through multiple trophic transfers (McClelland and Montoya, 2002; Chikaraishi et al., 2009; Germain et al., 2013). While the precise TEF values in different organisms remains under active investigation (e.g., Germain et al., 2013; Bradley et al., 2013, McMahon et al., 2015a, b), a wide variety of studies have now confirmed this fundamental behavior of the source vs. trophic AA groups (McMahon et al., 2013).

The unique behavior of trophic and source AA allows key issues with interpretation of bulk $\delta^{15}N$ TP estimation to be addressed using CSI-AA. First, an organism's TP can now be directly estimated using the offset in $\delta^{15}N$ values between selected source vs. trophic AA (McClelland and Montoya, 2002; Chikaraishi et al., 2009). Importantly, this CSI-AA approach to TP estimation is "internally normalized," meaning it does not require independent characterization of the $\delta^{15}N$ values of nitrate or primary producers in a system. At the same time, the baseline $\delta^{15}N$ value can also be directly estimated by measuring source AA. Together, these aspects mean that CSI-AA can for the first time de-couple the major individual factors underlying changes in $\delta^{15}N_{bulk}$. Further, relatively little sample is required (nanomolar amounts of nitrogen) for the nitrogen isotope analysis of AA by using gas chromatography combustion/isotope ratio mass spectrometry (GC/C/IRMS). Thus CSI-AA is now becoming widely used for more precise estimation of both TP and baseline $\delta^{15}N$ values, greatly extending the understanding of the actual structures of food webs, and also nitrogen source and flow in natural environments (Chikaraishi et al., 2009; Vohkshoori and McCarthy, 2014; Sherwood et al., 2014). Some examples of recent ecological studies using this method to clarify TP of

marine plankton include studies in the central Pacific (McCarthy et al., 2007), and near to Hawaii (Hannides et al., 2009). The majority of studies to date have used Phe as the best indicator of the baseline $\delta^{15}N$ value, and glutamic acid (Glu) as the most consistent indicator of trophic transfer (Chikaraishi et al., 2009).

Because CSI-AA can simultaneously supply information about TP, while also identifying the underlying N sources at the base of a food web, it is particularly well suited investigating N fixation in ecosystems, including the propagation of newly fixed N to higher trophic levels (McClelland et al., 2003; Sherwood et al., 2014). CSI-AA therefore offers a new approach to track inputs from atmospheric N to ecosystems, simultaneously corrected for change in TP. However, studies using planktonic CSI-AA in the subtropical N Atlantic were limited to a few samples from a single (smallest) zooplankton size class, and did not explicitly investigate CSI-AA derived TP, nor the potential to track relative diazotrophic contributions into multiple plankton size classes (McClelland et al., 2003).

Finally, CSI-AA has recently evolved very rapidly into multiple new applications, crossing ecology, paleoceanography and biogeochemical cycle research. There is growing interest in using CSI-AA parameters in different archives to reconstruct past planktonic ecosystem structure, as well as N fixation. Recent work has begun to address these questions in paleoarchives such as deep-sea corals (Sherwood et al., 2014), archived sperm whale tissues Ruiz-Cooley et al., 2015), and ocean sediments (Batista et al., 2014). Such work fundamentally depends, however, on understanding how CSI-AA parameters may be altered in primary vs. secondary export production, However, to date, such data is extremely limited; the most detailed examination of planktonic CSI-AA data has been mostly confined to primary producers (Chikaraishi et al., 2009; McCarthy et al., 2013) while relatively scarce data exists for natural zooplankton populations.

We present here a first examination of CSI-AA values and derived ecosystem parameters in multiple plankton size fractions, across a strong ocean-basin scale gradient in nitrogen fixation and associated plankton $\delta^{15}N_{bulk}$ values in the subtropical N Atlantic. The objectives are, first to compare estimations of trophic position in size-fractions of plankton along the transect, based on $\delta^{15}N_{AA}$, and second to use CSI-AA to estimate

diazotrophic N contribution to different size fractions of plankton, and compare these results with more common $\delta^{15}N_{bulk}$ approaches. To our knowledge, the relative importance of diazotrophic nitrogen input measured together with more precise CSI-AA trophic transfer values through a planktonic food web has never been directly evaluated, as in previous studies only one plankton size fraction was considered (McClelland et al., 2003). Finally, we use this unique data set of $\delta^{15}N_{AA}$ values to examine key CSI-AA parameters in diverse plankton sources. These new data should be invaluable to shape future hypotheses regarding CSI-AA parameter interpretation in both ecological and paleoceanographic studies.

4.2 MATERIAL AND METHODS

4.2.1 Sampling and bulk stable isotope analysis

Plankton samples were obtained during Leg 8 of the Malaspina-2010 expedition on R/V Sarmiento de Gamboa (January-March 2011), on a transect predominantly along 24ºN, between the Canary Island and Florida (Fig. 4.1). This cruise transect and detailed sample collection protocols has been described previously (Mompeán et al., 2013).

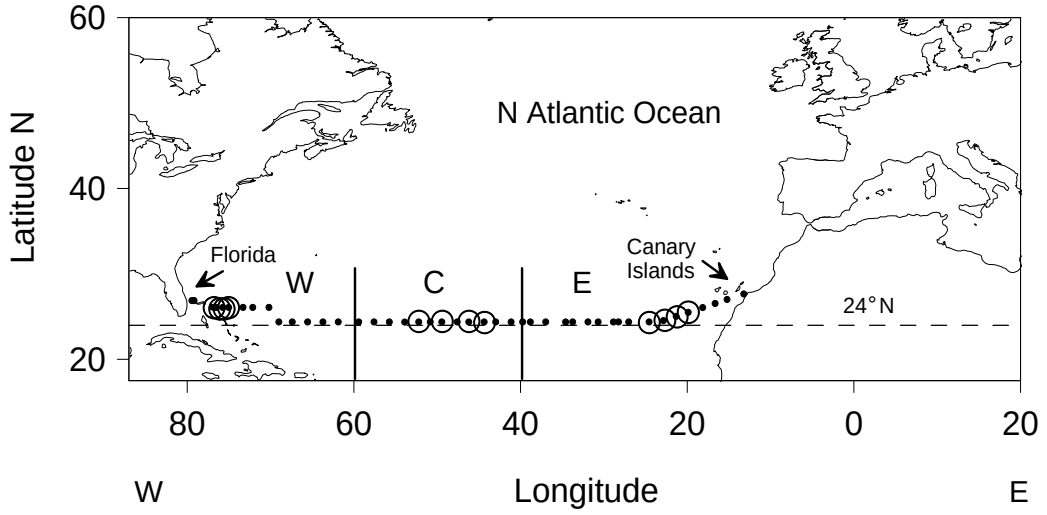

Figure 4.1 Location of plankton sampling stations during cruise Leg 8 of Malaspina-2010 expedition along 24 ºN crossing the Atlantic basin. Open circles indicate location of samples selected for compound-specific amino acid stable nitrogen isotope analysis in western (W), central (C) and eastern (E) zones.

Briefly, 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 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-weighed glass-fiber filters, dried (60ºC, 48 h) and stored in a desiccator before determination of biomass (dry weight), carbon and nitrogen content and natural abundance of stable carbon and nitrogen isotopes ashore. Nominal values of the individual size of organisms in each size fraction were estimated as the geometric mean of the values defining each size interval and expressed as carbon content (µg C) in a logarithmic scale (Rodriguez and Mullin, 1986).

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 analyzer (Carlo Erba CHNSO 1108) coupled to an isotope-ratio mass-spectrometer (Finnigan Mat Delta Plus). These measurements were reported as $\delta^{15}N_{bulk}$ and were already analyzed and discussed in Mompeán et al. (2013).

4.2.2 Compound-specific amino acid $\delta^{15}N$ analysis

Samples for CSI-AA were selected to span gradients in $\delta^{15}N_{bulk}$ values. We chose plankton from four sampling stations in each of the three zones (eastern, central and western regions). Individual samples were then pooled (quantitatively, so that each subsample was represented equally in the final composite) to have enough material in each size fraction for CSI-AA (Fig. 1). In total 15 samples in the transect were chosen for CSI-AA. Approximately 1 mg of total dry plankton material was then hydrolyzed for subsequent analysis.

The $\delta^{15}N$ values of individual AAs were measured via GC-IRMS, after 6 N HCl acid hydrolysis and the formation of TFA ester derivatives following previously published methods (e.g., McCarthy et al., 2013; Germain et al., 2013). We determined $\delta^{15}N$ values

for 12 AAs: glutamic acid + glutamine (Glx), aspartic acid + asparagine (Asp), alanine (Ala), isoleucine (Ile), leucine (Leu), proline (Pro), valine (Val), glycine (Gly), serine (Ser), lysine (Lys), phenylalanine (Phe), and threonine (Thr). Each AA was run four times on the GC-IRMS. Based on previous studies (e.g., McClelland and Montoya et al., 2002; Chikaraishi et al., 2009; Germain et al., 2013), AA values were categorized and presented in 3 groups, based on their relative $\delta^{15}N$ values and changes with trophic transfer: the source AAs (Gly, Ser, Lys, Phe), the trophic AAs (Glx, Asp, Ala, Ile, Leu, Pro and Val), and one "metabolic" AA (Thr).

4.2.3 Trophic position and related variables

To calculate CSI-AA based TP of plankton we used the most widely used current equation and TEF value, based on the isotopic offset between Glx and Phe (Chikaraishi et al., 2009):

$$TP = \left(\frac{(\delta^{15}N_{glu} - \delta^{15}N_{phe}) - 3.4‰}{7.6‰} \right) + 1$$

where $\delta^{15}N_{Glx}$ and $\delta^{15}N_{Phe}$ are measured values, +3.4‰ is the assumed isotopic difference between the Glx and Phe in primary producers, and +7.6‰ is the assumed ^{15}N enrichment in Glx relative to Phe with each trophic transfer from food source to consumer (TEF value). The standard errors in the estimation of TP, computed by propagation of analytical error in the individual AA determinations, did not exceed 0.1 TP.

The $\delta^{15}N$ value of total hydrolysable AAs ($\delta^{15}N_{THAA}$) is used as a proxy for total protein $\delta^{15}N$ value (e.g., McCarthy et al., 2007, 2013), and was estimated as the molar-weighted average of individual $\delta^{15}N$ values:

$$\delta^{15}N_{THAA} = \Sigma(\delta^{15}N_{AA} * mol_{AA})$$

where $\delta^{15}N_{AA}$ is the $\delta^{15}N$ value of each individual AA measured and $mol\%_{AA}$ is the molar percentage contribution of each AA. In our study we used the $\delta^{15}N$ value of each individual AA and $mol\%_{AA}$ were obtained from Lehman (2009).

The degradation index (ΣV), proposed by McCarthy et al. (2007) as a measure of the relative resynthesis of the original autotrophic AA pool in detritus or different organisms (plankton size fractions, in our case), was computed for each size individual fraction sample as the mean deviation of $\delta^{15}N$ of individual trophic amino acid, from their average:

$$\Sigma V = \Sigma \left| AA_i - Avg_{trp} \right| / n$$

where AA_i were individual $\delta^{15}N$ amino acid values, Avg_{trp} the average value and n the total number of trophic amino acids.

4.2.4 Diazotrophic N contribution

The impact of N fixation ($\%N_{fix}$) was estimated for each size-fraction using either measured $\delta^{15}N_{bulk}$ and/or $\delta^{15}N_{Phe}$, where the latter was applied as an alternate indicator of the baseline $\delta^{15}N$ values (source of N for primary producers; McCarthy et al., 2013). In all cases, final calculations were based on the model of Montoya et al. (2002):

$$\% N_{fix} = 100\left(\frac{\delta^{15}N_{bulk} - \delta^{15}N_{ref}}{\delta^{15}N_{diazo} - \delta^{15}N_{ref}}\right)$$

where $\delta^{15}N_{diazo}$ = -2‰ is the value determined for N-fixers in the N Atlantic (Montoya et al., 2002) and $\delta^{15}N_{ref}$ is the value measured in reference material in areas without significant influence of diazotrophy (Montoya et al., 2002; Landrum et al., 2011, Mompeán et al., 2013).

For all samples on which we measured CSI-AA, two alternate calculations were made: one based on the measured $\delta^{15}N_{bulk}$, and a second based on CSI-AA values for $\delta^{15}N_{Phe}$. In the first case, $\delta^{15}N_{bulk}$ was set equal to average $\delta^{15}N_{bulk}$ values previously reported in this same region (Mompeán et al., 2013) and $\delta^{15}N_{ref}$ values for each size class were those reported in Landrum et al. (2011) for regions without diazotrophy. For the CSI-AA based calculation of $\%N_{fix}$, the corrected $\delta^{15}N_{bulk}$ term was estimated from the measured $\delta^{15}N_{Phe}$ values as:

$$\delta^{15}N_{Bulk} = \delta^{15}N_{Phe} + \beta_{Phe}$$

where $\delta^{15}N_{Phe}$ is the measured value in each size fraction, and β_{Phe} is the average offset between phytoplankton bulk $\delta^{15}N$ and Phe values ($\delta^{15}N_{bulk}$ - $\delta^{15}N_{Phe}$), following the convention of Chikaraishi et al. (2009). Reported β_{Phe} values ranged between ~2‰ (Chikaraishi et al., 2009) and 3.4‰ (McCarthy et al., 2013). Because these values are relatively similar, we therefore used the average β_{Phe} value from both of these studies (+ 2.7‰) as the best estimate currently available. In the case of CSI-AA based estimates $\delta^{15}N_{ref}$ was set to +4.5‰, typical value for subsurface nitrate (Montoya et al., 2002).

The values of $\%N_{fix}$ were finally compared with several different proxies which should be related to the input of fixed atmospheric N: $\delta^{15}N_{Phe}$ (as an estimate of the isotopic signature of the source inorganic nitrogen for primary producers), N* (stoichiometric excess of nitrogen due to remineralization, Gruber and Sarmiento, 1997) and previously reported abundance of the colonial cyanobacterium *Trichodesmium* in our sampling regions. The index N* was computed as:

$$N^* = [(N - 16\ P) + 2.9]\ 0.87$$

where N and P were the subnitracline (down to 300 m depth) concentrations of nitrate and phosphate, respectively. As the values of N* are arbitrary, relatively high values indicate potential areas for N fixation where nitrogen rich organic matter is remineralized while low N* values are expected in areas where denitrification prevails (Gruber and Sarmiento, 1997). Values for N, P and *Trichodesmium* abundance for the same stations where $\delta^{15}N_{AA}$ was determined were obtained from data reported in Mompeán et al. (2013).

4.3 RESULTS

4.3.1 CSI-AA patterns across plankton size fractions

Across all size fractions, AA from in the samples from the central zone showed the lowest $\delta^{15}N$ values while those in the samples from the eastern zone showed the highest values (Fig. 4.2). Mean values of pooled (averaged) trophic and source AA were significantly correlated across all three zones ($r=0.635$, $P<0.05$, $n=15$), with a slope of 0.85. This finding is consistent with changes in the source of nitrogen (baseline $\delta^{15}N$ values) driving most of the variability in the sample set, given the relatively similar ranges of trophic positions in samples from the different zones (Table 4.1).

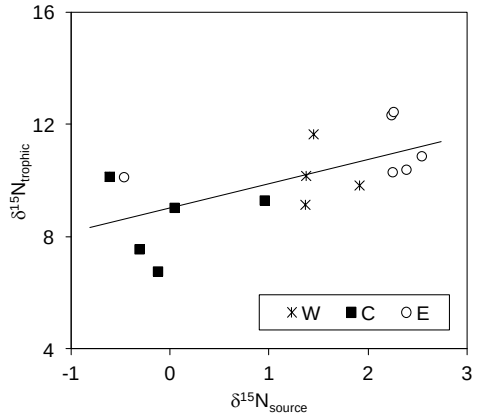

Figure 4.2 Relationship between average trophic vs. average source amino acid $\delta^{15}N$ (‰), averaged across all size classes sampled in west (W), central (C), and eastern (E) Atlantic. Regression line is significant ($P<0.05$), with corresponding Pearson correlation coefficient $r = 0.635$.

Table 4.1 Mean $\delta^{15}N$ (‰) of individual amino acids and bulk organic matter for five plankton size fractions analysed in the western (W), central (C) and eastern zones (E). Significant differences between fractions for each zone (ANOVA and Dunnet-C post-hoc tests, P<0.05) are indicated with different letters (a, b, c). Cases with no letters (e.g. Phe for C and E zones) indicate no significant differences within zones. Amino acids were grouped as trophic, source and metabolic (e.g. Germain et al., 2013). Trophic positions (TP) were computed using the $\delta^{15}N$ values of Phe and Glx (Chikaraishi et al., 2009) and the degradation index (ΣV, ‰) as the average deviation from the mean $\delta^{15}N$ for trophic amino acids (McCarthy et al., 2007).

Zone	Size fraction (μm)	$\delta^{15}N$ (‰)													TP	ΣV (‰)
		trophic							source				metab.	Bulk		
		Glx	Asp	Ala	Ile	Leu	Pro	Val	Gly	Ser	Lys	Phe	Thr			
W	40-200	11.9^{a}	8.7^{b}	12.3^{b}	8.7^{a}	8.5	8.6	10.2^{a}	3.4^{c}	2.7^{b}	1.8^{b}	-0.2^{b}	-10.5^{c}	2.6^{a}	2.2^{a}	1.4
	200-500	11.7^{a}	7.8^{a}	11.4^{a}	7.9^{a}	7.4	8.4	9.5^{a}	2.3^{b}	2.6^{b}	1.3^{b}	-0.8^{b}	-10.8^{c}	2.7^{a}	2.2^{a}	1.1
	500-1000	13.2^{b}	9.0^{b}	12.5^{b}	8.5^{a}	8.5	9.8	9.7^{a}	2.9^{c}	1.3^{a}	1.0^{b}	0.3^{b}	-11.6^{b}	2.8^{a}	2.2^{a}	1.4
	1000-2000	14.7^{c}	9.9^{c}	14.8^{c}	11.9^{c}	8.5	10.2	11.7^{b}	2.1^{b}	3.2^{b}	1.0^{b}	-0.4^{b}	-13.9^{a}	3.0^{a}	2.5^{b}	1.2
	>2000	12.4^{a}	8.6^{b}	12.0^{b}	9.9^{b}	8.9	9.3	9.9^{a}	-0.8^{a}	0.4^{a}	-0.2^{a}	-1.8^{a}	-14.5^{a}	4.3^{b}	2.4^{b}	1.6
C	40-200	8.6^{a}	6.0	8.8^{a}	5.7^{a}	5.8	5.9^{a}	6.6^{a}	1.4^{d}	-0.8^{a}	0.0	-1.1	-9.9^{d}	0.4^{a}	1.8^{a}	1.8
	200-500	10.3^{b}	6.7	9.7^{b}	6.0^{a}	5.9	6.7^{b}	7.6^{b}	0.4^{b}	0.8^{b}	-0.8	-1.6	-12.9^{c}	1.1^{a}	2.1^{b}	1.5
	500-1000	11.0^{c}	7.7	11.8^{c}	7.8^{b}	7.7	8.2^{c}	9.1^{c}	0.6^{b,c}	1.2^{b}	0.1	-1.8	-15.5^{b}	0.9^{a}	2.2^{b}	1.5
	1000-2000	11.6^{d}	6.4	12.6^{d}	8.2^{b}	4.3	8.6^{c}	8.7^{d}	0.8^{c}	1.5^{b}	2.3	-0.8	-15.1^{b}	1.3^{a}	2.2^{b}	1.4
	>2000	11.9^{d}	8.4	13.4^{e}	8.7^{b}	8.7	9.9^{d}	10.0^{e}	-1.4^{a}	0.7^{b}	-0.2	-0.9	-16.7^{a}	1.9^{b}	2.2^{b}	1.6
E	40-200	13.0^{a}	9.2^{a}	12.2^{a}	8.9^{a}	8.7^{a}	9.3^{a}	10.8^{b}	3.9^{c}	2.6	2.3^{a}	0.1	-9.8^{b}	3.1	2.2	1.4
	200-500	12.2^{a}	9.8^{b}	13.2^{b}	9.0^{a}	8.5^{a}	10.0^{a}	10.1^{a}	4.0^{c}	3.2	2.7^{a}	-0.3	-10.0^{b}	3.5	2.2	1.4
	500-1000	12.7^{a}	10.0^{b}	13.7^{c}	8.8^{a}	9.2^{b}	10.9^{b}	11.0^{b}	3.9^{c}	4.0	2.2^{a}	0.0	-10.2^{b}	3.8	2.2	1.4
	1000-2000	13.9^{b}	11.1^{c}	15.1^{d}	10.8^{b}	10.6^{c}	10.9^{b}	11.6^{b}	1.7^{a}	2.9	4.2^{b}	0.7	-12.3^{a}	4.1	2.4	1.3
	>2000	14.1^{b}	10.7^{c}	15.4^{d}	11.4^{b}	11.6^{d}	11.3^{b}	12.6^{c}	3.0^{b}	3.2	2.6^{a}	0.2	-12.6^{a}	3.9	2.4	1.1

The mean values of $\delta^{15}N_{Glx}$ (used as an indicator of TP) increased from the smallest to the largest size fractions in all zones (Table 4.1). Individual AA, however, displayed variability with size in the different zones. For instance, $\delta^{15}N$ of Leu, Pro, and Asp, showed small variation in their mean values within the western and central zones but $\delta^{15}N$ values clearly increased in larger size fractions in the eastern zone, following the general pattern for trophic AA. In contrast, the variability for source AA was relatively small within zones, but there were a few cases of significantly high or low values. Phe was the source AA with the lowest variability in $\delta^{15}N$ between size fractions within zones, consistent with assumptions that this AA is the best indicator of baseline $\delta^{15}N$ values. One exception was a single anomalous low value determined for the largest size fraction in the western zone. The $\delta^{15}N$ values of Thr consistently decreased with size fraction in all zones, consistent with prior indications that this unique "metabolic" AA has negative TEF values with trophic transfer (McClelland and Montoya, 2002; Germain et al., 2013). Values for $\delta^{15}N_{bulk}$ generally increased with size class (Table 4.1).

4.3.2 Trophic position estimates

Irrespective of the zone, the $\delta^{15}N$ of the average trophic AA increased significantly with organism size ($r=0.547$, $P<0.05$, $n=15$), while in contrast the average $\delta^{15}N$ values for source AA were not related to size (Fig. 4.3a). The relative change in trophic vs. source AA values, utilizing averages of the source and trophic AA groupings, represents the broadest measure of relative stability vs. change in trophic position (Popp et al., 2007; Sherwood et al., 2014). The increasing relationship of trophic AA values vs. the constant relationship of source AA with size class therefore indicates a consistent overall increase in TP with plankton size in all zones, expressed at the molecular level in individual AA $\delta^{15}N$ values.

Explicit CSI-AA based estimates of TP varied within a relatively narrow range, between 1.8 and 2.5 (Table 4.1). Within zones, TP increased significantly with size, except for within the eastern zone Considering all data, there was a significant trend of increasing in mean TP with size class across all zones ($r=0.675$, $P<0.01$, $n=15$, Fig. 4.3b). The agreement of the broader trends in average sources and average trophic AA values (Fig. 4.3a) with this trend in TP values strongly supports the validity of our TP estimates, despite being derived from $\delta^{15}N$ values of two specific diagnostic AA (Phe and Glu). Further, the standard errors in the estimation of TP were quite small, not exceeding 0.1 TP (Fig. 4.3b), indicating quite high precision in the ability of CSI-AA to resolve TP values between plankton size classes, despite the fairly narrow range in overall TP values.

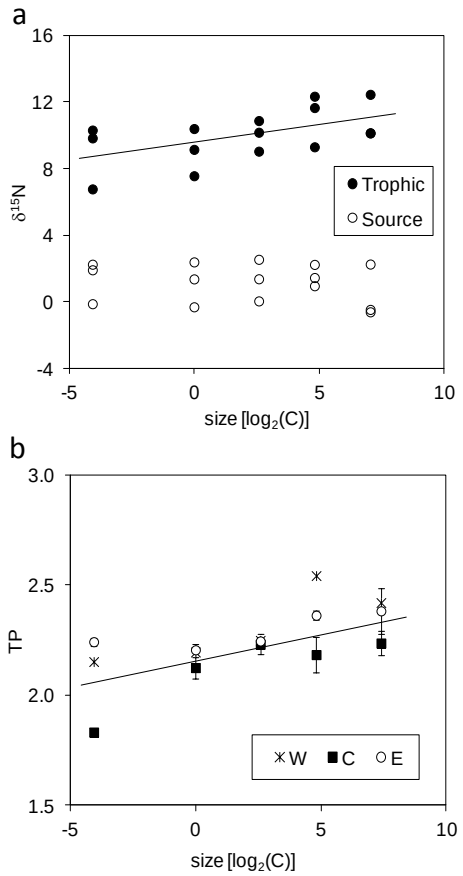

Figure 4.3 The relationship between compound-specific amino acid derived indications of trophic position, and plankton size. a) averaged trophic and source amino acid δ^{15}N (‰) values vs. plankton size class [$\log_2$(C), µg C indiv^{-1}]; the regression for only the trophic AA group is significant (P<0.05 ; Pearson r =0.547). b) Relationship between mean (±se) CSI-AA derived trophic position (TP) and plankton size class [$\log_2$(C), µg C indiv^{-1}] in samples from western (W), central (C) and eastern (E) zones. The overall regression line is significant (P<0.01; Pearson r= 0.45). Standard errors (se) of TP were estimated by error propagation using analytical variability for $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glx}$ (*see methods*).

4.3.3 Composition and degradation indices

The CSI-AA resynthesis index (ΣV) was low in all size fractions, with an average of 1.4 (range 1.1 to 1.8, sd= 0.7; Table 4.1). These values are similar to ΣV ranges previously reported for sinking particles, but are somewhat elevated vs. cultured phytoplankton (McCarthy et al., 2007), indicating the expected heterotrophic resynthesis with trophic transfers in zooplankton. In turn, $\delta^{15}N_{bulk}$ values were strongly correlated with $\delta^{15}N_{THAA}$ (r=0.965, P<0.001, n=14), with a slope ~1 and an intercept ~3.4‰ (Fig. 4.4a). There was also a significant correlation between $\delta^{15}N_{bulk}$ and the main source AA ($\delta^{15}N_{Phe}$) across zones (Fig. 4.4b), however with a much lower slope (~0.5).

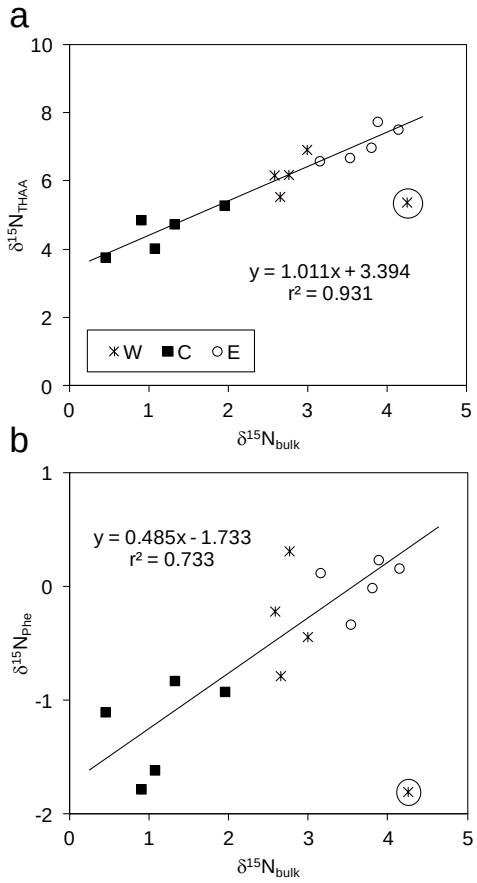

Figure 4.4 Relationship between $\delta^{15}N$ in bulk plankton ($\delta^{15}N_{bulk}$) and a) total hydrolizable amino acids ($\delta^{15}N_{THAA}$), a proxy for total protein $\delta^{15}N$ value, and b) Phe ($\delta^{15}N_{Phe}$) in samples from western (W), central (C) and eastern (E) zones. Both regression lines are significant (P<0.001; Pearson r values are 0.964 and 0.856, for panel a and b respectively). An outlier, corresponding to the anomalously low value of $\delta^{15}N_{Phe}$ in the western zone (Table 1), was not used in the regression computations and is indicated by a circle.

Threonine displayed unique behavior vs. all the other AA, with a strong negative relationship between $\delta^{15}N_{Thr}$ values and all proxies for trophic transfer. When normalized for changes in the isotopic signature of the nitrogen source, $\delta^{15}N_{Thr}$ values displayed linear decreases with TP (Fig. 4.5). While this same correlation obtained in all zones, there were also clear differences between the regressions lines found in different zones. For example, the central and eastern zones had offset regression lines for ($\delta^{15}N_{Thr}$-$\delta^{15}N_{Phe}$) vs. TP, having very similar slopes (-14.928, -18.908 respectively), but significantly different intercepts (ANCOVA, P<0.05). The regression line for the western zone (excluding the outlier) had a lower slope than the other zones, also was not statistically significant at the 95% confidence level.

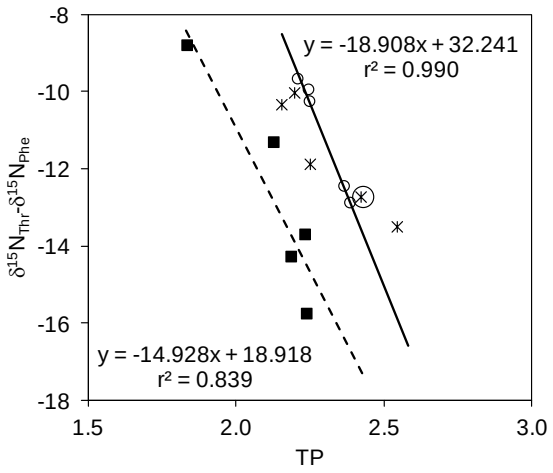

Figure 4.5 Relationships between trophic position (TP) and δ^{15}N values of Thr, normalized to Phe δ^{15}N, as a proxy for baseline δ^{15}N values ($\delta^{15}N_{Thr}$ - $\delta^{15}N_{Phe}$). Regression lines fitted independently to central (dashed line) or eastern (continuous line) zones are shown (both significant; P<0.05; r = 0.915 and 0.995, respectively). The corresponding regression for the western zone (line not shown) has a slightly higher slope (-8.44), but was not statistically significant. As in prior figures, the outlier $\delta^{15}N_{Phe}$ value not used in the regression computations is indicated by a circle.

4.3.4 Impact of diazotrophy

The estimation of contribution of nitrogen fixation to zooplankton ($\%N_{fix}$) based on the measured $\delta^{15}N_{bulk}$ values indicated generally similar contributions from diazotrophy for all size classes in the central and western zones, where δ^{15}N enrichments were relatively low (Fig. 4.6a). In contrast, the eastern zone displayed a marked increase of $\%N_{fix}$ with size, consistent with values reported in Mompeán et al. (2013). The CSI-AA derived estimates of nitrogen fixation, based on corrections for baseline values using $\delta^{15}N_{Phe}$ (*see methods*), produced slightly different results (Fig. 4.6b). While in both bulk and CSI-AA based data $\%N_{fix}$ reached almost 50% in the central zone, $\%N_{fix}$ from the CSI-AA in the eastern and western zones were higher than $\%N_{fix}$ values derived from $\delta^{15}N_{bulk,}$ reaching between 20-30%. Moreover the overall pattern of $\%N_{Fix}$ values vs. size class was different between the two calculations, particularly for eastern and western zones. Estimates using CSI-AA indicated approximately equal contributions of diazotrophy in all size classes for all zones, while the bulk approach showed increases of %Nfix with size out of the central zone. We note that the single very high value for the largest size fraction in the western zone (indicated by a circle in Fig. 4.6b) is likely an artifact, generated by a single anomalously low value for $\delta^{15}N_{Phe}$ noted above (Table 4.1).

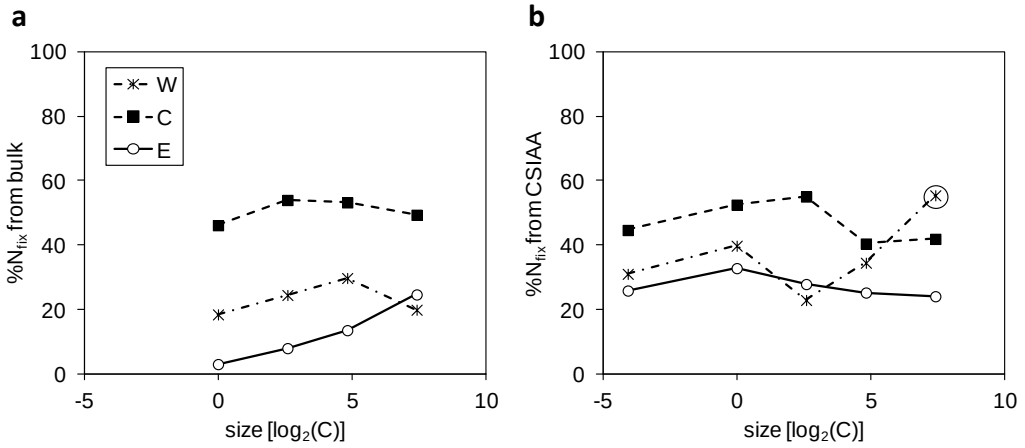

Figure 4.6 Compression of estimates of contribution to N fixation (%N_{fix}) to total N in different plankton size samples from western (W), central (C) and eastern (E) zones. Plankton size classes are represented by the mean individual size [log_2(C), µg C $indiv^{-1}$]. Estimations of %N_{fix} were computed using the mixing model of Montoya et al. (2002) and either (a) measured $\delta^{15}N_{bulk}$ (from Mompeán et al., 2013) or (b) using $\delta^{15}N_{Phe}$ as a proxy for correcting $\delta^{15}N_{bulk}$ for baseline $\delta^{15}N$. Note that very high value for largest size class in W zone (indicated by circle) is likely an artifact, due to the anomalous $\delta^{15}N_{Phe}$ in this sample noted in pervious figures. No estimates were made using $\delta^{15}N_{bulk}$ for the smallest size class as reference values for no fixation areas were available only for plankton larger than 200 µm (Mompeán et al., 2013).

The zonal variation of the new CSI-AA based estimates for %N_{fix} across all size fractions were also strongly consistent with the relative patterns in a number of ancillary measurements that would be expected to correlate with diazotrophy (Fig. 4.7). The $\delta^{15}N_{Phe}$ varied inversely with %N_{fix} in both the CSI-AA estimate (Fig. 4.7a), and the fully independent estimate derived from %N_{bulk} (not shown). Similarly, the relative offsets in N* and the abundance of *Trichodesmium*, varied inversely with values of $\delta^{15}N_{Phe}$. These comparisons indicate that the highest impact of diazotrophy was actually in the central zone, consistent with the largest potential for remineralization of nitrogen rich organic matter indicated by N*, along with the highest abundance of *Trichodesmium*. Indeed, pair wise differences in %N_{fix} and $\delta^{15}N_{Phe}$ between zones were not as clear as for the environmental variables associated with N fixation (ANOVA and Dunnet-C post-hoc tests, $P<0.05$).

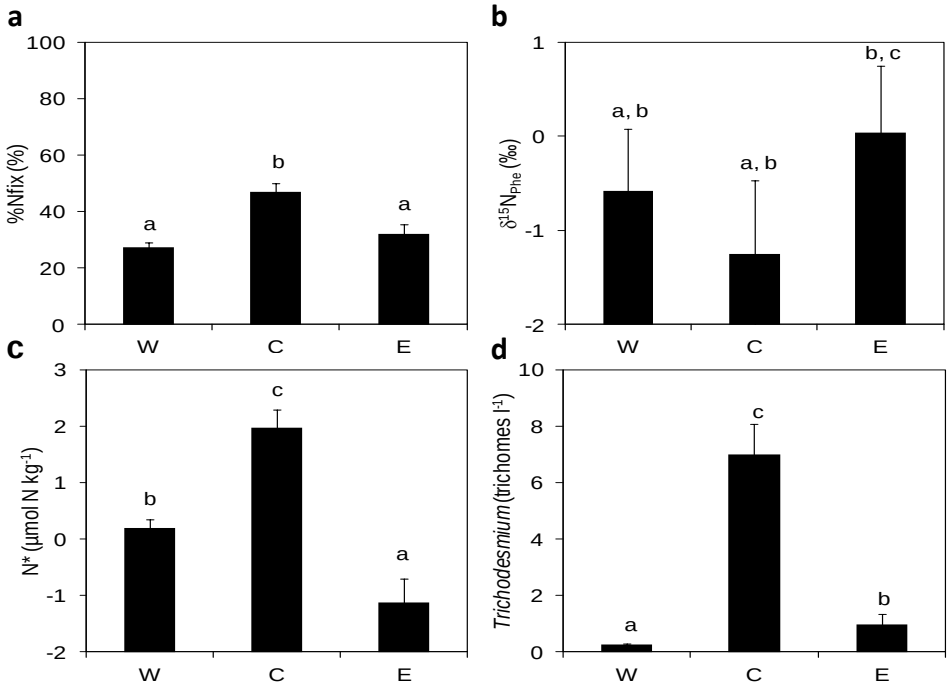

Figure 4.7 Nitrogen fixation estimates compared with related parameters from western (W), central (C) and eastern (E) zones. Mean (±se) values of % N_{fix} determined using the corrected value of $\delta^{15}N_{bulk}$ as in Fig. 6b (panel a), $\delta^{15}N_{Phe}$ values (‰, panel b), average N* values (panel c, µmol N kg^{-1}) and the average abundance of *Trichodesmium* (panel d, trichomes l^{-1}). N* (Gruber and Sarmiento, 1997) was determined from nitrate and phosphate concentrations in the surface layer (0-125 m), extracted from results reported in Mompeán et al. (2013) as were *Trichodesmium* abundance data. Significant differences between zones (ANOVA and Dunnet-C post-hoc tests, P<0.05) are indicated with different letters (a, b, c).

4.4 DISCUSSION

4.4.1 Plankton size and trophic position

Trophic positions in this study were computed with the most commonly applied CSI-AA model, based on Glu and Phe (e.g. Chikaraishi et al., 2009). The accuracy of TP estimates depends on two main factors: first, the assumed offsets between Glu and Phe in primary producers (as noted above, the β value), and second the assumed $\delta^{15}N$ change with trophic transfer (Trophic enrichment factor; TEF). Recent studies have shown that TEF values widely assumed for Glx (+7.6‰ increase with each trophic step) are likely not constant across all trophic levels, and so can result in underestimates of TP for higher level consumers. High TP consumers have now been widely reported as having low trophic enrichment factors (Popp et al., 2007; Germain et al., 2013; Bradley et al., 2014), and one recent controlled feeding study has indicated that change in TEF is

biochemically predictable, linked largely to diet composition changes commonly associated with increasing trophic level (McMahon et al., 2015a). A recent meta-analysis of Nielsen et al. (2015) pooling data from many sources is consistent with this, and has suggested ranges of TEF values with increasing TP. Germain et al. (2013) first proposed that a multi-TEF equation was necessary for use in marine mammals, and more recent work has confirmed that a very similar multi-TEF formulation is likely needed for higher TP carnivores generally, testing it explicitly in fish and birds (McMahon et al, 2015a,b; Nielsen et al., 2015).

However, for lower TP organisms, and for oceanic plankton in particular, the TEF issues documented for higher trophic level carnivores are less clear. Specifically, much of the early work which determined the "classic" TEF values for Glu and Phe were specifically conducted on plankton (e.g., McClelland and Montoya, 2002; Chikaraishi et al., 2009). Subsequent CSI-AA TP elaborations applying these TEF values to sinking marine particles and plankton tows have confirmed that they yield reasonable values (McCarthy et al., 2007; Batista et al., 2014), and these higher TEF values for the first steps of marine food webs are also supported by multi-TEF approaches (Germain et al., 2013). It is therefore not surprising that the use of the "classic" $TEF_{Glu\text{-}Phe}$ (7.6) yields reasonable TP estimates for these plankton samples, nor that applying instead the meta-analysis values would result in very little change in our plankton TP values (application of the meta-analysis values would result in an increase of ~ 0.3 TP overall, or a total TP range 2.1 to 2.8). Therefore, based on literature for lower TP organisms, we have elected to use here the earlier TEF values for these plankton samples. Of course, no matter what TEF values are applied, the spatial and size-related TP patterns would remain unchanged.

The relatively small range of TP found in this study is typical of marine zooplankton estimates using CSI-AA approaches (Hannides et al., 2009; Nakatomi et al., 2013; Hannides et al., 2015). Even maximum values for the largest size fraction were <3 TP, suggesting a large dependence from organic matter directly derived from primary producers, rather than from carnivory. This result is consistent with current ideas about omnivorous feeding for most zooplankton (e.g., Calbet, 2001), but also with the fact that these were size fractionated mixed tows, resulting in the presence of primary producers in some of the sampled size fractions. For instance, the smallest size fraction (40-200µm)

sampled in this study include almost equivalent abundance of phytoplankton and zooplankton in all zones (Mompeán et al., 2013). Moreover, the presence of filaments of *Trichodesmium* cannot be discarded even in the largest size fractions. This could be a factor at least in the central zone, where this cyanobacterium was abundant (Fig. 4.7d), and where the lowest values of TP were observed (Table 4.1).

However, low TP values could also result from the low isotopic fractionation in some trophic steps. While the average $\delta^{15}N$ increase between subsequent trophic steps for entire food webs is well established (Post, 2002), increases observed for either top consumers (Hussey et al., 2014) or in microbial food webs (Gutiérrez-Rodriguez et al., 2014) are comparatively smaller. Therefore, the application of average TEF values could cause an underestimation of the actual number of trophic steps for individual species in areas with microbial-loop dominated food webs. In our study, the significant regression between $\delta^{15}N_{bulk}$ (after correction for variation in baseline $\delta^{15}N$, via $\delta^{15}N_{Phe}$values), and TP implies a constant trophic enrichment of ca. 3‰, and thus within the range reported in the literature (Post, 2002). This supports the validity of the estimations of TP for the range of organisms collected in the different fractions, even when the inclusion of entire food webs, from microbes to top consumers, might require the application of specific TEF values.

Overall, our data shows that organism size is the key variable most closely linked to TP, as expected in size-structured marine food webs (Jennings et al., 2008). Even when the increase in TP between size fractions was small (Table 4.1), there were still significant correlations with $\delta^{15}N$ in source AA (Fig. 4.3a), and with also CSI-AA TP values (Fig. 4.3b), with plankton size class across all zones. This result also underscores the precision of CSI-AA TP estimates. Early work by McCarthy et. al. (2007) suggested that CSI-AA TP estimates were likely accurate to <0.5 TP, however the strong correlations we have observed in these plankton size classes suggests that much smaller fractional variations in average CSI-AA TP data are clearly meaningful. However, at the same time there was also variation in specific TP relationships between individual zones. Taken in isolation, among the three zones only the central zone showed an obvious relationship between amino acids $\delta^{15}N$ and size class (Table 4.1), likely due to the very low values in the smallest size fraction in this region. However, it is most likely that relatively low CSI-AA

data density accounts for this, and we hypothesize a larger data set would reveal the same trends in each zone that are clear in the larger total data set. It is also possible that the mixture of organism types (autotrophic and heterotrophic) with different TP size fractions, particularly in the eastern and western zones, may have played a role in blurring relationships between isotope value and TP.

4.4.2 CSI-AA parameters in oceanic plankton

Our data set of CSI-AA values across multiple plankton size classes represents a unique opportunity to examine CSI-AA parameters in natural, mixed plankton end members crossing an entire ocean basin. These data have important implications for a wide range of oceanographic work. For example, rapidly expanding paleoceanographic CSI-AA applications, as in sediments (e.g., Batista et al., 2014; Larsen et al., 2015) and deep sea corals (e.g., Sherwood et al., 2014; Schiff et al., 2014), rest on assumptions about the CSI-AA signatures in planktonic sources. However, published data for natural plankton populations, and in particular zooplankton, is extremely limited.

The ΣV parameter (McCarthy et al., 2007) has now been widely applied as a proxy for degree of diagenetic resynthesis of amino acids (e.g., Calleja et al., 2013; Hannides et al., 2013; Batista et. al., 2014). This parameter has typically been used as an indicator of relative bacterial AA resynthesis. However, while ΣV has been hypothesized to also increase somewhat with metazoan heterotrophy, only a few prior data points exist for ΣV in zooplankton or mixed plankton tows (McCarthy et al., 2007). These basin-wide observations represent, to our knowledge, the first comprehensive data on ΣV in natural mixed plankton sources. The relatively narrow ΣV range observed across our entire data set (mean±sd = 1.4±0.19) therefore is somewhat remarkable. This value for mixed zooplankton, crossing very different ocean regions, establishes a first clear threshold for ΣV values in natural zooplankton, corresponding closely with previously hypothesized values (1.0 to 1.5). These data grounds the idea that at least in oceanic material, ΣV values over ~ 2 represent a clear threshold for major microbial alteration (McCarthy et al., 2007).

The THAA parameter represents a proxy for $\delta^{15}N$ value of total proteinaceous material (e.g., McCarthy et al., 2013). The significant linear regression with slope of ~1between the isotopic signature of total hydrolyzable proteins ($\delta^{15}N_{THAA}$) and that of bulk organic matter $\delta^{15}N$ (Fig. 4.4a) is therefore consistent with all size fractions having very similar bulk biochemical composition. This further indicates, as would be expected for these samples, that analyzed organic matter is "fresh", derived from local primary production sources, without substantial input of allochthonous detritus. This inference is also supported by the significant correlation between $\delta^{15}N_{Phe}$ and $\delta^{15}N_{bulk}$ (Fig. 4.4b), which shows that all plankton size fraction $\delta^{15}N$ values co-vary with baseline $\delta^{15}N$ signatures. In addition to confirming similar bulk nitrogenous biochemical composition, this observation also confirms that our CSI-AA TP estimates can be confidently interpreted as representing local plankton.

The quantitative relationships between THAA, $\delta^{15}N_{Phe,}$ and $\delta^{15}N_{bulk}$ in ocean plankton sources represent critical information to make both paleoceanographic interrelations from CSI-AA data (e.g., Batista et al, 2014; Sherwood et al., 2014), as well to construct precise isoscapes based on CSI-AA data in modern organisms (Vokhshoori and McCarthy, 2014; Vokhshoori et al., 2014). The significant regressions we observed for both parameters in plankton sampled across the entire Atlantic basin confirm that both, THAA or $\delta^{15}N_{Phe}$ can be reasonably used to reconstruct $\delta^{15}N$ values of plankton sources. The regression intercepts (3.4‰ and -1.7‰, for THAA and $\delta^{15}N_{Phe}$ respectively) represent the key correction factors for deriving baseline $\delta^{15}N$ values from either living organisms or paleoarchives. In particular, the 3.4‰ intercept for THAA vs. bulk $\delta^{15}N$ regression is very similar to the offset found for both phytoplankton and bacteria (McCarthy et al., 2013; Batista et al., 2014). These results imply that, on average, the differential isotope fractionation between total protein and all other cellular nitrogenous molecules (nucleic acids, amino sugars, chlorophyll, etc) is remarkably similar across multiple oceanic fresh biomass sources. The very strong correlation observed in all samples (r^2 =0.93; Fig. 4.4a) further indicates a remarkable similarity of this $\delta^{15}N$ offset across plankton groups and size classes. Because $\delta^{15}N_{Phe}$ does not change appreciably with trophic transfer, $\delta^{15}N_{Phe}$ has often been taken as a direct proxy for baseline $\delta^{15}N$ (e.g., Vokhshoori and McCarthy, 2014; Sherwood et al., 2014). The slightly lower regression coefficient observed for

$\delta^{15}N_{Phe}$ vs. $\delta^{15}N_{bulk}$ (r^2 =0.73) is consistent with variability in TP for the different plankton size classes sampled. The offset indicated by our ocean-wide regression intercept (-1.7) is therefore expected, based on increase in $\delta^{15}N_{bulk}$ with TP in zooplankton. Nevertheless, the surprisingly strong general relationship across all size classes suggests that this value might be explored to reconstruct average zooplankton $\delta^{15}N$ values from $\delta^{15}N_{Phe}$ values measured in sinking POM or paleoarchives.

4.4.2.1 Threonine

Understanding the $\delta^{15}N$ isotopic values of Thr is currently one of the frontiers of CSI-AA work. Originally classified as a "source" AA based on early results from zooplankton (McClelland and Montoya, 2002), Thr is now recognized as unique, displaying "inverse" $\delta^{15}N$ fractionation behavior with trophic transfer. While it seems clear that Thr has novel tracer potential, its systematics are currently very poorly understood. Values of $\delta^{15}N_{Thr}$ have been suggested to represent an alternate (but inversely fractionating), indicator of trophic position (McMahon et al., 2015a; Bradley et al., 2015), a metabolic indicator for physiological stress or starvation (Hare et al., 1991), and a possibly unique tracer for the metabolism of specific organism groups (e.g. marine mammals; Germain et al., 2013). Our data set measuring $\delta^{15}N_{Thr}$ across plankton size classes therefore represents a unique opportunity to examine the systematics of $\delta^{15}N_{Thr}$, directly in the context of changing TP, across ocean basin-scale populations of similar organisms.

The depletion in the $\delta^{15}N_{Thr}$ with increasing TP of plankton (Fig. 4.5) supports results from other consumers (e.g. Styring et al., 2010; Sherwood et al., 2011; Germain et al., 2013), consistent with a fundamental difference between the biochemical transamination pathways for Thr vs. all other AA. Specifically, the strong inverse linear relationships with TP are consistent with a possible inverse isotope effect hypothesized for Thr transamination (McMahon et al., 2015a), leading to $\delta^{15}N_{Thr}$ values that are inversely correlated with TP in all zones. Our data also indicates surprising large negative TEF values for Thr (~-14 to -16‰, based on Phe normalized regressions), which are far greater than those originally reported for zooplankton. McClelland and Montoya (2002) originally reported TEF_{Thr} values of ~ -1‰ in limited plankton feeding experiments. Such modest TEF values were the reason for the initial classification as a "source" AA, but

were also consistent with early observations of $\delta^{15}N_{Thr}$ values in sinking particles from the equatorial Pacific (McCarthy et al., 2007). However, the regressions calculated for ($\delta^{15}N_{Thr}$-$\delta^{15}N_{Phe}$) vs. TP in our data (Fig. 4.5) indicate negative TEF values for zooplankton heterotrophy at least an order of magnitude greater. The offset between the two regression lines in Fig. 4.5 is mainly a reflection of the offset in baseline values between the central zone (with maximum N fixation) vs. the other zones. The predicted $\delta^{15}N_{Thr}$-$\delta^{15}N_{Phe}$ values at TP= 1 (3.99 and 13.33 ‰, respectively for the central and eastern zones) should indicate the average offset of $\delta^{15}N$ values of Thr vs. Phe in autotrophic primary production (i.e., the relative β values of these AA) in each zone. The values in the central zone are very similar to values observed in algal growth experiments across multiple species (~3.5‰; McCarthy et al., 2013), however the intercept in the eastern zone is inconsistent with any previous culture data, and so seems less reasonable.

While the regression results for the eastern zone suggest caution in comparing exact values, the significance of the two regressions at least implies that characteristically different TEF_{Thr} values may exist between ocean regions. If correct, this would imply that specific TEF_{Thr} values may be linked to planktonic ecosystem structure. As noted above, recent feeding experiments in fish (McMahon et al., 2015a) have suggested that variations in amino acid TEF values are strongly mechanistically linked to diet composition (in particular to relative protein content), as opposed to being linked to trophic level per se. In that data, Thr had one of the most extreme ranges in TEF values of any amino acid, (~ -2 to -10 ‰, depending on diet composition). The higher end of this TEF_{thr} range found in teleost fish is in fact generally similar to the TEF_{thr} implied by in our data regressions, making the very large differences in TEF_{thr} vs. the original McClelland and Montoya (2002) work puzzling. However, the apparent differences between zones do imply that $\delta^{15}N_{Thr}$ values in plankton (or sinking particles, and in turn the sedimentary record) may be linked to local food webs, suggesting future work is required to explore interpretation of $\delta^{15}Nt_{Thr}$ values in specific oceanographic regimes.

Overall, this unique data set offers several novel conclusions, but also points to a number of major uncertainties for future research. It seems clear from both regressions and directly measured values that that TEF_{Thr} values in zooplankton consumers are in fact not universally low, as was indicated by early research (McClelland and Montoya,

2002, McCarthy et al., 2007). The approximate TEF_{Thr} values derived from our regressions with TP instead are more similar to the highest TEF_{Thr} values recently reported in controlled feeding experiments with fish (McMahon et al., 2015a). Together with apparent offsets in TEF between ocean zones, this may indicate far more variability in TEF_{Thr} than for other AA, suggesting food-web specific interpretation of Thr values may ultimately be required. Such differences may also underlie the large ranges in $\delta^{15}N_{Thr}$ values observed in different organisms with similar trophic levels (e.g., deep sea corals; Sherwood et al., 2014a; vs. harbor seals; Germain et al., 2013), as well as differences in region-specific $\delta^{15}N_{Thr}$ values in plankton tows and sinking particles (McCarthy et al., 2007; Batista et al., 2014).

4.4.3 Propagation of diazotrophic N along the planktonic food web

Our new computations of $\%N_{fix}$ using CSI-AA resulted in a major increase of estimated importance of diazotrophy for the oligotrophic N Atlantic in the west and east zones, while the values estimated the CSI-AA vs. bulk $\delta^{15}N$ approaches in the central zones are both fairly similar (Fig. 4.6a, b). Previous estimations using $\delta^{15}N_{bulk}$ indicated than up to 65% of nitrogen in zooplankton could be derived from biological fixation of atmospheric nitrogen in the central zone, but in west and east average zonal values based on $\delta^{15}N_{bulk}$ were slightly lower (Montoya et al., 2002; Landrum et al., 2011; Mompeán et al., 2013, Fernández et al., 2014). These earlier estimates were based on the comparison of $\delta^{15}N_{bulk}$ of areas with and without significant rates of nitrogen fixation, and thus critically dependent on assumptions about $\delta^{15}N$ values for subtropical areas, rather than actual measured data. CSI-AA based $\%N_{fix}$ estimates in this study reproduced the main observation and also the values of highest impact of N fixation in the central zone found in previous studies, however the differences in eastern and western zones suggest that $\%N_{fix}$ estimates based on broad assumptions of $\delta^{15}N_{bulk}$ values may be slightly less accurate than those based on $\delta^{15}N_{Phe}$, used as an internal, molecular level, record of baseline $\delta^{15}N$ values.

As noted above (*see Materials and methods*) the exact values for our CSI-AA based $\%N_{fix}$ approach also depend on the offset between $\delta^{15}N_{bulk}$ and $\delta^{15}N_{Phe}$ in autotrophic sources (β_{Phe}). While a single exact value of β_{Phe} cannot be definitively known for such broadly

distributed natural samples, the use of $\delta^{15}N_{Phe}$ as an internal, direct proxy for baseline $\beta^{15}N$ value for every sample should yield more accurate $\%N_{fix}$ patterns. Further, variation in β_{Phe} between currently published averages would mainly slightly shift the total $\%N_{fix}$ estimates either higher or lower, but it should not affect the pattern of intra-sample offsets. Therefore, we hypothesize that CSI-AA based estimates are fundamentally more precise in their ability to indicate intra-sample variations in $\%N_{fix,}$ between different plankton size classes, or samples from different regions. The sample-specificity of correcting for baseline $\beta^{15}N$ in the CSI-AA approach is therefore in direct contrast to $\delta^{15}N_{bulk}$ based estimates, which must apply sweeping assumptions about end member $\beta^{15}N$ values to entire sample sets.

These new CSI-AA estimations of $\%N_{fix}$ in fact suggest a fairly constant transmission of the diazotrophic signal up the food web in all zones, as the $\%N_{fix}$ values showed little variation with plankton size within zones. In contrast, the estimates using the traditional $\delta^{15}N_{bulk}$ approach showed an almost uniform contribution of diazotrophic nitrogen across plankton size fractions only in the central zone while there was an apparent increase of $\%N_{fix}$ with size in the other zones. While the increase of the diazotrophic signal in large plankton was attributed in previous studies to the effect of migrations and differences in turnover time (Landrum et al., 2011; Mompeán et al., 2013, Fernández et al., 2014) our results agree with a uniform transmission of nitrogen sources up the food web.

Finally, we note that averaging the impact of $\%N_{fix}$ across size classes in the different zones, our overall results correspond closely with several independent indicators of nitrogen fixation (Fig. 4.7). The filament-forming *Trichodesmium* was abundant mostly in the central zone, however also was present in some stations of the eastern zone (Mompeán et al., 2013). However, there were also other organisms responsible for nitrogen fixation, as the study of Benavides et al. (2103) showed significant nitrogen fixation rates in the <10µm plankton size fraction across the same transect. We therefore hypothesize that such small nitrogen fixers are most likely responsible for the relatively low $\delta^{15}N_{Phe}$ measured in the western zone, which clearly indicates the an isotopically low baseline nitrogen source. Another indicator of the importance of nitrogen fixation, N*, clearly pointed to the central zone as a region with high potential

for N fixation, in close agreement with our minimum $\delta^{15}N_{Phe}$ and maximum $\%N_{fix}$ values. It is also possible that low $\delta^{15}N$ values in the source nitrogen can also result from a strong fractionation of inorganic nitrogen during phytoplankton uptake (e.g., Waser et al., 1998), or from combined nitrogen in atmospheric deposition (Knapp et al., 2010). However, isotopic fractionation does not occur when dissolved nitrogen concentrations are very low (i.e., conditions of essentially complete inorganic N utilization), as in our study area (Mompeán et al., 2013), while atmospheric deposition of inorganic nitrogen is expected to be lower in the open ocean than in areas near the continents.

Overall, our data further supports the increasing evidence of a larger prevalence of nitrogen fixation in most of the subtropical N Atlantic than has been previously appreciated (Benavides et al., 2013; Fernández et al., 2014), in contrast to prior studies focused mainly on the western region with maximum abundances of *Trichodesmium* (Montoya et al., 2002, 2007; McClelland et al., 2003; Luo et al., 2012). These new results also point to the key role of zooplankton in the transmission of the diazotrophic nitrogen up the food web.

4.5 OVERVIEW AND CONCLUSIONS

The measurements in this basin-scale sample set represent, to our knowledge, the most extensive data for CSI-AA patterns in oceanic plankton. The strong correspondence in $\delta^{15}N$ values between bulk organic matter and protein-AA indicated that the relative $\delta^{15}N$ value offsets between different nitrogenous biochemical classes in plankton is remarkably homogeneous across ocean regions, and also across plankton size classes spanning several orders of magnitude. The specific offsets identified between bulk and both total protein $\delta^{15}N$ and $\delta^{15}N_{Phe}$ values also provide new and well-founded calibration relationships, necessary for reconstruction of baseline $\delta^{15}N$ values from CSI-AA data measured in paleoarchives (Sherwood et al., 2011; 2014a,b; Batista et al., 2014). Similarly, the narrow range observed in the ΣV parameter across all samples confirms previous hypotheses about changes in this parameter in zooplankton (McCarthy et al., 2007), while establishing a firm threshold for evaluating ΣV as a degradation indicator in

sinking particles and sediments. Finally, the rapid decrease of $\delta^{15}N$ values in Thr with plankton size class indicates TEF_{Thr} values far lower than previously reported for zooplankton, similar in fact to those reported in top consumers. Together with apparent offsets in TEF_{Thr} between our ocean zones, these data also implies that food web specific interpretation of Thr values should be investigated. We suggest that our new plankton data should be crucial in mechanistically understanding $\delta^{15}N_{Thr}$ systematics, and that $\delta^{15}N_{Thr}$ may be a useful new parameter to compare trophic structure of communities driven by different nitrogen sources.

While previous studies used single species or size classes (McClelland and Montoya, 2002; McClelland et al., 2003; McCarthy et al., 2007), our analysis showed for the first time that CSI-AA of ocean-basin scale natural zooplankton populations can represent a realistic indicator of the sources of nitrogen at the base of the food web, and also of the relative trophic position of plankton size classes. Our CSI-AA based estimates also revealed far more homogeneous spatial patterns of nitrogen fixation relative to bulk $\delta^{15}N$ estimates. We hypothesize that because CSI-AA based estimates of N fixation are internally normalized to $\delta^{15}N$ baseline values for every sample, they are inherently more precise, removing uncertainties associated with the broad assumptions generally required with bulk $\delta^{15}N$ values, CSI-AA in plankton size fractions indicated that atmospheric nitrogen inputs affected biological production in larger areas of the subtropical N Atlantic than previous estimates have suggested. Even in regions influenced by upwelling of nutrient-rich deep waters (as in the eastern basin), or in the absence of common blooms of nitrogen-fixing organisms (as in the western zone), the isotopic signal effectively traced the relative magnitude of nitrogen sources most likely from atmospheric origin. With a trophic structure largely related to organism size rather than nitrogen source, this implies that zooplankton integrate the inputs of nitrogen fixation across time and space, ultimately transferring diazotrophic N to top consumers.

CAPÍTULO 5

Síntesis

A lo largo de esta tesis hemos utilizado los isótopos estables como principal herramienta en el estudio del plancton oceánico. Se realizó un amplio muestreo durante la campaña Malaspina-2010 por los tres grandes océanos del planeta (Atlántico, Pacífico e Indico), donde los buques oceanográficos Sarmiento de Gamboa y Hespérides surcaron aguas de tres de los cuatro biomas oceánicos (Trades, Coastal y Westerlies) y 16 provincias biogeográficas (Longhurst, 2007).

Este gran número de observaciones sugiere que las relaciones encontradas podrían ser aplicadas de forma general en los ambientes pelágicos no polares. Las zonas muestreadas incluyen un ámplio rango de valores de producción primaria, desde zonas de afloramiento altamente productivas (ej. Benguela) hasta los giros oligotróficos subtropicales. En el Atlántico Norte subtropical además se tiene en cuenta la variabilidad temporal, ya que esta zona fue muestreada en dos estaciones del año diferentes.

Así se resolverá el objetivo principal que se plantea en esta tesis que es analizar los patrones de variabilidad en las fuentes de nutrientes y la complejidad de la red trófica planctónica a distintas escalas espaciales. Por la mayor rapidez en el análisis de las muestras se comenzó con el estudio de la abundancia natural de isótopos estables de nitrógeno y carbono en distintas clases de tamaño individual de los organismos del plancton de la zona del Atlántico Norte (Capítulo 3, publicado como Mompeán et *al* 2013) donde también se pudieron comparar los resultados de isótopos con otras variables ambientales medidas en esta campaña como temperatura, salinidad y concentración de nutrientes inorgánicos. Éstos primeros resultados a una escala regional mostraban una relación en la distribución de los isótopos con el tamaño del plancton, lo que dio lugar a realizar el estudio a gran escala con todos los datos medidos en las distintas fases de la campaña Malaspina 2010. Así se calcularon los espectros de tallas centrándonos en el isótopo del nitrógeno, que nos daba una información más clara en esta relación de tallas con abundancia isotópica. Por el gran volumen de datos que

supuso esta campaña, la mayor campaña oceanográfica de los últimos tiempos, no se pudo disponer a tiempo de todos los datos de nutrientes medidos en laboratorio, como los nitratos o fosfatos empleados en la zona del Atlántico Norte, para hacer una comparativa global con los datos de isótopos estables. Como alternativa se utilizó otro estimador del enriquecimiento de nutrientes como son los valores de concentración de clorofila medidos *in situ*. Así en el Capítulo 2 se estudian los patrones espaciales en la estructura de la red trófica a gran escala aplicando modelos continuos de distribución por tamaños de la biomasa y el $\delta^{15}N$ para el plancton de las capas superficiales del océano.

Por último en el Capítulo 4 de esta tesis, para corroborar la importancia de la fijación de nitrógeno en el Atlántico Norte (Capítulo 3) y estudiar la posición trófica y el estado metabólico del plancton, se utilizó una herramienta muy útil y que nos da mucha información con un volumen muy pequeño de muestra, que es el análisis de isótopos estables en aminoácidos.

5.1 ESPECTRO DE TALLAS Y FUENTE DE NUTRIENTES

Como hemos introducido en esta síntesis por el volumen de muestra primero se hicieron los análisis de un transecto en la región subtropical en el Atlántico Norte, que se engloba en el bioma Westerlies, una escala geográfica en la que se observó una gran variabilidad geográfica en la composición isotópica del plancton de distintos tamaños (Capítulo 3). Un análisis posterior de estos resultados mediante el cálculo de los espectros de tamaño normalizados reveló que en esta región del Atlántico Norte tanto el intercepto y pendiente del espectro de biomasa como el intercepto del $\delta^{15}N$ eran bajos en comparación con otras regiones (Capítulo 2). En contraste, en el resto de biomas muestreados los parámetros que definen el espectro de tallas presentaron menor variabilidad. Esta homogeneidad en el plancton del océano abierto está relacionada con las diferentes fuentes de nutrientes en estas zonas que controlan la productividad. En la mayor parte del océano la productividad depende de los nutrientes disponibles en la capa superficial o los que provienen de las zonas de afloramiento, sin embargo en las regiones con giros oligotróficos la productividad es escasa pero se producen entradas

locales de nutrientes que favorecen, por ejemplo, fenómenos como la fijación de nitrógeno atmosférico.

Para la comparación de los resultados de isótopos estables con datos de nutrientes, para el primer estudio (Capitulo 3) en el Atlántico Norte se pudieron utilizar los valores analizados de nitratos y fosfatos. Los fosfatos no aportaron mucha información ya que se encontraron valores bastante bajos y homogéneos en toda la columna de agua analizada y a lo largo del transecto. Sin embargo los nitratos aunque en la capa superficial fueron escasos presentaron un aumento importante a partir de 75-100m (Figura 3.2) y sobre todo en los extremos del transecto. Estos datos sugieren que en estas zonas se produce una mayor entrada de NO_3^- por advección de las zonas profundas. Este NO_3^- está más enriquecido en ^{15}N (Montoya et al. 2002) y su utilización por el plancton explicaría los elevados valores de $\delta^{15}N$ observados en el plancton muestrado en estas zonas en este estudio.. Por el contrario, los bajos valores de $\delta^{15}N$ planctónico en la zona central sugieren un mayor uso de formas de nitrógeno regenerado (principalmente amonio) o derivado de la fijación de N_2 atmosférico, ya que estas formas de nitrógeno están menos enriquecidas en ^{15}N (Montoya 2008).

En el estudio de todos los océanos (Capitulo 2) al no poder disponer a tiempo de los resultados de nitratos, estimamos la importancia de las entradas de nutrientes desde las capas más profundas por medio del DCM (profundidad del máximo de clorofila), la capa donde se acumula la biomasa fitoplanctónica que resulta más superficial en zonas de afloramiento (Capítulo 3). La importancia de las entradas de nutrientes desde las capas profundas se refleja en los interceptos de los espectros de tamaño de la biomasa (C_a), un índice de la magnitud de la biomasa en los productores primarios (Zhou 2006), pues la biomasa de fitoplancton es mayor en zonas de afloramiento que en los giros oligotróficos con un menor aporte de nutrientes desde las capas profundas (Behrenfeld et al. 2006). Este estudio representa la primera aplicación del espectro de tamaño normalizado de biomasa utilizando isótopos estables. En este caso los cambios en el intercepto del espectro de tamaños están relacionados con el origen de la fuente de nitrógeno dominante para los productores primarios, mientras que la pendiente indica el fraccionamiento isotópico a lo largo de la red trófica. Por tanto se esperaran bajos interceptos en zonas donde se producen entradas de nitrógeno atmosférico, ya sea por

presencia de organismos diazotrofos, o por entradas de nitrógeno antropogénico, teniendo estas zonas valores de $\delta^{15}N$ cercanos a cero (Montoya 2008, Morin et al. 2009). Así se encuentran valores de $\delta^{15}N_a$ más bajos en el bioma Westerlies que en los biomas Trades o Coastal (Tabla 2.1), lo que concuerda con la mayor importancia de la fijación de nitrógeno atmosférico en el primer bioma (Capítulo 3). Por el contrario la pendiente $\delta^{15}N_b$ no tuvo diferencias significativas entre biomas lo que sugiere que la transferencia de nitrógeno en la red trófica es similar en todos los océanos. Por tanto, el cálculo de estos parámetros nos proporciona una herramienta clave para conocer las fuentes de nitrógeno dominantes a escala local o regional.

5.2 ESPECTRO DE TALLAS Y ESTRUCTURA TRÓFICA

Los estudios previos no siempre han encontrado relaciones lineales significativas entre $\delta^{15}N$ y el tamaño del plancton, lo que se ha atribuido a factores que incrementan la variabilidad isotópica local como el desfase temporal entre productores y consumidores (Fry & Quinones 1994, Rolff 2000, Bode et al. 2003, Mompeán et al. 2013). Esta variabilidad limita la comparación de comunidades. Sin embargo en nuestro estudio el proceso de normalización permite obtener parámetros característicos (el intercepto y la pendiente del espectro) en todas las situaciones, a pesar de la variabilidad local del $\delta^{15}N$, lo que proporciona una herramienta mejor para comparar diferentes comunidades. Los resultados de pendiente del espectro de isótopos sugieren la existencia de un patrón macroecológico común en la composición isotópica del plancton en función del tamaño, incluso cuando las fuentes de nutrientes (nitrógeno) son diferentes, se puede explicar porque la transmisión hasta la red alimentaria se hace por reacciones bioquímicas similares para todas las comunidades planctónicas.

En las comunidades tropicales y subtropicales que viven en aguas oligotróficas el principal mecanismo de transmisión de nitrógeno a niveles tróficos superiores requieren el reciclaje de materia orgánica disuelta a través de las comunidades microbianas y el uso de los nutrientes derivados por el fitoplancton y protozoos que los hacen accesibles al zooplancton (McClelland et al. 2003, Mulholland 2007). En contraste, el caso de las

comunidades boreales (no consideradas en esta tesis) esta transmisión se produce principalmente por herbivorismo y depredación por grandes organismos (Basedow et al. 2010, Stowasser et al. 2012).

Así sea cual sea el proceso de adquisición o pérdida de nutrientes en todos los ecosistemas, la aplicación del espectro normalizado de $\delta^{15}N$ en toda la red trófica, incluyendo peces y consumidores superiores, puede ser utilizado para analizar su variabilidad considerando los intercambios de nitrógeno.

5.3 BIOMASA PLANCTÓNICA Y NUTRIENTES EN EL ATLÁNTICO SUBTROPICAL

Como ejemplo de comunidad subtropical en nuestro estudio se empleó un transecto longitudinal del Atlántico Norte donde se observó un descenso gradual de biomasa desde los márgenes, cercanos a los continentes (zonas de afloramiento), hacía la zona central (giro oligotrófico). El giro oligotrófico del Atlántico Norte ocupa la mayor parte de la zona central de éste estudio y se caracterizó por los bajos valores de biomasa planctónica en todas las clases de tamaño, especialmente en el macrozooplancton (> 2000μm). Esta zona es conocida por la baja advección de nutrientes desde zonas profundas, que explica los bajos niveles de producción primaria (Behrenfeld et al. 2006). Se esperaría una correlación negativa entre la biomasa planctónica y la temperatura o salinidad de la capa superficial (SST, SSS) ya que la estratificación en estas zonas se produce por la capa superficial cálida. Sin embargo en este estudio sólo el macrozooplancton se correlacionó con SST y el plancton de tamaño pequeño (<500 μm) con SSS, lo que sugiere que la biomasa planctónica no sólo está relacionado con la estratificación (y por tanto con las limitaciones a las entradas de nutrientes desde las capas profundas), sino que estarían implicadas otras entradas de nutrientes.

Los isótopos de nitrógeno y carbono muestran una gran variación a lo largo del transecto. Se podría relacionar la cantidad de $\delta^{13}C$ con la presencia de diatomeas en la zona siendo así más elevados estos valores en las zonas de afloramiento de las islas Canarias. Sin embargo en este estudio no fue la composición de la comunidad la primera causa de variación de $\delta^{13}C$, sino que los valores de $\delta^{13}C$ medidos parecen reflejar la

fuente principal de carbono inorgánico para la producción primaria: CO_2 atmosférico en la zona central y de advección vertical en las zonas laterales, ya que el CO_2 en las aguas que afloran está empobrecido en ^{13}C (Gruber et al. 1999).

La correlación lineal entre isótopos de carbono y nitrógeno encontrada, refleja la importancia de los procesos biogeoquímicos frente a la composición del plancton. Tanto los valores de la pendiente como los coeficientes de correlación fueron mayores en clases de talla <1000 µm que en las clases de talla superiores, lo que podría indicar que la presencia ocasional de grandes organismos (como salpas y pterópodos) puede causar la gran variabilidad en $\delta^{13}C$ del macrozooplancton, mientras que el zooplancton <1000 µm (compuesto en gran parte por copépodos, Tabla 3.2) mostró menor variabilidad en $\delta^{13}C$.

Los bajos valores de $\delta^{15}N$ medidos en el plancton apoyan la hipótesis de que la fijación de N_2 atmosférico o el uso de nitrógeno regenerado, como el amonio, serían la fuente principal de nitrógeno en la zona central. Esta interpretación se corresponde con la correlación negativa determinada entre la abundancia de *Trichodesmium* y los valores de $\delta^{15}N$ en todas las clases de tamaño del plancton (Fig 3.9). En contraste, los valores más elevados de $\delta^{15}N$ encontrados en los extremos del transecto indican el uso de NO_3^- por advección, ya que el NO_3^- de aguas profundas está más enriquecido en ^{15}N (Montoya et al. 2002).

5.4 IMPACTO DE DIAZOTROFÍA EN EL ATLÁNTICO NORTE SUBTROPICAL

Los resultados obtenidos en esta tesis indican que la fijación de nitrógeno es una de las fuentes principales de nitrógeno para el ecosistema pelágico en esta región. Los datos de alta resolución espacial (Capítulo 3) revelaron que la influencia de la diazotrofía es alta en la región central de los giros subtropicales (>50 %), pero sigue siendo importante en los bordes de los giros (20-38 %) (Fig. 3.10), mientras que los estudios anteriores destacaron el mayor impacto de diazotrofía en la zona oriental (Montoya et al. 2002, 2007). Posteriormente en el estudio de isótopos en aminoácidos se vio que esos valores

de fijación de nitrógeno eran similares en la zona central y ligeramente superiores en las zonas este y oeste del transecto (Fig. 4.6b).

En el análisis a partir de la medida global de $\delta^{15}N$ (Capítulo 3) la contribución de la diazotrofía fue casi uniforme a través de las distintas fracciones de tamaño del plancton en la zona central mientras que había un aparente incremento de $\%N_{fix}$ en las otras zonas, incluso en plancton de gran tamaño (Fig. 4.6a,b). En estudios anteriores este incremento de la diazotrofía en el plancton de mayor tamaño se atribuyó a las migraciones y a los diferentes tiempos de renovación de la biomasa en las distintas clases de tamaño (Landrum et al., 2011; Mompeán et al., 2013, Fernández et al., 2014). Sin embargo los últimos resultados del análisis de $\delta^{15}N$ en aminoácidos (Capítulo 4) sugieren una transmisión uniforme de las fuentes de nitrógeno a lo largo de la red trófica en todas las zonas.

Las causas de los bajos valores en $\delta^{15}N$ global, que como consecuencia se traducen en elevados porcentajes de diazotrofia ($\%N_{fix}$), pueden ser: la presencia de *Trichodesmium* y otros organismos fijadores de nitrógeno en las fracciones de tamaño superiores, o la existencia de compuestos orgánicos poco enriquecidos en ^{15}N en estas clases de tamaño. Así el uso del valor isotópico de nitrógeno del aminoácido fenilalanina ($\delta^{15}N_{Phe}$) como indicador del origen del nitrógeno de los productores primarios permite precisar mejor el grado de diazotrofia al reducir la incertidumbre en los valores de referencia de $\delta^{15}N$ para las zonas sin fijación de nitrógeno.

5.5 ANÁLISIS DE ISÓTOPOS ESTABLES DE NITRÓGENO EN AMINOÁCIDOS EN EL ATLÁNTICO NORTE

Con el estudio de isótopos estables en aminoácidos en el Atlántico Norte (Capítulo 4) se consigue mucha información a partir de una pequeña cantidad de muestras. Así además de afinar en el estudio de la influencia de la fijación de nitrógeno atmosférico en esta zona se estiman propiedades de la red trófica (ej. relación de tamaños, diversidad trófica, estado metabólico) a partir de la composición isotópica de los aminoácidos del plancton.

Con este análisis en aminoácidos se encontró una relación lineal positiva entre la señal isotópica de las proteínas ($\delta^{15}N_{THAA}$) y el valor isotópico en las diferentes fracciones de tamaño del plancton ($\delta^{15}N_{bulk}$) que indica que la materia orgánica analizada contiene bajo contenido en detritus, baja degradación y baja influencia de entradas no marinas. En esta relación el valor de la pendiente (= 1) indica una composición similar en todas las clases de tamaño mientras que el valor del intercepto (= 3,4) es similar al obtenido en estudios anteriores en fitoplancton y bacterias (McCarthy et al., 2013; Batista et al., 2014) y es coincidente con el valor promedio del enriquecimiento entre niveles tróficos (Post 2002). Además la correlación encontrada entre el valor de $\delta^{15}N_{Phe}$ y la posición trófica (TP) indica que todas las fracciones de tamaño covarían con los valores de $\delta^{15}N$ de las fuentes de nitrógeno para productores primarios, como requieren las asunciones de esta aplicación metodológica (McClelland y Montoya 2002, Chikairaishi et al. 2009).

El cálculo de la posición trófica por medio del análisis de aminoácidos mostró un estrecho rango de valores (TP<3 incluso en las clases de mayor tamaño), lo que sugiere una gran dependencia de la materia orgánica directamente derivada de productores primarios. El aumento de TP entre tallas fue muy pequeño (tabla 4.1), y sólo fue significativo en la zona central (figura 4.3). Esto puede ser explicado por la presencia de organismos con diferente TP en las diferentes fracciones de tamaño, sobre todo en las zonas extremas (este y oeste) del transecto.

Por último la relación de TP con el valor isotópico de la treonina ($\delta^{15}N_{Thr}$) confirma por primera vez en el plancton a escala oceánica el comportamiento especial de este aminoácido, que se incrementa de forma acusada en las posiciones tróficas más elevadas (Sherman et al. 2013, Batista et al. 2014). Un resultado interesante surge al restarle a $\delta^{15}N_{Thr}$ el valor de la fuente de nitrógeno, representado por $\delta^{15}N_{Phe}$, encontrando que en la zona central que todas las clases de tamaño contienen una alta proporción de materia orgánica derivada del fitoplancton (bajos valores de TP) comparado con otras zonas, esta diferencia no está causada por la fuente de nitrógeno de productores primarios. Esto sugiere la utilidad que puede tener la medida de $\delta^{15}N_{Thr}$ en futuros estudios de la estructura de redes tróficas ya que podría resultar un indicador de TP más preciso que los indicadores más utilizados actualmente basados en $\delta^{15}N_{bulk}$ o $\delta^{15}N_{Glx.}$

CAPÍTULO 6

Conclusiones

1. Los espectros de tamaño de isótopos estables de nitrógeno nos ofrecen una nueva herramienta para caracterizar la estructura de la redes tróficas planctónicas a través de los océanos.

2. Estos espectros de tamaño permiten calcular parámetros (como el intercepto $\delta^{15}N_a$ y la pendiente $\delta^{15}N_b$) que permiten inferir las fuentes de nitrógeno más relevantes y su transmisión por el plancton a escala local o regional.

3. Con estos espectros se ha encontrado que el bioma Westerlies tuvo valores de $\delta^{15}N_a$ más bajos que concuerdan con una mayor importancia de la fijación de nitrógeno en este bioma, sin embargo los valores de la pendiente $\delta^{15}N_b$ fueron parecidos en todos los biomas lo que sugiere que la transferencia de nitrógeno en la red trófica es similar en todos los océanos.

4. En el Atlántico Norte subtropical los resultados obtenidos amplían la extensión geográfica de la región en la que existe una gran dependencia de la red trófica planctónica de la fijación de nitrógeno atmosférico. La mayor influencia de la diazotrofía corresponde a la región central del giro subtropical donde se encontró además una correlación negativa entre la abundancia de *Trichodesmium* y los valores de $\delta^{15}N$ en todas las clases de tamaño del plancton.

5. Este estudio muestra por primera vez a escala regional que el análisis de isótopos en aminoácidos de las poblaciones naturales de plancton es un indicador de las fuentes de nitrógeno en la base de la red trófica y de su posición trófica.

6. Con el estudio de isótopos estables en aminoácidos se confirma la importancia de la fijación de nitrógeno en la mayor parte del Atlántico subtropical, y se reduce la variabilidad en las estimaciones del impacto de la diazotrofia en las distintas clases de tamaño del plancton.

7. La buena relación entre los valores de $\delta^{15}N$ de la materia orgánica y las proteínas en aminoácidos indica que la composición bioquímica del plancton oceánico es muy homogénea a través de los océanos, independientemente de la composición de especies y de la estructura de tallas de los organismos.

8. Las medidas de isótopos estables en aminoácidos en al Atlántico Norte indican una transmisión uniforme de las fuentes de nitrógeno a lo largo de la red trófica en todas las zonas del oceáno, en concordancia con la interpretación de las medidas de la pendiente del espectro normalizado de $\delta^{15}N$.

9. El rápido descenso de ^{15}N en treonina (Thr) a lo largo de la red trófica planctónica sugiere que las mediciones de $\delta^{15}N$ en Thr pueden mejorar la comparación de la estructura trófica de comunidades que utilizan diferentes fuentes de nitrógeno.

10. La extrapolación del espectro normalizado de $\delta^{15}N$ a toda la red trófica, incluyendo peces y consumidores superiores, puede ser utilizada para analizar su variabilidad y estructura considerando los intercambios de nitrógeno.

CAPÍTULO 7

Resumen

7.1 INTRODUCCIÓN

La estructura del plancton oceánico ha sido estudiada previamente a partir del tamaño de los organismos, se creía a los herbívoros como red trófica dominante en los ecosistemas marinos, hasta que se vio que la red trófica microbiana era el componente más importante de los sistemas acuáticos.

Estudios como Gasol et al. (1997) y Morán et al. (2004) muestran la relación de heterótrofos y autótrofos en aguas oceánicas, sugiriendo que en aguas abiertas el control de los consumidores se realiza de arriba abajo (top-down) y en las zonas de costeras al contrario (botton-up). Aunque una excepción clara a estos patrones aparece en zonas oligotróficas donde la limitación de nutrientes y luz es la que controla la producción. Este ejemplo nos muestra la complejidad de las redes tróficas y de los ciclos de carbono y nutrientes en los ecosistemas planctónicos sobre todos en sistemas oligotróficos de océano abierto.

Así, Longhurst (Longhurst 2007) propone una división de los océanos en cuatro biomas: Polar, Westerlies, Trades y Coastal. Esta división propone que los diferentes mecanismos de producción pelágica en los océanos se pueden agrupar en seis modelos simples que dependen de la limitación de nutrientes, la luz, la presencia de vientos,…, en las diferentes zonas oceánicas. En esta tesis el muestreo cubre tres de los cuatro biomas, todos a excepción del bioma Polar. Surcando así zonas con diferentes modelos de producción.

Esta producción primaria en los océanos puede estar limitada por elementos como P, Fe,…, aunque el nitrógeno es el principal factor limitante en grandes áreas de los océanos (Ryther & Dunstan 1971, Graziano et al. 1996, Moore et al. 2008). La producción primaria no se sostiene sólo por formas de nitrógeno orgánico regenerado siendo necesario un aporte de nitrógeno externo que compense las pérdidas por sedimentación (Capone et al., 2005; Ward et al., 2007). Este nitrógeno alóctono puede

entrar en grandes cantidades por mezcla vertical (ej. en invierno) o advección en zonas de afloramientos (upwelling o, en menores cantidades por difusión (McGillicuddy et al. 1998), deposición atmosférica (Duce, La Roche, et al. 2008) o por fijación biológica del N2 atmosférico (Mahaffey et al. 2005, Capone et al. 2005, Karl & Letelier 2008).

En las zonas oligotróficas como el giro del Atlántico Norte la fijación de nitrógeno es el aporte principal de nitrógeno para la producción primaria, y se produce por organismos fijadores (diazotrofos) como la cianobacteria *Trichodesmium*. Estos organismos debido a su toxicidad, transfieren este nitrógeno recién fijado al resto de la red trófica por exudación en forma de nitrógeno orgánico disuelto.

Así para cuantificar el flujo de nitrógeno en las redes tróficas, identificar las posiciones tróficas de los organismos e investigar la estructura de dichas redes ha sido utilizado el análisis de isótopos estables, en particular la composición de isótopos del nitrógeno (Hobson & Welch 1992, Yoshii et al. 1999, Ogawa et al. 2001, Fry 2006).

La relación de isótopos estables de nitrógeno ($\delta^{15}N$) se puede usar como estimador de la posición trófica porque el $\delta^{15}N$ de un consumidor está enriquecido con respecto a su dieta (Deniro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 2012). Aunque surgía un problema al estimar el valor de $\delta^{15}N$ en productores primarios debido a que incorporan el nitrógeno de varias fuentes (N_2, NO_3^-, NH_4^+) y al mismo tiempo son organismos con periodo de vida corto (Bronk & Glibert 1993, Rolff 2000, Dore et al. 2002). Rolff (2000) encontró un rango anual con valores de $\delta^{15}N$ para fitoplancton de 0‰ a 7‰ y un rango para zooplancton de 3‰ a 10‰.

Así este método fue mejorado con el análisis del isótopo de nitrógeno en aminoácidos (McClelland & Montoya 2002, McClelland et al. 2003, Chikaraishi et al. 2007).

Una de las mayores ventajas del método de análisis de isótopos en aminoácidos es que la posición trófica se estima a partir de dos aminoácidos de un sólo organismo, por tanto no se requiere la caracterización de los valores de $\delta^{15}N$ de los productores primarios. Además el método de los aminoácidos facilita la estimación de los niveles tróficos incluso en muestras pequeñas, lo que permite avanzar en la comprensión de las

estructura de las redes tróficas y el flujo de nitrógeno en ambientes naturales (Chikaraishi et al. 2009).

7.2 MÉTODOS

El muestreo se realizó durante la campaña de circunnavegación MALASPINA-2010 que a bordo de dos buques oceanográficos: BO Sarmiento de Gamboa y BIO Hespérides se recorrieron los océanos Atlántico, Índico y Pacífico. La campaña MALASPINA-2010 es un estudio multidisciplinar en el que se tomaron muestras físicas, químicas y biológicas del océano profundo y superficial, tanto para su estudio inmediato como para servir de material científico a generaciones futuras. En el BO Sarmiento de Gamboa (Enero-Marzo 2011) se realizó un transecto a lo largo del paralelo 24^{o}N entre las Islas Canarias y Florida (leg 8), y en el BIO Hespérides (Diciembre 2010-Julio 2011) se realizó un muestreo desde Cádiz hasta Cartagena (Murcia) recorriendo a lo largo de 7 tramos (legs) los océanos Atlántico, Indico y Pacífico.

Se tomaron muestras en 145 estaciones oceánicas a lo largo de tres cuencas oceánicas y 16 provincias biogeográficas, situadas en tres de los cuatro grandes biomas: Trades, Westerlies y Coastal, únicamente quedaría fuera de este muestreo el Bioma Polar. Para nuestro estudio en los dos buques oceanográficos se recolectaron muestras de plancton cada 24 horas en horario diurno en los primeros 200m y las propiedades estimadas del agua a través de lances de CTD en los primeros 300m desde la superficie.

Las muestras de plancton se dividieron en 5 fracciones de tamaño (40-200, 200-500, 500-1000, 1000-2000 y >2000μm) que se secaron a bordo y se utilizaron posteriormente en laboratorio para el análisis de biomasa, utilizando un analizador elemental (Carlo Erba CHNSO 1108) y para la abundancia natural de isótopos estables (δ^{13}C y δ^{15}N) que fue determinada usando un espectrómetro de masas (Finnigan Mat Delta Plus) acoplado a un analizador elemental. Además se conservó una alícuota en formol para la determinación posterior de abundancia de Trichodesmium a través de un análisis de imagen (FlowCam). Los detalles del muestreo y análisis se encuentran en Mompeán et *al.* (2013) y Moreno-Ostos et *al*. (2012).

De las muestras de plancton recopiladas en el leg 8 del Atlántico Norte se seleccionaron 5 muestras de cada parte del transecto (este, central y oeste) y se analizaron los isótopos estables en aminoácidos utilizando un espectrómetro de masas de relaciones isotópicas acoplado por combustión a un cromatógrafo de gases (gas chromatography /combustion/isotope ratio mass spectrometry GC/C/IRMS). Se analizaron los valores de $\delta^{15}N$ de 12 aminoácidos esenciales que se dividen en 3 grupos: aminoácidos fuente (glicine, Gly, serina, Ser, lisina, Lys y fenilalanina, Phe), aminoácidos tróficos (ácido glutámico, Glu, ácido aspártico, Asp, alanina, Ala, isoleucina, Ile, leucina, Leu, prolina, Pro y valina, Val) y un aminoácido "metabólico" (treonina, Thr). Con estos resultados se pudieron calcular parámetros como: la posición trófica (TP) a partir de los valores de Glu y Phe, la cantidad de proteínas, el estado metabólico o la fijación de nitrógeno atmosférico.

Las variables hidrográficas medidas en cada estación diaria incluyeron: temperatura, salinidad, fluorescencia, clorofila *a*, la profundidad del máximo de clorofila, la estratificación por la frecuencia de Brunt- Väisälä y nutrientes como el nitrato y fosfato (utilizado en este estudio sólo del Leg 8). También se han empleado bases de datos de satélite disponibles *on-line* para obtener información sobre la producción primaria anual media y la deposición de polvo atmosférico en la cercanía de las estaciones muestreadas.

7.3 RESULTADOS

La distribución de los espectros de biomasa y $\delta^{15}N$ es homogénea en todos los océanos, excepto en el Atlántico Norte subtropical (bioma Westerlies) donde los interceptos para ambos espectros fueron más bajos (C_a y $\delta^{15}N_a$) y las pendientes diferentes: más pronunciada la del espectro de biomasa (C_b) y menos la de $\delta^{15}N$ ($\delta^{15}N_b$) que en otras regiones.

Esta baja variabilidad en la mayor parte del océano refleja una gran homogeneidad en las fuentes de nitrógeno y la estructura de la red trófica en los océanos abiertos, a pesar de la variabilidad local en la biomasa. Los mayores cambios en las fuentes de nitrógeno

estuvieron relacionadas con entradas de nitrógeno atmosférico, lo que concuerda con una mayor abundancia de la cianobacteria diazotrófica *Trichodesmium* en el bioma Westerlies.

En el estudio más detallado de este bioma (Capítulo 3) se encontró que la zona central tiene una biomasa planctónica baja, y más alta en ambos extremos, este y oeste. Por otro lado los resultados de isótopos de nitrógeno mostraron una distribución espacial simétrica en todas las fracciones de tamaño, con los valores más bajos en la zona central ($\delta^{15}N<1‰$). Esta distribución está inversamente correlacionada con los valores obtenidos de isótopos estables de carbono ($\delta^{13}C$) y con la abundancia del fijador de nitrógeno *Trichodesmium*. Mayores valores de $\delta^{15}N$ del plancton en los extremos del transecto comparados con la zona central es consistente con una mayor entrada de NO_3^- por advección de las aguas marinas profundas, ya que este NO_3^- de aguas profundas está más enriquecido en ^{15}N (Montoya et al. 2002).

Con el estudio del espectro de tallas, se encontró baja variabilidad en las pendientes de $\delta^{15}N$ sugiriendo que la estructura trófica es similar a través de las diferentes comunidades planctónicas. A través del análisis de aminoácidos realizado en el Atlántico Norte subtropical se confirma un estrecho rango (1.8 a 2.5) en las estimaciones de la posición trófica (TP), con los valores más bajos en la zona central y un incremento general esperado en las clases de tamaño superiores.

Otros resultados interesantes del análisis de aminoácidos fueron las relaciones encontradas entre los valores de $\delta^{15}N$ de la materia orgánica total con el de las proteínas ($\delta^{15}N_{THAA}$) o el de Phe ($\delta^{15}N_{Phe}$), que pueden ser utilizadas para reconstruir los valores de $\delta^{15}N$ de las fuentes planctónicas.

Por último la relación de TP con el valor isotópico de la treonina ($\delta^{15}N_{Thr}$) confirma por primera vez en el plancton a escala oceánica el comportamiento especial de este aminoácido que disminuye de forma acusada en las posiciones tróficas más elevadas (Sherman et al. 2013, Batista et al. 2014).

7.4 DISCUSIÓN

El análisis comparativo de los espectros de tamaños de biomasa y $\delta^{15}N$ resultó una herramienta útil para estimar las fuentes de nitrógeno inorgánico y su transferencia en la red trófica como se muestra a grandes escalas espaciales con el plancton oceánico. Las diferencias entre los espectros de tamaños de biomasa y $\delta^{15}N$ sugieren homogeneidad en la transferencia neta de nitrógeno hacia niveles superiores de la red trófica.

Con el estudio de los aminoácidos, la buena relación entre los valores de $\delta^{15}N$ de la materia orgánica y las proteínas en aminoácidos indica que la composición bioquímica del plancton oceánico es muy homogénea a través de los océanos, independientemente de la composición de especies y de la estructura de tallas de los organismos.

En la zona del Atlántico Norte subtropical se ha encontrado que las fuentes de carbono y nitrógeno atmosférico prevalecen sobre las fuentes de aguas profundas. Además la zona influida por la diazotrofía es mayor de lo encontrado en estudios previos. Confirmado posteriormente con el estudio de aminoácidos incluso en regiones influidas por afloramientos de aguas profundas enriquecidas en nutrientes (como la zona este), o en ausencia de bloom de organismos fijadores de nitrógeno (como la zona oeste), la señal isotópica muestra la mayor influencia de fuentes de nitrógeno de origen atmosférico. Con una estructura trófica relacionada más con el tamaño de los organismos que con la fuente de nitrógeno, el zooplancton integra las entradas de nitrógeno atmosférico en el tiempo y el espacio, y transfiere el N diazotrófico a consumidores superiores.

Por último en relación a los resultados encontrados para el aminoácido Thr, el rápido descenso de los valores de $\delta^{15}N$ en Thr con las diferentes clases de tamaño indican que los valores del factor de enriquecimiento trófico para este aminoácido (TEF_{Thr}), son los más bajos que se han obtenido hasta ahora en zooplancton, similares de hecho a los obtenidos en consumidores superiores. Esto implica que la interpretación de la red trófica a través de los valores de Thr debería ser investigada. Así sugerimos que nuestros datos de plancton son cruciales en la comprensión de este parámetro, y que $\delta^{15}N_{Thr}$ sería

un nuevo parámetro útil para comparar la estructura trófica de comunidades impulsadas por diferentes fuentes de nitrógeno.

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